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Session 1:

TECHNETIUM CHEMISTRY AND RADIOPHARMACEUTICALS – I

Technetium radiopharmaceuticals, current situation and perspectives

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Technetium-99m has been the key radionuclide in Nuclear Medicine since four decades now and is still maintaining this privileged position in most departments, although the situation might change in the coming years. It owes this favourable role to its continuous availability, the convenient half-life of 6.02 h, its low radiation dose to patients and manipulators, the efficient and high resolution detection of this gamma radiation by conventional gamma cameras and the possibility to incorporate it as a transition metal in a wide variety of complexes.

Most of the first-generation ^{99m}Tc-radiopharmaceuticals are structurally not well characterized (e.g. complexes of ^{99m}Tc with diphosphonates, DTPA, soluble and denatured albumin, …) but they survive on the basis of a proven clinical usefulness. However, due to more stringent requirements with respect to manufacturing methods of labelling kits (GMP) and quality of starting matials, a tendency of discontinuation of some of these preparations has started, especially for products of biological origin or with a limited volume of sale.

In the eighties and the nineties, an intensive search for new ^{99m}Tc-radiopharmaceuticals based on a rational approach (e.g. cationic complexes for myocardial perfusion agents, neutral lipophilic compounds for brain perfusion tracers, …) and a steadily growing knowledge of Tc-complexation chemistry has resulted in efficient tracer agents for measurement of kidney function (^{99m}Tc-mertiatide), myocardial perfusion (^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin) and brain perfusion (^{99m}Tc-exametazime, ^{99m}Tc-bicisate). In the case of the heart agents, however, the designed rational approach appeared not the correct starting idea and the successful development of the tracers was more a lucky shot. On the other hand, several similar tracer agents with approriate characteristics for clinical use did not reach commercial availability (^{99m}Tc-ethylene dicysteine, ^{99m}Tc-NOET, ^{99m}Tc-furifosmin, ^{99m}Tc-HL-91) or were discontinued (^{99m}Tc-BATOs), mainly for reasons of economics.

The intensive search for ^{99m}Tc-radiopharmaceuticals also lead to the development of several ligand systems for the formation of stable ^{99m}Tc-complexes, such as Tc(V)O and Tc(V)dioxo complexes with a wide variety of tetraligands and diligands, Tc(V)nitrido complexes, Tc(I)-hexa-isonitriles, complexes of Tc with hydrazinonicotinic acid (Hynic), and most recently, the promising $Tc(I)(CO)_3$ complexes with numerous triligands. This knowledge has been exploited for the design of conjugates of a Tc-binding ligand with biomolecules (proteins, peptides, drugs binding to receptors, transporter proteins, enzymes, RNA) in order to allow labelling of the bioactive compounds with ^{99m}Tc and use of the radiolabelled agents for diagnosis and follow-up of specific diseases, especially cancers.

As derivatization with a ^{99m}Tc-chelate increases the molecular weight with at least 300 Da, it mostly seriously alters the biological properties of small compounds, rendering the radiolabeled biomolecule useless for the intended purpose (e.g. ^{99m}Tc complexes of conjugates of glucose, flumazenil, benzylguanidine, benzothiazoles,…). This constitutes a major challenge to the successful development of small ^{99m}Tc labelled receptor agents. The only positive example in this series is ^{99m}Tc-TRODAT-1, based on a conjugate of a BAT

tetraligand and a tropane, with preserved (although reduced) affinity for the dopamine transporter.

However, in the case of relatively large biomolecules such as peptides and proteins, derivatization with a ^{99m}Tc-chelate is relatively well tolerated without compromising too much the affinity and specificity for the target structure. Examples of clinically useful radiopharmaceuticals designed in this way are ^{99m}Tc-Hynic-tricine-annexin for imaging of apoptosis, ^{99m}Tc-EDDA/HYNIC-Tyr(3)-octreotide and ^{99m}Tc-N4-[Tyr3]octreotate for visualisation of neuroendocrine gastro-entero-pancreatic tumours, complexes with peptides in which a sequence is incorporated to provide a donor atom set which allows stable complexation of technetium-99m such as ^{99m}Tc-P587 and ^{99m}Tc-P829 for somatostatin receptor imaging and ^{99m}Tc-apcitide for thrombus imaging.

Also for the latter tracer agents, the development into commercially available radiopharmaceuticals and the successful introduction into clinical practice are hampered by several factors, such as:

- the fact that radiopharmaceuticals have to follow now the same developmental pathway as non-radioactive drugs, implying a very high cost associated with toxicity studies and clinical trials which may surpass in many cases the expected revenues;
- the competition of positron emission tomography (PET) which offers a better spatial resolution and a more accurate quantitative measurement, and is better suited for screening the whole body for cancer and other diseases. Moreover, bioactive compounds can simply be labelled with carbon-11 or fluorine-18 without serious alteration of their structure, resulting in tracer agents with (almost) fully preserved biological properties.
- The competition of other imaging modalities which yield images with superior spatial resolution such as spiral CT and magnetic resonance imaging (MRI). In addition, with modern MRI instruments, also regional physiological and metabolic activity can be measured in various sites in the body using functional MRI techniques, contrast agents and nuclear magnetic spectroscopy.

These and still other factors constitute a serious challenge and obstacle, not only for a further successful development of new technetium-99m labelled tracer agents but also for maintaining the up to now monopolistic position of nuclear medicine procedures for functional and metabolic imaging.

^{99m}Tc-labelled minigastrin for tumour targeting: Optimization of labelling and peptide sequence

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Gastrin/CCK receptors are overexpressed in a number of tumours such as medullary thyroid carcinoma (MTC), neuroenteropancreatic tumours (NET) and small cell lung caner (SCLC). Therefore Gastrin analogues binding to the CCK-2 receptor are promising candidates for Nuclear Medicine imaging. Recently a Minigastrin derivative (eEEEEEAYGWMDF) has been labeled with ^{131}I, ^{111}In and ^{90}Y and evaluated in patients. However, due to its availability and optimal decay properties ^{99m}Tc would still be the radionuclide of choice for diagnostic applications. This paper describes the development of a ^{99m}Tc analogue and the optimisation of radiolabelling approaches and peptide sequence for targeting Gastrin/CCK receptor positive cells in vivo.

Two MG sequences, eEEEEEAYGWMDF (MG0) and eAYGWMDF (MG11) were derivatized both with HYNIC at the aminoterminus as well as MG0 with a HIS derivative ((N_α-His)Ac) for Tc-Carbonyl labelling. Labelling was perfomed at high specific activities (>1Ci/µmol) using Tricine and EDDA as coligands for HYNIC-MG0, HYNIC-MG11 and $[^{99m}Tc(OH_2)_3(CO)_3]^+$ for (N_α-His)Ac-MG0.

Stability experiments were carried out by RP-HPLC analysis in PBS, serum, histidine- and cysteine-solutions as well as rat liver and kidney homogenates. Plasma protein binding was determined by use of size exclusion chromatography using Microspin columns.

Competition experiments of unlabeled conjugates on CCK-2 receptor positive AR42J membranes versus [^{125}I]-Tyr^{12}-Gastrin I were used to determine receptor affinity for unlabelled conjugates under study. Receptor binding and internalisation experiments with the radiolabelled derivatives were performed also using AR42J rat pancreatic cells. Biodistribution experiments were carried out in nude mice carrying AR42J tumours by injection of ^{99m}Tc-labeled peptide with or without coinjection of 50µg cold MG.

At specific activities >1Ci/µmol both MG1 and MG11 could be labelled with yields >95% independently of the labelling approach and peptide sequence. Lipophilicity as determined by HPLC was in the order ^{99m}Tc-HYNIC-MG11>^{99m}Tc-HYNIC-MG0<^{99m}Tc-(N_α-His)Ac)-MG0.

Stability experiments of all ^{99m}Tc labeled conjugates revealed a high stability of the label in PBS and serum as well as towards challenge with histidine and cysteine. Only Tricine/HYNIC-labelled derivatives showed a somewhat decreased in vitro stability. Incubation in kidney homogenates resulted in a rapid degradation of all conjugates with <20% intact peptide after 60 minutes (min) at 37°C and no considerable differences between the radiolabeled conjugates. A somewhat lower degradation rate was seen in liver homogenates. Protein binding varied considerbly with lowest levels for ^{99m}Tc-EDDA/HYNIC-MG11. Plasma protein binding was lower for HYNIC derivatized peptides, but also for ^{99m}Tc-MG11 compared to ^{99m}Tc-MG0.

All peptides revealed high affinity for the MG receptor in the nanomolar range. Internalisation behaviour was very rapid for all labeled conjugates in the order of ^{99m}Tc-(N_α-His)Ac-MG0 > ^{99m}Tc-EDDA/HYNIC-MG0 >^{99m}Tc-EDDA/HYNIC-MG11>^{99m}Tc-Tricine/HYNIC-MG with maximum values >10%.

Overall biodistribution was comparable with rapid renal excretion and very low unspecific retention in most organs, revealing very promising distribution and excretion patterns with low blood and liver levels and low intestinal excretion. ^{99m}Tc-EDDA HYNIC derivatives matched the pharmacokinetic properties of the corresponding ^{111}In-labelled analogues. Maximum tumour uptake was found 4 hours (h) p.i. with highest tumour uptake levels of 7.11±0.22% ID/g for ^{99m}Tc-EDDA/HYNIC-MG11 and 8.09±1.87 ID/g for ^{99m}Tc-EDDA/HYNIC-MG0. These values were reduced by more than 80% by coinjection of cold MG. ^{99m}Tc-Tricine/HYNIC-MG0 (2.2%ID/g) and ^{99m}Tc-(N_α-His)-Ac-MG0 (1.2%ID/g) showed much lower levels of specific tumour accumulation. Kidney uptake and retention was dependent both on peptide sequence and labelling approach with very high levels for ^{99m}Tc-HYNIC-MG0 derivatives (up to (>100%ID/g), but much lower levels for ^{99m}Tc-EDDA/HYNIC-MG11(1.9% ID/g) and ^{99m}Tc-(N_α-His)-Ac-MG0(1.2%ID/g).

Tumour/organ ratios were found to be superior for ^{99m}Tc-EDDA/HYNIC derivatives, with best overall levels for ^{99m}Tc-EDDA/HYNIC-MG11 especially in kidneys.

For labelling of peptides targeting specific receptors both peptide sequence and labelling approach have to be optimized in the development of a suitable radiopharmaceutical. Our results in ^{99m}Tc-labelling of Minigastrin derivatives show that a high stability and hydrophilicity is necessary for suitable pharmakokinetics, but labelling strategy also considerably influenced both tumour uptake and kidney retention. ^{99m}Tc-HYNIC-MG11 showed advantages over ^{99m}Tc-MG0 analogues with much lower kidney retention without impaired uptake in tumours tissue. ^{99m}Tc-EDDA/HYNIC-MG11 seems to be a promising candidate for imaging CCK-2 receptor positive tumours.

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Design and synthesis of isoniazide mimetic conjugated with DTPA, potential ligand of novel radiopharmaceutical and contrast agent for medical imaging, Bis (amide) of diethyelene triaminepentaacetic acid: DTPA-Bis(INH)

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The structure of isonicotinic acid hydrazide was coupled to diethylenetriaminepentaacetic acid (DTPA) via amide linkage. The Overall yield of three steps synthesis starting from DTPA is above 80%. The new ligand binds with infection site through specific interaction with gene *iniA*, *iniB* and *iniC* under pathological conditions. In particular, complexation of DTPA moiety with ^{99m}Tc and Gd^{+}III showed excellent results as metallopharmaceutical for medical imaging.

Primary object of the present invention is to propose a novel INH derivative based on DTPA, which form stable complexes with most of lanthanides and transition metals in periodic table. Another object of the present invention is to introduce a chelating group without compromising its biological activity for early diagnosis of infection using nuclear medicine and MR techniques.

The DTPA-Bis(INH) was synthesized in high yield using simple synthetic procedure. Radiochemical purity was ascertained chromatographically using different solvent system. Blood kinetics in rabbits and biodistribution in mice was studied. The ability of DTPA-Bis(INH) to target infection site in vivo was assessed in gamma scintigraphic studies of normal rabbit and a rabbit with induced tuberculosis.

The DTPA-Bis(INH) was characterized by Mass spectroscopy in ESI positive mode, $M+H^{+}$ was found to be 632.2.The complex was successfully labeled with ^{99m}Tc radionuclide with more than 95% labeling efficiency.It was found stable up to 24h. Blood kinetics showed rapid first pass clearance with biological half life $t_{1/2}$ (F) = 11 min. Imaging of a normal rabbit and a rabbit with induced tuberculosis was carried out. An appreciable activity was visualized in liver and kidneys. In diseased rabbit similar pattern was observed with the accumulation of activity at the tubercular site at 24 h post injection. Biodistribution revealed major accumulation in liver 6.30±0.58%ID/g at 1 h and 6.25±0.11 at 4h and in kidneys 7.65±0.52%ID/g at 1h and 6.70±0.28% ID/g at 4h. Radiotracer uptake was also seen in bone 1.32±0.180%ID/gat1h. From the present work it can be concluded that the radiolabeled DTPA-Bis(INH)accumulate at the site of infection.

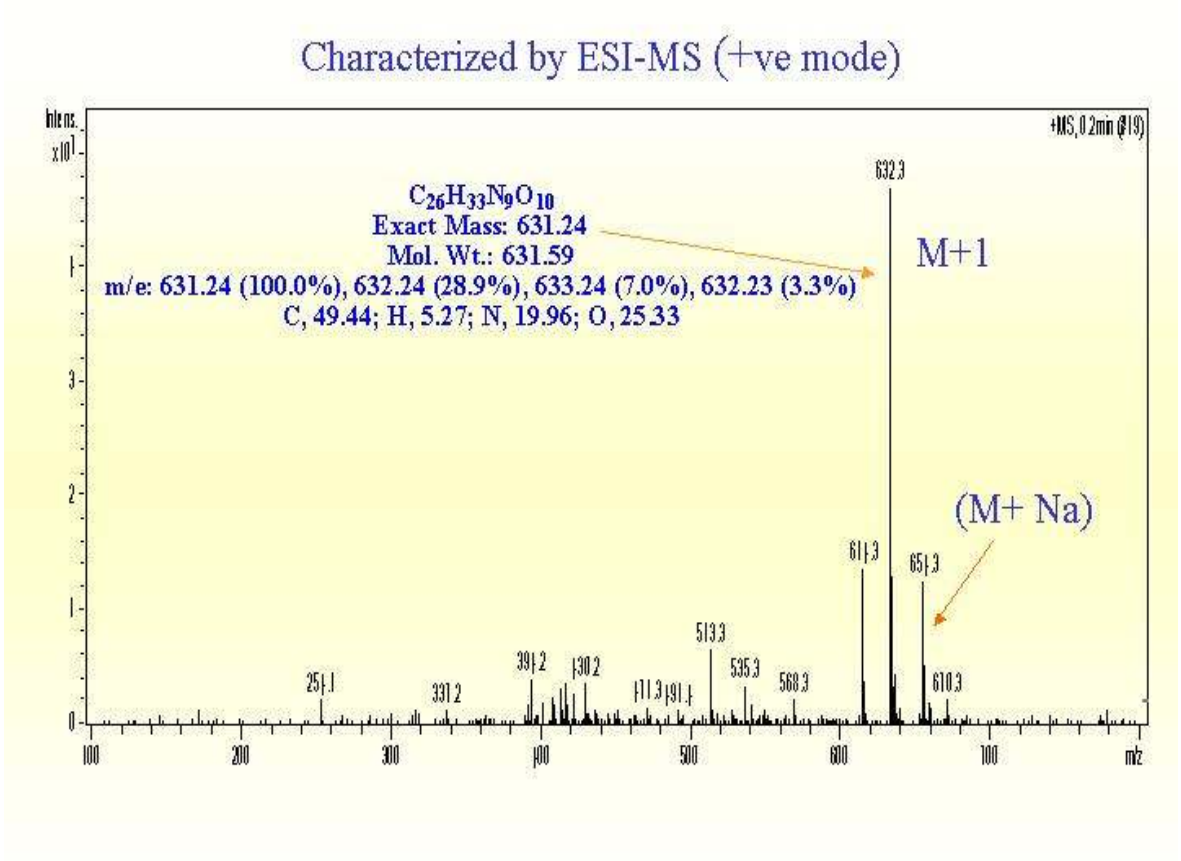

FIG 1. Mass Spectrum of DTPA-Bis(INH) ESI positive mode.

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Antimicrobial peptide ^{99m}Tc-UBI 29-41 for infection diagnosis: kit formulation and a preliminary clinical study

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Antimicrobial peptides that show specific binding to pathogens are potential precursors for the design of radiopharmaceuticals to be used in differential diagnosis of infections. For this study, the ubiquicidin derived peptide UBI 29-41 (TGRAKRRMQYNRR; 1,693 Da), was investigated. A lyophilized kit was developed and after labelling quality control was performed including its differential uptake in infected tissues in mice as well as the binding to viable bacteria. Based on the promising pre-clinical results kit-formulated ^{99m}Tc-UBI 29-41 was studied in a patient with hip prosthesis.

Each lyophilized kit contains 50.0 μg UBI 29-41, 13.2 μg SnF_2, 40.0 μg $Na_2P_2O_7.10H_2O$, 100 μg KBH_4 and 180 μg NaCl sealed under vacuum. Labelling was done by adding 370-1850 MBq of ^{99m}Tc-pertechnetate to the kit and variables such as times and temperatures of incubation were optimized. As control, a non-kit direct labelling of the peptide was done by adding ^{99m}Tc-pertechnetate (200-700 MBq) to a vial containing 100 μl UBI 29-41 (1 mM in 0.01M acetic acid pH 4.0), 40 μl SnCl2.2H2O (0.5 mg/ml) and 20 μL KBH4 (10 mg/mL). Radiochemical purity (RCP) of the radiolabelled peptide was evaluated using ITLC-SG, RP-HPLC and by Sep-Pak C18 reverse phase extraction. Biological controls were done in an animal model. Infections and sterile inflammations were induced in mice by intramuscular injection of 0.1 mL saline containing 108CFU/mL *Staph. aureus* or ~109 caloric killed bacteria, respectively. Labelled peptides were injected intravenously 24 h later and biodistribution studies were performed at 1 h after injection of the tracer. Activity uptake in the different organs/tissues was expressed as% of injected dose (% ID), and accumulation of the tracer in the infected muscle was expressed as a target to non-target ratio (T/NT). The presence of viable bacteria was determined using microbiological techniques and the accumulation of the tracer was expressed as function of the number of colony forming units.

To evaluate its potential as an infection-imaging agent in humans we have studied the kit formulated ^{99m}Tc-UBI 29-41 in a patient with a history of three reinterventions of right hip due a reiterated infected prosthesis within five years. At the moment the patient is suspected for infected prosthesis (great pain and high temperature local with elevated CRP and ESR).

After injection of 333 MBq a dynamic study was performed of pre determined areas of interest near both hips to quantify accumulation of the tracer in these areas. Additional imaging was performed to obtain optimal localization of the right hip prosthesis (target) and its corresponding contralateral normal area (non -target) at 15, 30, 60 min and 2, 3, 4 and 24 h. The T/NT were calculated at various intervals in order to determine the ideal imaging time. Whole-body anterior and posterior images were also acquired at 3 h to study biodistribution.

All chromatographic techniques did not indicate radiochemical impurities higher than 7%. Incubation for one hour at room temperature or for 5 min at 100^{o}C was required to reach highest labelling yields and labelled peptides were stable for at least 6 h post labelling.

In vitro binding to bacteria showed an increase in specific binding as function of number of bacteria reaching 32.7% for *S. aureus* (5.08E+06 cfu/mL). In mice, for ^{99m}Tc-UBI 29-41 renal clearance was >60% ID within 1 h after administration. In infected mice T/NT ratios were 2.3±1.0 (n=7) and 1.2±0.6 (n=2) for sterile inflamed tissues. Presence of viable bacteria, was confirmed in infected muscle (103-106 CFU/g tissue) and a good correlation (y=3.108x – 5.108; R=0.6652) between the ratio of accumulation and number of viable bacteria was calculated. Data were comparable to the (non-kit) control tracer.

In early dynamic images of the patient we observed equal perfusion in both hips. Accumulation in the probable infected hip was highest between 30 and 60 min after injection. The focus of accumulation was diagnosed at the proximal bone sector of the femur near the prosthesis.

The direct labelling of UBI peptides is rapid, efficient and stable showing specific uptake in infected tissues. We can conclude that ubiquicidin derived peptide UBI 29-41 labelled with technetium-99m is a promising tracer for bacteria specific imaging. The kit formulation allows the easy and safe use in nuclear medicine clinics. It was similar to that observed by Akhtar, et al. [1].

ACKNOWLEDGEMENT

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Session 2:

TECHNETIUM CHEMISTRY AND RADIOPHARMACEUTICALS - II

Preparation and evaluation of third generation technetium-99m radiopharmaceuticals

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The preparation and evaluation of three different technetium-99m labeled peptides as third generation radiopharmaceuticals were studied. Lys3-Bombesin ([Lys3]BBN), Ubiquicidin 29-41(UBI 29-41) and Tyr3-octreotide (TOC) were prepared as instant kit formulations to be labeled by direct or indirect methods with ^{99m}Tc in order to evaluate in vivo prostate malignancies, infection processes and lung cancer respectively.

[Lys3]BBN and TOC were conjugated to HYNIC [1] and UBI 29-41 used as a free ligand [2-3]. Structures of the corresponding peptides were built and the optimized structures, in the best stable configurations, were calculated by molecular mechanics and quantum-mechanical calculations. The Tc cation was added and the potential energy of the final structure evaluated. In order to correlate the calculated and experimental results, in vitro stability tests with cysteine challenge, human serum, dilution in saline solution and binding assays to bacteria or receptor specific cells were performed for each labeled peptide. The components of the lyophilized kits were selected to produce a direct ^{99m}Tc labeling for UBI 29-41 and ^{99m}Tc-EDDA/HYNIC-peptide for [Lys3]BBN and TOC. In vivo studies involved infected mice or implanted tumour cells in athymic mice. Whole body images from patients with suspected infection or lung cancer or prostate cancer were acquired.

Molecular mechanics and quantum-mechanical calculations were essential tools in explaining experimental results associated with molecular recognition and stability. For [Lys3]BBN, it was possible to demonstrate that the only site available to introduce HYNIC as Tc chelator was Lys3, obtaining a very high thermodynamic stability without interference in the stereospecificity of the C-terminal eight residues which are believed to contain the domain responsible for receptor recognition (Fig. 1). In the case of UBI 29-41, a peptide without cysteine residues, Lys and Arg7 could be the specific site to coordinate ^{99m}Tc in the UBI structure, in which the Arg7 amino group has a structural arrangement facing the Lys amino group and forming a good chelating cage for the technetium cation.

^{99m}Tc-EDDA/HYNIC-[Lys3]BBN, ^{99m}Tc-UBI 29-41 and ^{99m}Tc-EDDA/HYNIC-TOC obtained from lyophilized kits, showed radiochemical purities over 93%, high in vitro and in vivo stability and preservation of the molecular recognition after a simple kit reconstitution without further purification. Lyophilized formulations showed high stability during the storage at 4°C for 3-6 months. ^{99m}Tc-UBI 29-41 prepared by a direct method has adequate biokinetic properties and ability to detect infection foci in humans. ^{99m}Tc-EDDA/HYNIC-TOC has been useful in patients with lung cancer.

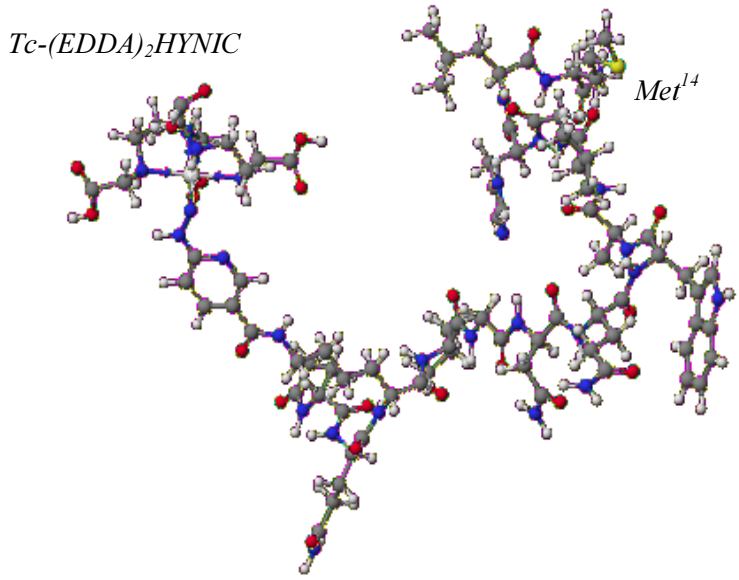

FIG. 1. Calculated structure of Tc-(EDDA)$_2$HYNIC-BBN (E= 105 Kcal/mol).

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Detection of DVT in experimental animal model using radioactive label tirofiban – GPIIb/IIIa inhibitor

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Keywords: DVT, tirofiban, GPIIb/IIIa receptor inhibitor, experimental animal model.

Detection of acute deep venous thrombosis (DVT) based on the bimolecular behaviour of components of the clotting process including the platelets and their specific expressed receptors have suggested like a new approach in nuclear medicine. The new venue has focused on the use of small peptide or peptidomimetic ligands with high specificity for this receptors and incorporation of a convient nuclide for imaging purpose.

Tirofiban (N-(Butylsulfonyl)-4-O-(4-(4-piperidyl)-L-tyrosine is a non-peptide tyrosine derivate, highly selective, short acting inhibitor of fibrinogen binding to platelet glycoprotein IIb/IIIa.

The **aim** of our study was to introduce Tirofiban as a specific imaging agent to GPIIb/IIIa receptor in the case when we have activated platelets during the process of the platelet aggregation and thrombus formation in the experimental animal model and to evaluate his radiochemical and biological behaviour.

Iodine-125 – Tirofiban labeling was performed using Iodo-gen method and the quality control of the labelled product was checked, using TLC technique in 1mol/L HCl like solvent. The percentage of labelling was more than 95% without purification. Technetium-99m-Tirofiban labeling was in the presence of reducing agent (Sn^{2+}). The quality control was done by Paper Chromatography (PC) and Instant Thin Layer Chromatography (ITLC) using two solvents – methylethylketone and 0.9%NaCl. The percentage of the obtained complex was more than 95% and $^{99m}TcO_4^-$ less than 5%. The labelled product was stable without changing the percent of labeling 2 h at room temperature.

The labelled Tirofiban has a fast blood clearance in the normal rat model (without induced thrombosis). More than 80% of injected dose (for both of used products) was eliminated from the circulation in the first hour after injection.

The biodistribution and visualisation of the labelled molecule was carried out using experimental model of thrombosis in male Wistar rat. Planar images were obtained 30 min, 2 and 24 h after application of 2–6×10^6cpm in 50–100 μm ^{99m}Tc-Tirofiban or 1.6–2.1×10^6cpm in 50μm of iodine ^{125}I-Tirofiban in rat's tail vein. The sensitivity and specificity were determined using ratio" left leg positive for DVT" and" right leg negative for DVT". By using ROI technique and biodistribution studies of scarified animals we quantified left thrombotic/right nothromotic leg ratio. The obtained ratio was 1.76 after 30 min, 1.99 after 2 h and 2.06 after 24 h. These values were considered as positive in the detection of acute DVT.

Our results from experimental studies showed that radiolabeled Tirofiban could be helpful in the further clinical investigation in the patients with acute deep venous thrombosis and that he has some diagnostic potential in nuclear medicine.

Preparation of in-house dextran and its clinical applications in sentinel node studies, Siriraj Hospital

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From 2003, an in-house Dextran for sentinel node study has been prepared at Division of Nuclear Medicine, Siriraj Hospital. The technique is the 'Pramongkutklao modified method'. Main materials: Dextran powder (M.W. 70000, Sigma), Stannous chloride dihydrate crystals ($Sn_2Cl_2.2H_2O$, Sigma), water-for-injection (WFI), HCl, and NaOH. Procedure: Dextran was dissolved in WFI. $Sn_2Cl_2.2H_2O$, 4% in HCl was added to the Dextran solution of which the pH was subsequently adjusted to 6.5 –7.0 using NaOH. The final volume was reached via WFI and the solution was passed through a 0.22 μ Millipore-filter into N_2-purged vials, 0.5 ml in each aliquot. They were then kept in a freezer (-20 ^{0}C) for up to 6 months.

For each lot, the bacteriological and radiochemical purity were checked at the Department of Microbiology and at the Division of Nuclear Medicine, respectively. The bacteriological testing showed no growth after two weeks of incubation. The radiochemical purity study was performed using 2 systems – paper chromatography/ Acetone, and ITLC-SG/ Methyl-Ethyl-Ketone (MEK) with 8-cm strips giving the radiochemical purity of approximately 95% or above, as shown in Fig. 1. However, the first system is routinely used because it is much cheaper. The stability of the labelled compound or bench-life was done three times showing the radiochemical purity of above 95% (paper/ Acetone) through out 6-7 h after labelling. Periodically, the particle size of Dextran in the kit was checked using the Sub-Micron Particle Analyser Coulter® Model N4 MD at the Faculty of Pharmacology, Mahidol University. It came out to be around 100 nm.

For clinical applications in Siriraj Hospital, at the moment ^{99m}Tc-Dextran is requested by two surgeons to use in two main groups of the patients – those with breast cancer and those with skin cancer (either malignant melanoma or squamous cell carcinoma).

For breast cancer, two categories are used: non-imaging and imaging. The former is done to localize a small tumour using a gamma-probe in the Mammogram Unit without prior imaging. The latter is done firstly at the Nuclear Medicine to localize the mass via a static imaging and it can be viewed at about 45 minutes after injection. The patient is then transferred to the operating room where another administer of ^{99m}Tc-Dextran with the use of a gamma-probe and the confirmation via blue dye are performed before the removal of the sentinel node (SN). In both cases, 400 μCi of the labelled compound was required. In most cases, a good agreement between blue dye and ^{99m}Tc-Dextran could be achieved.

For skin cancer, the activity of 4–5 mCi for injection was preferred. A static as well as a dynamic imaging were carried out and the node could be shown within 10 minutes after

injection. A good agreement with the blue dye was also obtained with the better results for the ^{99m}Tc-Dextran technique by which even deep SNs could always be shown.

The localization of the sentinel node via this ^{99m}Tc-Dextran is satisfactorily helpful and effective especially for micro-metastasis.

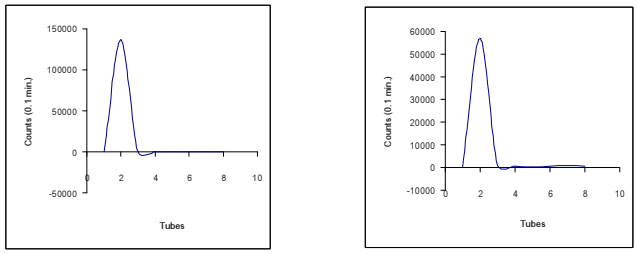

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FIG. 1. a. ^{99m}Tc-Dextran: paper chromatography and acetone, b. ITLC-SG and MEK.

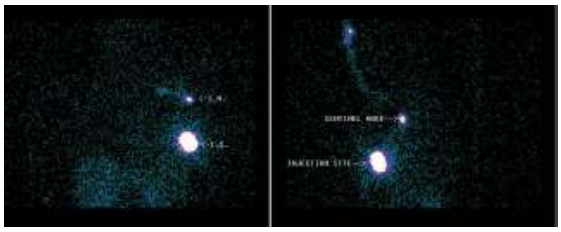

FIG. 2. Localization of sentinel node in breast cancer.

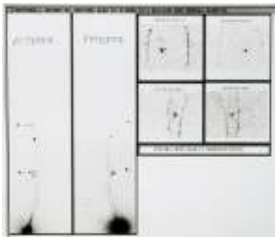

FIG. 3. Localization of sentinel node in malignant melanoma.

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Comparative study of ^{99m}Tc-EMB and native EMB and role of ^{99m}Tc-EMB in resistant mycobacterial imaging

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Introduction: Tuberculosis is a dreaded infectious disease causing greatest number of mortalities. Therapeutic potential of anti-tubercular drug Ethambutol is being utilized for the treatment of this granulamatous, opportunistic disease. Existing conventional diagnostic modalities (microbiological, molecular biology, chromatography, etc.) have their own proven utility but they suffer from certain loopholes. So, radionuclidic emission based Nuclear Medicine modality utilizing radiolabeled EMB was used in the study for early detection and localization of resistant tubercular lesion.

Materials and method: Radiocomplexation of EMB with ^{99m}Tc was done and standardized using stannous mediated reduction of ^{99m}Tc. In vitro studies i.e. serum and blood stability with ITLC was done. In vitro pharmacological studies like Uptake studies, MIC studies and CFU assays were done to know the effect of radiocomplexation in biological activity of drug. In vitro pharmacological studies and organ distribution of ^{99m}Tc-EMB were studied in New-Zealand white rabbit and balb/c mice respectively at different time intervals up to 24 h and compared with native EMB. Imaging was done in animal model (thigh model with resistant tubercular lesion) and subsequently in humans for further confirmation.

Result: Labeling efficiency of ^{99m}Tc-EMB was found to be >95%. Only 3-4% of ^{99m}Tc leached out from complex till 24 h of incubation in blood and serum confirming its high stability. Biological studies indicated the similarity in the behaviour of labeled and native EMB. Drug uptake was observed in resistant bacilli also. Blood kinetic studies exhibited V_d= 474ml, $t_{1/2}$= 17.7 h, Cl= 18.5ml/h which was comparable to native EMB as indicated in Therapeutic drugs, Collin Dollery. Organ distribution indicated renal and hepatobiliary route of excretion. Scintigraphic studies under Gamma camera in both animal model and humans indicated normal biodistribution and efficacy of tracer to localize in the resistant tubercular lesion.

Conclusion: ^{99m}Tc-EMB is highly specific and sensitive tracer for the resistant tubercular detection.

Session 3:

TECHNETIUM RADIOPHARMACEUTICALS APPLICATIONS

Technetium radiopharmaceuticals: Applications in nuclear cardiology

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Single photon emission tomography with technetium labelled perfusion tracers is a consolidated tool for coronary artery disease diagnosis and for risk stratification. ^{99m}Tc labelled myocardial perfusion tracers have allowed a significant evolution in myocardial perfusion scintigraphy. Technical improvements in scintigraphic data acquisition, processing and analysis, such as single photon emission tomography (SPECT) and gated-SPECT can be applied in current clinical practice as a consequence of the availability of perfusion tracers with optimal physical characteristics for gamma camera technology.

Since their introduction more than fifteen years ago, ^{99m}Tc labelled myocardial perfusion tracers have allowed a significant evolution in myocardial perfusion scintigraphy. Technical improvements in scintigraphic data acquisition, processing and analysis, such as single photon emission tomography (SPECT) and gated-SPECT can be applied in current clinical practice as a consequence of the availability of perfusion tracers with optimal physical characteristics for gamma camera technology.

Despite the maturity of SPECT imaging and the more favourable physical properties of ^{99m}Tc compared with ^{201}Tl, at present none of the ^{99m}Tc-labeled agents that have been approved for clinical use has ideal biodistribution properties. A ^{99m}Tc labeled myocardial perfusion tracer with ideal biological characteristics is still the goal of many investigators. Favourable biological properties can be summarized in: 1) myocardial uptake proportional to blood flow with linear relationship at both low and high flow, 2) high extraction fraction (ideal =1) 3) favourable myocardial uptake and retention with minimal wash out and/or redistribution allowing appropriate timing for SPECT acquisition, 4) fast blood clearance with high heart to background ratio for early imaging, 5) fast lung clearance, 6) fast liver clearance and minimal residual abdominal activity interfering with scintigraphic acquisition, 7) easy kit preparation, 8) in viitro and in vivo stability.Many tracers have been syntethized including ^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin, ^{99m}Tc-teboroxime, ^{99m}Tc-N-NOET, ^{99m}Tc-furifosmin. The principal properties of commercially available tracers are analysed.

Imaging oncogene expression in breast cancer with receptor specific peptides and peptide nucleic acids

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This year, breast cancer (BC) will attack approximately 210, 000 and will take the lives of 40,000 women in the U.S. Standard screening with breast self-examination and mammography, recommended to minimize BC morbidity, miss 10-20% (up to 40% in young women) of breast cancer. Moreover, if an abnormality is found, an invasive diagnostic procedure is required to determine if the breast contains hyperplasia, atypia, or cancer. Approximately 80% of invasive procedures detect a benign pathology.

BC cells express a gene product, cell surface receptor VPAC1, so named because the endogenous growth hormones Vasoctive Intestinal Peptide (VIP) and Pituitary Adenylate Cylcase Activating Peptide (PACAP) bind to VPAC1 receptors with high affinity. VPAC1 receptors are overexpressed on 100% of human breast cancer cells. Cyclin D1 is a key regulator of the cell cycle and overexpressed in 50% to 80% of breast cells, whereas it is low or absent in normal breast tissues. The human breast cancer cell line MCF7 displays elevated levels of CCND1 mRNA, encoding cyclin D1, and an elevated level of IGF1R mRNA, encoding insulin-like growth factor 1 receptor. We hypothesed that ^{99m}Tc or ^{64}Cu labeled VIP analogues, or a peptide nucleic acid (PNA) chimera specific for IGFI receptor and CCND1 mRNA, will permit us to early image breast cancer by planar, SPECT or PET imaging.

We synthesized, characterized and administered i.v. ^{99m}Tc-AcGly-D (Ala)-Gly-Gly-aminobutanoyl-VIP (TP3654), ^{64}Cu diaminodithiol-aminobutanoyl-VIP (TP3982), ^{99m}Tc-AcGly-D(Ala)-Gly-Gly-PNA-D(Cys-ser-lys-Cys) chimera (WT4185) and Cu-64-DOTA-PNA-D(cys-ser-lys-cys) (WT4348). A 12mer, CTGGTGTTCCAT nucleic acid sequence served as the PNA and 3 or 4 mer mismatched PNAs as negative controls.

Using ^{99m}Tc-TP3654 we have successfully imaged human breast cancers not detectable by current modalities. In athymic, nude mice bearing MCF-7 human breast cancer xenographs, Cu-64-TP3982 tumour uptake was 85 times greater than ^{99m}Tc-TP-3654 and with ^{64}Cu-WT4348 twice as high as that of ^{99m}Tc-WT4185. PET imaging was performed using MOSAIC (Philips) animal PET scanner. Fusion imaging was facilitated by ImTek animal CT scanner. ^{64}Cu was obtained from Mallindrodt Institute of Radiology, Washington University, St. Louis, Mo.

Work was supported by NIH CA-109231 and DOE-ER63055.

Synthesis and evaluation of ^{99m}Tc-kanamycin and ^{99m}Tc-isoniazid for infection imaging

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There is no recognized "gold standard" for imaging sites of infection or inflammation. Many technetium complexes have been put forward as being efficacious in the detection of inflammation and infection [1]. Synthesis of ^{99m}Tc-Kanamycin and ^{99m}Tc-Isoniazid was investigated and evaluated as infection imaging agents in animal models. The direct method of labeling of Kanamycin and Isoniazid with technetium-99m was exploited which is simple, rapid, efficient and does not require bifunctional chelating agents.

Kanamycin is an anti-infective used for treatment of infections when penicillin or other less toxic drugs cannot be used. Kanamycin was labeled with technetium-99m pertechnetate using $SnCl_2 \cdot 2H_2O$ as reducing agent. Labeling efficiency and radiochemical purity and stability were assessed by a combination of ascending paper chromatography and instant thin layer chromatography on silica gel. The labeling efficiency depends on ligand / reductant ratio, pH, and volume of reaction mixture. At low pH (2-5) the minimum labeling efficiency is 75, while at pH 6-7 the labeling efficiency of ^{99m}Tc-Kanamycin is > 97%. In basic media at pH 8 the labeling efficiency is decreased (60-72%). The complexation of ^{99m}Tc with Kanamycin is not rapid and maximum labeling efficiency is achieved after 30 minutes. The resulting complex of ^{99m}Tc-Kanamycin is quite stable and labeling efficiency of ≥ 98% is maintained for up to 6 h. The final formulation for the radiotracer ^{99m}Tc-Kanamycin was: Kanamycin 4mg; $SnCl_2$ $2H_2O$ 20 μg; pH 6-7; ^{99m}Tc 370-500 MBq; reaction mixture volume ~2 ml; and incubation time 30 min at room temperature.

In vitro binding of ^{99m}Tc-Kanamycin to S. Aureus bacteria were assessed by the method described Welling, et al., [2]. For comparison purposes binding of ^{99m}Tc-Ciprofloxacin to bacteria were also performed. In vitro binding of ^{99m}Tc-Kanamycin to bacteria was in the range of 40-50% (Fig. 1), while binding of ^{99m}Tc-Ciprofloxacin ranged from 40 to 65% (n=4).

Biodistribution studies of ^{99m}Tc-Kanamycin were performed in rats/rabbits. Saline (0.3 ml) containing 2×10^8 cfu viable S. aureus ATCC 25923 was injected into the right thigh muscle of each animal followed by scintigraphy after 48 h, when significant swelling was visible at the injection site. A significantly higher accumulation of ^{99m}Tc-Kanamycin was seen at sites of S. aureus infected animals (rat/rabbit).

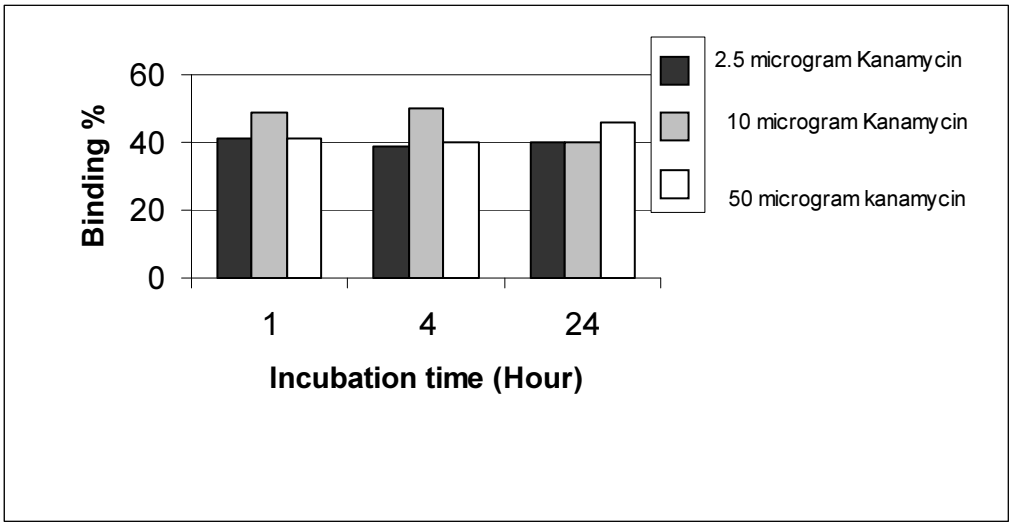

FIG. 1. In vitro binding of the ^{99m}Tc-Kanamycin to viable S. aureus (n=4 per experiment).

The antitubercular drug Isoniazid has also been labeled with ^{99m}Tc for use as a specific radiopharmaceutical in the early and correct diagnosis of tuberculosis. The maximum radiolabeling yield 95% was obtained with reaction mixture containing 2 mg Isoniazid; $SnCl_2 \cdot 2H_2O$ 100 µg; pH ~7; ^{99m}Tc 370-500 MBq; and reaction mixture volume ~2 ml. The reaction mixture was incubated for 25 min in a boiling water bath. Scintigraphy in animals was carried out after intravenous administration of ^{99m}Tc-Isoniazid in the dorsal ear of rabbits. Imaging was performed at different time intervals after administration till 24 h. ^{99m}Tc-Isoniazid is able to concentrate specifically and irreversibly in vivo bacterial model in rabbit tubercular infection. The uptake of directly labeled ^{99m}Tc-Isoniazid in tubercular lesion is comparable to indirectly labeled ^{99m}Tc-Isoniazid [3].

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Preparation of ^{99m}Tc-pentavalent DMSA and its uptake by benign bone diseases

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^{99m}Tc-(V)DMSA is a tumour seeking agent that is known for its ability to detect medullary thyroid carcinoma and to be suitable in detection of tumoural recurrence and or its metastasis. However, ^{99m}Tc-(V)DMSA could accumulate also in soft tissue tumours, in lung cancer, metastatic diseases, brain tumours and some benign bone diseases. ^{99m}Tc-(V)DMSA could reliably prepared by addition to the DMSA(III) additional of concentrated $NaHCO_3$. A simple and efficient chromatography analysis uses an ITLSC-SG/MEC in butanol /acetic acid/ water (3:2:3) to calculate the ratio DMSA(V)/DMSA(III) and the amount of free pertechnetate. Its mechanism of accumulation has been thought to be related to its avidity to some cancer cells but also related to the glucose mediated acidosis. The uptake of DMSA by benign bone disease has been studied in 68 patients with a known primary cancer including patients with thyroid medullary carcinoma. An uptake of the ^{99m}Tc-(V)DMSA by the benign bone diseases was noticed in more than 80% of the 68 patients, and that could lead to a misinterpretation of the ^{99m}Tc-(V)DMSA total body scan and make an increased number of false positive in the detection of metastasis or recurrence. This was confirmed also in patients with thyroid medullary carcinoma.

Key words: DMSA V, preparation, benign bone disease, metastatic diseases.

Session 4:

NOVEL TECHNETIUM CHEMISTRY AND RADIOPHARMACEUTICALS - I

Which role for Technetium-99m radiopharmaceuticals in the age of molecular imaging?

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Molecular imaging is a new paradigm that is currently modifying our common approach to the study of fundamental biological processes. In this representation, the behaviour of a single cell in a living tissue is thought to be the result of the entanglement of a number of basic biochemical pathways, which are tightly grouped together to form a final biological network extended through the whole organism.

Nuclear imaging is a subfield characterized by the use of radiolabeled single-molecule probes, which are specifically designed for monitoring selected biomolecular processes belonging to a particular biological network. After the advent of a new generation of small animal scanners having a submillimiter resolution, Single Photon Emission Computed Tomography (SPECT) is receiving a growing interest brought about by the observation that, unlike Positron Emission Tomography, there is no intrinsic physical limit to the resolution that could be achieved when single-photon emitting radiolabeled probes are employed.

Technetium-99m is still recognized as the γ-emitting radionuclide having the most ideal nuclear properties and, therefore, ^{99m}Tc radiopharmaceuticals may play a significant role in molecular imaging, particularly if novel categories of tracers exhibiting superior imaging characteristics will be developed using advanced chemical methods. At present, a number of fundamental biological processes can be successfully monitored using ^{99m}Tc agents, and they will be shortly reviewed in this paper. Examples range from the use of $[^{99m}TcO_4]^-$ for monitoring gene expression, to the labeling of a large number of different peptides targeting receptors expressed in various disease states and processes such as tumour proliferation, inflammation, angiogenesis and formation of atherotic plaques.

Problems and perspectives in the design of imaging agents for the central nervous system will also be discussed.

^{99m}Tc EDDA/HYNIC-TOC is a suitable radiopharmaceutical for radioguided surgery of neuroendocrine tumours

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Commercially available ^{111}In-DTPA-Octreotide is a well established radiopharmaceutical for imaging of neuroendocrine tumours (NET) expressing somatostatine receptors. Its analog [Tyr^3] octreotide can also be successfully radiolabelled with ^{99m}Tc. Compared to ^{111}In labelled preparation, the advantages of ^{99m}Tc labelled [Tyr^3] octreotide are: energy of gamma rays more suitable for registration using gamma detectors, shorter half life, allowing use of higher activities and lower tracer uptake by the kidneys. Its pharmacokinetical properties does not differ significantly from comercially available ^{111}In-DTPA-Octreotide (1).

For reasons stated above we propose ^{99m}Tc labelled [Tyr^3] octreotide for radioguided surgery of NET.using gamma probe.

Four patients, two with clinical and biochemical signs and symptoms of gastrinoma, one with biochemical evidence of insulinoma and the other with those of insulinoma underwent surgery. In all patients markedly increased ^{99m}Tc labelled [Tyr^3] octreotide uptake by the tumour (grade III and IV) was clearly seen on planar scintigraphy and SPECT while all investigations, including contrast CT, MRI and endoscopical US were negative or inconclusive in all patients except the patient with carcinoid, where the tumour was seen also on CT.

[^{99m}Tc-ethylendiaminediacetic acid-hydrazinonicotinamide-D-Phe^1 Tyr^3] octreotide (^{99m}Tc-EDDA/HYNIC-TOC) was prepared according to protocol suggested by E. Von Guggenberg (2) Radiochemical purity was determinate using reversed phase gradient HPLC (a 125×4 mm HP ODS hypersil column, 0,02 M Phosphate buffer pH 6,2/acetonitrile at a flow rate 1 ml/min). Indicative retention time of ^{99m}Tc peptide complex was at 14 min. Radiochemical purity above 95% was achieved in all cases. Following radiolabelling the radiopharmaceutical was sterilized by filtration using 0,22 μl filter.

10 μg of peptide labelled with 600 MBq of ^{99m}Tc were injected i.v. 4 h prior to surgery.

Using gamma probe the surgeons were able to localize tumours successfully in all cases. The intraoperative count rate over the tumour was more than three times higher than the radioactivity detected elsewhere in the operating field. After removal of the tumours high radioactivity of the excised tissue was confirmed ex vivo by gamma probe and gamma camera measurements, and the nature of the tumours was confirmed by histology.

All patients were clinically, biochemically and scintigraphically disease free on follow-up three months after surgery.

We conclude, that ^{99m}Tc labelled [Tyr^3]octreotide is the radiopharmaceutical of choice for radioguided surgery of NET due to its physical and biological properties. In the hands of

surgeons experienced in the use of gamma probe, this approach seems to be superior to traditional surgical technique, making surgery of NET shorter and easier, as well as increasing success of operative treatment of NET.

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Comparative in vitro and in vivo evaluation of a series of novel bombesin-like peptides

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Small neuropeptides labeled with gamma and/or beta emitting radionuclides, are currently being investigated for their ability to bind to cell-surface receptors, over-expressed in a wide variety of malignant tissues. Particular attention has been focused on the amphibian peptide bombesin (BN) and its mammalian counterpart gastrin releasing peptide (GRP) [1-3].

New bombesin-like peptides, of the general formula X-spacer-QRLGDQWAVGHLM, were synthesized by the introduction of amino acids containing electron donor groups capable of complexing reduced ^{99m}Tc, into the basic Bombesin structure. The derivatives under study are: BN1:Cys-*Aca*-BN[2-14], BN1.1:Gly-Gly-Cys-*Aca*-BN[2-14], BN1.2:MeGly-Gly-Cys-*Aca*-BN[2-14], BN1.3:$(Me)_2$Gly-Gly-Cys-*Aca*-BN[2-14], BN1.4:Cys-Gly-Cys-*Aca*-BN[2-14] where Aca represents 6-amino-n-hexanoic acid.

Peptide synthesis was performed according to the Fmoc strategy. The complexes with $^{185/187}$Re were formed via the precursor rhenium gluconate. The peptide derivatives, as well as the Rhenium complexes, were identified by HPLC, ESI-MS and ^{1}H- and ^{13}C-NMR. Radiolabeling with ^{99m}Tc was performed via the precursors ^{99m}Tc-gluconate and ^{99m}Tc-MDP, while radiolabeling with ^{186}Re via the precursor ^{186}Re-gluconate. In all cases, the radiolabeling conditions (pH, concentration of the reducing agents and of the peptide derivatives, radioactivity added) were assessed, so that the final radiolabeled product is obtained with the maximum possible specific activity. The ability of the new peptides to tag cancer cells was comparatively evaluated in epithelial prostate cancer cells (PC3), by investigating whether the BN-derivatives inhibit [^{125}I-Tyr^{4}]-BN binding. Comparative biodistribution studies of the ^{99m}Tc-labeled peptides were performed in normal Swiss mice in order to examine the in vivo behaviour and the pharmacokinetic properties of the ^{99m}Tc-labeled derivatives. Further on, biodistribution and scintigraphic studies of the most promising radiolabeled peptide, were assessed in prostate cancer models (female nude mice).

Spectroscopic data of rhenium-185/187 complexes indicated the formation of a dimer for BN1, while for the other derivatives the rhenium complexes were found to be identical to the proposed theoretical structures, i.e. complexes of a 1:1 metal to ligand ratio. Chromatographic analysis showed that ^{99m}Tc- labeling led to the formation of a single derivative, in each case, in high yield (>98%), which was found to be stable with time. From the preliminary in vitro assays we can conclude that all the new derivatives present specificity for the prostate (PC-3) cancer cells, with IC50 values ranging from 0.51 to 1.36 nM (Fig. 1). The in vivo behaviour of the radiolabeled products, as evaluated in normal mice, presented high uptake in pancreas, an organ over-expressing Bombesin receptors. The elimination of the derivatives takes place mainly via the urinary system, and to a lesser extent from the hepatobiliary system. For the ^{99m}Tc-BN1.1, satisfactory tumour images were obtained 1 h p.i., the experimentally induced

prostate cancer being clearly delineated. Biodistribution data showed a tumour to non-tumour ratio of 30.

The preliminary results mentioned above warrant further investigation of the ^{99m}Tc-BN1.1 bombesin derivative, as a possible candidate for diagnostic cancer studies, while labeling of this derivative with Re-186/188 could provide a promising therapeutic biovector, with high in vivo specificity.

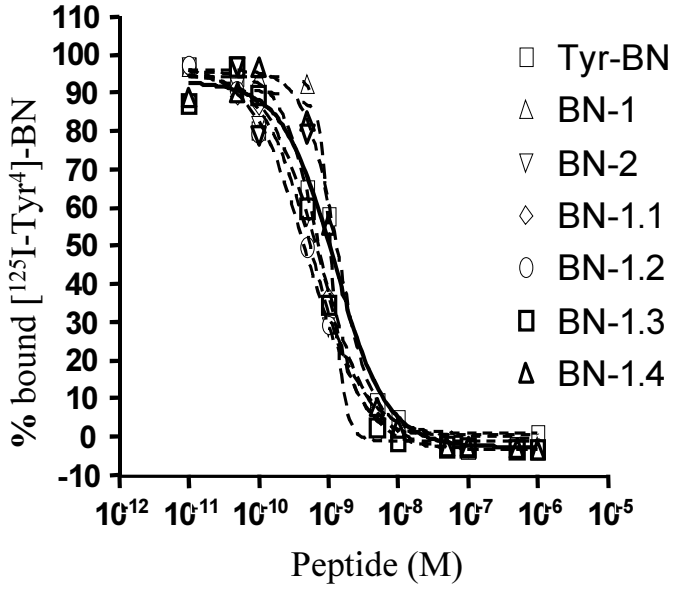

FIG. 1. Displacement of binding of [^{125}I-Tyr^4]bombesin from PC-3 cells by Tyr^{4}BN, BN1.1, BN1.2, BN1.3, BN1.4.

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[^{99m}TcN]-N-benzyl piperidine dithiocarbamate: A potential sigma receptor imaging agent

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High expression of sigma receptors in central nervous system suggest their possible role in drug design for diseases such as psychiatric and movement disorders, amnesia, depression and cancer. Herein we report an attempt to target sigma receptors localized in the brain using an analogue of piperidine, a molecule known to exhibit affinity for the brain. 4-amino-N-benzyl piperidine was derivatized to its dithiocarbamate (DTC), known to be a suitable chelating system for complexation with the $[^{99m}TcN]^{2+}$ precursor. The radiolabeled complex, [^{99m}TcN]-4-dithiocarbamato-N-benzyl piperidine, was obtained by addition of 50μg(2×10^{-4}M) of the dithiocarbamate to the preformed ^{99m}TcN precursor and characterized by HPLC.

Since the sigma receptors are also over-expressed in a large variety of human tumours such as glioma, neuroblastoma, melanoma, breast cancer etc., the radiolabeled piperidine analogue was tested for its affinity towards cancer cells. In vitro studies in the breast cancer cell line MCF-7 and fibrosarcoma cells were carried out by incubating ~10^5 cells with ~20 ng of [^{99m}TcN]-4-dithiocarbamato-N-benzyl piperidine. Specificity of [^{99m}TcN]-4-dithiocarbamato-N-benzyl piperidine was ascertained by inhibition studies with 15 μg pentazocine, a sigma-receptor specific ligand. Pharmacokinetics of the product was studied in normal Swiss mice.

^{99m}TcN complex of piperidine could be radiolabeled in >98% yield. HPLC pattern revealed single species with retention time of 15 min. In vitro binding with MCF-7 was ~1% and there was ~75% inhibition after pre-incubation of cells with pentazocine whereas with fibrosarcoma the values were 2% and 55%, respectively. In biodistribution studies in normal mice, brain uptake of ~0.6 ID/gm at 5min.p.i. was observed which reduced to 0.3%ID/g at 2h.p.i. Significant uptake in other vital tissues such as heart, lungs, liver and kidneys expressing sigma receptors was also observed. Administration of Pentazocine one hour prior to [^{99m}TcN]-4-dithiocarbamato-N-benzyl piperidine resulted in nearly 30% reduction in radioactivity associated with these organs at 5 min.p.i. There was >95% decrease in accumulation of radioactivity in the brain following pretreatment with pentazocine. Displacement of the radiolabeled complex by known sigma receptor binding ligand in brain, heart, kidneys and other organs indicate that [^{99m}TcN]-4-dithiocarbamato-N-benzyl piperidine acts primarily as a sigma receptor in vivo and thus has a potential for sigma receptor tissue imaging.

^{99m}Tc-labelled peptide F11: A new potential αvβ3, integrin antagonist for scintigraphic detection of tumours

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Integrins are cell surface receptors that are involved in cell-cell and cell-matrix interactions. They are heterodimeric glycoproteins consisting α and β chains that are non-covalently linked on the cell surface.

The $\alpha_v\beta_3$ integrin is implicated in many pathological processes, such as osteoporosis, misregulated angiogenesis, tumour growth and tumour metastasis. This receptor is highly expressed on different malignancies such as osteosarcomas, neuroblastomas, glioblastomas, lung carcinomas, breast neoplasms, gastric carcinoma, prostate cancer, bladder carcinomas and invasive melanomas. Many integrins, including $\alpha_v\beta_3$ receptor, act by recognising the specific tripeptide sequence Arg-Gly-Asp (RGD) of their substrates. Targeting of this receptor may provide information about its status on tumour and help specific therapeutic planning. Thus, a variety of radiolabelled RGD derivative peptides have been proposed for the diagnosis and therapy of malignant diseases. The aim of the present work was to develop a peptide with the sequence Arg-Gly-Asp-Ser and label it with ^{99m}Tc to obtain scintigraphic images in an animal model.

Peptide F_{11} was obtained by solid phase synthesis, containing a linear sequence Arg-Gly-Asp-Ser to recognize αvβ3 receptor and other linear sequence (Ala-Gly-Gly-Gly) at the N-terminal to chelate ^{99m}Tc. Peptide was analysed by mass-spectrometry and its purity determined by reverse-phase HPLC. The purity of obtained peptide was higher 95%.

Labelling procedure was carried out at neutral pH, employing stannous fluoride as reducing agent. The influence of molar ratio F_{11}: Sn^{+2} (1:0.5, 1:1, 1:2 y 1:4) on radiochemical purity of radiopharmaceutical was assessed. Results were represented by using a chart. Using a 1.3 fold molar excess of stannous floride was attained a ^{99m}Tc-labelling yield of 95.2±2.1% and recovery from HPLC was >92%. The stability of the radiolabelled peptide was tested by its incubation in 0.1M PBS pH=7.2, in a solution containing 30 fold molar excess of L-cysteine and in fresh human plasma, at room temperature up to 24 h. Samples were analysed by HPLC.

In the absence of cysteine, radiolabelled peptide resulted stable, with more than 90% of the activity remaining bound to the peptide at 24 h. Challenging the label with 30 fold molar excess with L-cysteine was enough to transchelate about 40% of the metal after 24 h incubation. A shift in retention time of ^{99m}Tc-F_{11} was observed when peptide was incubated in plasma suggesting a sensitivity to the action of chelating agents (such as glutathione and

cysteine) and peptidases in plasma. A significative binding to plasma proteins was seen by size-exclusion HPLC.

One μg (27.5-29.0 MBq) of ^{99m}Tc-F_{11} was injected through ocular plexus of C57BL6 male mice to determine the biokinetics up to 24 h. Scintigraphic images were acquired after administration of 13 μg (67-74 MBq) of ^{99m}Tc-F_{11} to C57BL6 mice bearing B16 melanoma tumours and nude mice with A431 tumours. Renal uptake was from 9.5%ID/g at 1 h to 4.0%ID/g at 24 h, thus it could be main elimination pathway. The rest of the organs showed an uptake lower 2.0%ID/g. Serum pharmacokinetics was fixed to a bicompartmental model with a $T_{1/2\alpha}$ = 43.1±13.5 min and $T_{1/2\beta}$=327±149 min. Scintigraphic images showed and intense tumour uptake of the radiopharmaceutical.

Conclusion: ^{99m}Tc-Labeled peptide F_{11} could be a promising radiopharmaceutical for scintigraphic detection of tumours.

Nuclear imaging of amyloid deposits based upon thioflavins

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Alzheimer's Disease (AD) is a chronic neurodegenerative disorder characterized by the presence of amyloid deposits and neurofibrillar tangles in the brain. Direct assessment of local changes of amyloid deposits in vivo would greatly facilitate the diagnosis and therapeutic treatments of AD. The goal of this study is to develop small-molecule probes that can be used to follow amyloid deposition in vivo in patients with neurodegenerative diseases.

Over the past years, we set out to develop a series of small molecules based on thioflavins as radiotracers for use in nuclear imaging modalities such as positron emission tomography and single photon emission computed tomography. The potential of these amyloid-imaging agents for in vivo studies of amyloid deposition has been evaluated based on the following methods: 1) spectrophotometric binding assays with synthetic amyloid-β (Aβ) fibrils and AD brain homogenates; 2) fluorescent staining of brain tissue sections to evaluate specificity of binding to amyloid deposits; 3) fluorescent microscopy in mouse models to determine the brain permeability and characterize the binding specificity in vivo, and 4) PET studies in human subjects diagnosed with AD and age-matched control subjects.

To date, we have identified some lead compounds as molecular probes with specificity towards amyloid deposits. The in vitro and in vivo binding properties of these compounds have been demonstrated in the following ways: 1) they selectively binds to Aβ fibrils; 2) they selectively stains amyloid deposits in AD brain tissue sections; 3) they readily penetrates the blood-brain barrier, selectively detects amyloid deposits in vivo in living mice; and 4) one of these compounds has been successfully used in PET studies in human subjects.

In conclusion**,** amyloid-imaging probes have been developed that could be used to monitor amyloid load in vivo. Applications of the probes are under investigation for potential pathophysiology studies and efficacy evaluation of anti-amyloid therapies currently under development.

Keywords: amyloid, positron emission tomography, Alzheimer's disease, imaging.

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HYNIC-TOC labelled with ^{99m}Tc via an instant kit formulation: Preliminary results

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Imaging somatostatin receptor positive tumour with Indium-111-diethylene triamine-pentacetic acid-D-Phe-octreotide (^{111}In-DTPA-octreotide) has become a widely used diagnostic procedure in clinical nuclear medicine. This technique permits the localization and staging of tumours that express the appropriate somatostatin receptors, the most important of which is receptor subtype 2 (SSTR2).

However, ^{99m}Tc can be considered as the radiolabel of choice with daily availability from a generator and the ^{99m}Tc-EDDA/HYNIC-TOC is a promising new radiopharmaceutical with the potential to replace ^{111}In-DTPA-OCT as the radiopharmaceutical for somatostatin receptor scintigraphy.

The aim of this work was the development of an instant kit formulation based on a ^{99m}Tc labelling of HYNIC-TOC using a coligand exchange from tricine to EDDA. The preparation of the labelled conjugate was achieved at elevated temperature and under optimized conditions of pH, EDDA concentrations and stannous ion. The radiochemical purity of this labelled solution was always higher than 95%.

Several experiences were carried out to obtain the best kit formulation considering the parameters of the wet labelling solution. Three formulations were evaluated : two of them containing EDDA, HYNICTOC, tricine, stannous chloride and mannitol, and the other one without tricine. EDDA and tricine were dissolved in various solutions of different pH such as water, 0.1N NaOH and 0.2M phosphate buffer pH 6. Mannitol was the bulking agent (50 mg/vial) in all formulations. TABLE I shows the content of 1.2 ml of dispensed solution in sterile vials immediately before the lyophilization process.

TABLE I. DIFERENT KITS FORMULATIONS FOR LABELLING HYNICTOC WITH ^{99m}Tc

EDDA	TRICINE	$SnCl_2.2H_2O$	MANNITOL	HYNICTOC
10 mg/water (gently heated)	30 mg/water	20µg/ClH 0.1N	50 mg/water	20 µg/ethanol 10%
10 mg/NaOH 0.1N	30 mg/phosphate buffer	20µg/ClH 0.1N	50 mg/phosphate buffer	20 µg/ethanol 10%
10 mg/water (gently heated)	_	20µg/ClH 0.1N	50 mg/water	20 µg/ethanol 10%

Labelling of the kit was performed adding 0.5 ml of 0.2M phosphate buffer pH 6 and the necessary activity of ^{99m}Tc pertechnectate, freshly eluted, in 1.5 ml of saline for the first formulation(I). The second one(II) was labelled adding pertechnectate in saline. In the case of the third formulation(III) the tricine was dissolved in 0.5ml of 0.2M phosphate buffer pH 6 and added to the vial before the pertechnectate. Finally all the formulations were incubated in boiling water during 10 min.

Quality control tests were carried out to evaluate different parameters such as radiochemical purity (RP) (Waters 600 HPLC with radiometric and UV detectors and a Deltapak C18 column) , dissolution time, pH. The stability of the kits during storage at 4ºC was performed during three months. The results of the experiments are shown in Table II. In all cases impurities such as ^{99m}Tc colloid and $^{99m}TcO_4^-$ were lower than 3% (assayed by ITLC).

TABLE II. RADIOCHEMICAL PURITY OF THE THREE FORMULATIONS OVER A PERIOD OF THREE MONTHS

KIT	pH	HYNICTOC%			
I	4.5	87.7 t=0	90.8 t=1	70.4 t=2	50,8 t=3
II	7.5	89.4 t=0	64.4 t=1	72.9 t=2	85.0 t=3
III	4.5	80.5 t=0	51.0 t=1	89.0 t=2	61.2 t=3

Formulation I was stable for one month and then dropped to 70%. The other two formulations showed non-reproducible results. It will be necessary to produce more batches of the formulation I and to test it over a longer period of time (including the internalization assay) in order to find the best kit formulation with clinical grade quality.

Session 5:

INDIGENOUS CAPACITY BUILDING IN RADIOPHARMACEUTICALS

Capacity building in radiopharmaceuticals: Saudi Arabia experience

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Undoubtedly, easy availability of radiopharmaceuticals is a key element in application of radioisotopes in health care. And, creating self-sufficiency within the country and the geographical region in manufacturing these time-limited products further enhances this prospect. For obvious reasons, on demand availability and timely distribution of products bodes well for a regional programme.

At King Faisal Specialist Hospital & Research Centre in Riyadh, Saudi Arabia, one such programme began in early 1980s with the installation of the CS-30 (26.4 MeV) cyclotron, with an intention not only to make available the cyclotron products for medical imaging, but also to establish a contemporary research programme in radiotracer development as exemplified by simultaneous installation of a Tomogram (PET scanner) at the time when PET scanning was in its infancy.

The first beam on target in 1982 produced the first batch of ^{67}Ga citrate radiopharmaceutical, followed by an addition of various other cyclotron based products over the years. Presently, the Cyclotron Facility routinely produces six cyclotron isotopes (^{201}Tl, ^{67}Ga, ^{81m}Kr, ^{123}I, ^{18}F and ^{13}N) which are subsequently formulated into nine different radiopharmaceuticals. Weekly, approximately 25 batches of radiopharmaceuticals are manufactured supporting 35 nuclear medicine facilities within the country and the geographical region.

A key motivating and driving force for our Centre has been the goal of becoming a comprehensive radiopharmaceuticals manufacturing facility. Consequently, we introduced in Year 2000 the ^{131}I based products for diagnosis as well as for therapy, including on-request manufacturing of ^{131}I labeled mIBG.

Good Manufacturing Practice is the cornerstone of any radiopharmaceuticals manufacturing program. KFSH&RC is a perfect example of how this operational and guiding principle has been applied and evolved over the years, culminating into an effective quality management system for manufacturing radiopharmaceutical products consistently conforming to specifications.

The programme building has been “work in progress” from the onset and continues to be so, particularly in establishment and implementation of strict operational philosophy of GMP and ISO quality management system. We also realized that people are the most important component of any viable program. For efficient functioning, the staff must be well qualified and appropriately trained to achieve the mission of the organization. This has been achieved through staff selection based upon educational background, followed by extensive on-the-job training, as well as didactic education. Consequently, our facility has had a good mix of young and experienced staff. Furthermore, we have availed of the various IAEA’s programmes in specific training and fellowships for in depth exposure to other centres. With embarkation upon new programs, continuing education remains a central theme in ultimate success of the entire program.

The experience gained over two decades of continuous operation breeds confidence in the staff to achieve the goal of making Saudi Arabia self-sufficient in all its’ radiopharmaceuticals needs.

Continuing with our commitment to make available to the peoples of the country the most contemporary imaging modality, PET scanner was installed at KFSH&RC in 1995. Along with the

routine PET work, we have established a team of scientists to perform research work in developing new radiotracers.

KFSH&RC's cyclotron facility has continually focused on an overwhelming goal of becoming a comprehensive radiopharmaceuticals manufacturing facility. To this end, the year 2005 is the beginning of establishing just one such facility through expansion of the program that entails: a new building; a state-of-the-art cyclotron (30 MeV; plus a small cyclotron dedicated for PET isotopes production); advanced clean rooms; more importantly, the establishment of the Tc-99m Generators and Cold Kits manufacturing programs.

Presentation will entail past, present and future of radiopharmaceuticals manufacturing at King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia encompassing programme building and striving for self sufficiency.

Trends in indigenous radioisotope and radiopharmaceutical production in Bangladesh

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There are 17 nuclear medicine centres (NMC) in Bangladesh which are distributed all over the country. The objective of RIPD is to produce short lived radioisotopes and radiopharmaceuticals for these NMCs.

Iodine-131 generated during irradiation of natural TeO_2 in the 3 MW TRIGA research reactor is separated by dry distillation method. Recently two independent dry distillation apparatus were installed in the ^{131}I production plant for alternate use at RIPD. Since July 2003 more than ^{11}Ci of ^{131}I solution has been produced. At present, on average, 300mCi of ^{131}I is produced on a weekly basis by irradiating about 38.5g of TeO_2 at 2.5 MW for 15 h of interrupted irradiation. Due to the limitation in reactor operation time and target size, RIPD meets only a part of country's demand for ^{131}I. Increase of reactor operation time and installation of more dry central thimble (DCT) in the reactor to irradiate more than one target at a time is under active consideration of the authority.

Equipment for diagnostic and therapeutic ^{131}I capsule production has recently been installed at RIPD. Test production of diagnostic ^{131}I capsules has been done successfully. Therapeutic capsule production will be started when ^{131}I solution with required radioactive concentration will be available.

RIPD started its activity with the production of instant ^{99m}Tc by solvent extraction method by irradiating natural molybdenum (as MoO_3) target in 1987. In 1988 the Division produced ^{99m}Tc-sublimation generator by irradiating titanium molybdate in the reactor.

A facility for the production of chromatographic ^{99m}Tc-generator was installed at RIPD under IAEA TC Project BGD/4/014 in 1997. In this facility four ^{99m}Tc-generators per batch can be produced. So far 100 batches of 15GBq ^{99m}Tc-generators have been produced from imported fission ^{99}Mo. Yearly production of ^{99m}Tc-generatos is shown in Fig.1. Users comment regarding the quality and performance of the locally produced ^{99m}Tc-generator are quite satisfactory [1]. At present, RIPD meets 20% demand of ^{99m}Tc-generators in the country. Generator production at RIPD is cost effective. The cost of the local product is less than 50% with respect to that of the imported generators.

In order to meet the country's entire demand for ^{99m}Tc-generators by local product, a Technical Cooperation Project (BGD/2/010) with the IAEA is under implementation. A ^{99m}Tc generator plant with a capacity of producing 50 generators per batch will be installed at RIPD soon. When the new plant goes into operation, RIPD will be able to replace the import by local product.

The demand of in vivo ^{99m}Tc kits in Bangladesh is more than 6 000 vials per year. A programme to establish a kit preparation facility at RIPD has been approved under the government's three year (2005-2008) rolling plan.

In conclusion, RIPD is working on building up indigenous capability for production and supply of main radioisotopes and radiopharmaceuticals used in nuclear medicine in Bangladesh.

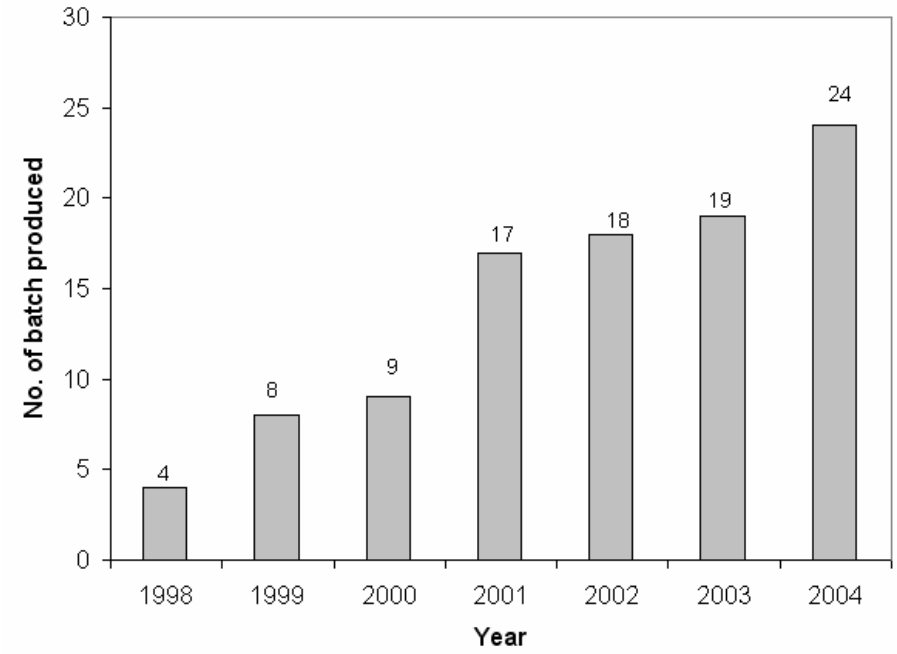

FIG. 1. Yearly production of ^{99m}Tc-generatos at RIPD.

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Experience on the production of Pakgen ^{99}Mo-^{99m}Tc generators

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A facility for the production of ^{99}Mo-^{99m}Tc generators (GPL) in clean room system was provided by the IAEA under Technical Cooperation Project. Regular production of Pakgen ^{99m}Tc generators has been started from January 2003. The GPL is provided with A, C and D categories of clean room specifications and various materials used for generator preparation are transferred through airlocks while dress change rooms are provided for personnel. More than 1800 Pakgen ^{99m}Tc generators with calibrated activities (five days reference date) ranging from 300 to 600 mCi of ^{99}Mo have been supplied to various nuclear medical centres in Pakistan. Before preparation, the generator bodies and various sections of GPL have been cleaned and disinfected. During the preparation, required calculated activity of ^{99}Mo has been loaded on the columns of pre assembled generators by prewash, loading and postwash steps and quality of the generator has been checked by elution efficiency and molybdenum breakthrough of eluate obtained after an hour from postwash step by comparing the measured activity with the theoretical activities. All these calculations have been carried out using computer programme MOGEN-I developed in Visual Basic 6 software; this software is suitable for recording and acquitting commercial data, monitoring production and quality control. Mathematical equations for theoretical calculation of generator parameters using ^{99}Mo-^{99m}Tc-^{99}Tc decay schemes have been used in this software; otherwise these calculations are laborious and time consuming.

During the elution, theoretical prediction of the performance of a ^{99}Mo-^{99m}Tc generator, in terms of the activity of ^{99}Mo, ^{99m}Tc on the column, activity of ^{99m}Tc in the generator eluate, the generator elution efficiency and the total mass of technetium (^{99m}Tc +^{99}Tc) present in a given eluate, have been carried using another software MOGEN-II. The input variables are ^{99}Mo activity, generator elution efficiency, number of elutions, the growth time between elutions, the decay time between elution and the use of ^{99m}Tc. The output of the program for each elution gives the activities of ^{99}Mo and ^{99m}Tc, atom numbers of ^{99}Mo, ^{99m}Tc and ^{99}Tc and the ^{99}Tc/^{99m}Tc atom ratio for the generator immediately before elution, for fresh eluate, for decayed eluate and for the generator after elution. This software will be useful for judging the quality of Pakgen ^{99m}Tc generators for a week or so.

Pakgen ^{99m}Tc generator provides, by elution, ^{99m}Tc-sodium pertechnetate in sterile, isotonic solution ready for intravenous or oral administration or for aseptic preparation of ^{99m}Tc labelled radiopharmaceuticals. All Pakgen ^{99m}Tc generators are test eluted before shipment. Quality tests performed are elution efficiency, molybdenum breakthrough, radiochemical purity, chemical purity, radionuclidic purity, sterility and apyrogenicity. The eluate fulfils the quality control criteria (Table I) prescribed by International Atomic Energy Agency and various Pharmacopoeias like United States, British and European (Table I) for sodium pertechnetate ^{99m}Tc injection [1-3]. In hospitals, the Pakgen ^{99m}Tc generators showed optimum performance in terms of elution yields, molybdenum breakthrough, labelling yields and quality of gamma scans.

TABLE I. CHARACTERISTICS OF SODIUM PERTECHNETATE (^{99m}Tc) INJECTION (FISSION)

Quality	EP Limits	Methodology	Our results
Characteristic	A clear, colourless solution, ^{99m}Tc with half-life 6.02 h emits gamma radiation 140 keV	Visual Gamma counting	A clear, colourless solution
PH	4-8	pH meter or pH paper	5.5-6.5
Radionuclidic purity	^{99}Mo 0.1% ^{131}I $5\times10^{-3\%}$ ^{103}Ru $5\times10^{-3\%}$ ^{89}Sr $6\times10^{-5\%}$ ^{90}Sr $6\times10^{-6\%}$ α-emitters $1\times10^{-7\%}$ other γ-emitters 0.01%	γ-Spectrometry β-Spectrometry α-Spectrometry	Below permissible limits
Radiochemical purity	Not less than 95% as pertechnetate	TLC/ paper chromatography	More than 98%
Chemical purity	Al < 10μg/ml	Chemical test	Al < 4 μg/ml
Yield of ^{99m}Tc	90-110%	Ionization Chamber	90-110%

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Biont – a new centre for PET radiopharmaceuticals production in Central Europe

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BIONT was established in 2005 as a part of the Cyclotron Centre of the Slovak Republic project. All its facilities represent the most state of the art technology for research and radionuclides production. The layout of the facility, comprising an IBA Cyclone 18/9 that delivers high beam currents of 100 or 45 μA, respectively, and a GMP zone for producing PET radiopharmaceuticals. Cyclone 18/9 is equipped with five targets. Two aqueous targets for $^{18}F^-$ (Nb and Ag targets) one gas target for $^{18}F_2$ production, aqueous target for ^{13}N, and gas target for $^{15}O_2$ and $^{11}CO_2$. IBA Cyclone 18/9 is equipped with external beam target Irradiation on COSTIS (COmpact Solid Target Irradiation System). This system has especially been developed for SPECT compatible PET radionuclides using small cyclotrons. COSTIS is designed for irradiation of solid targets requiring helium cooling of the targets front side. External target offers a new possibility for radionuclides suitable for medical application.

The production area includes six lead-shielded boxes with new modules for ^{18}F-FDG and ^{18}F-DOPA synthesis, a dry methylation module for preparation of ^{11}C-methyliodide precursor, and system designed for ^{13}N-NH_3 and ^{15}O-H_2O production, and an automatic dispensing unit, and all necessary auxiliary equipment. A quality control laboratory has been built in accordance with principles of GPCL and equipped with gas chromatography, HPLC, a TLC scanner, an UV-VIS spectrometer, a gamma spectrometer and a LAL test.

The Radiopharmaceuticals R&D laboratory is equipped with two shielded chambers, a Mini Cell for synthesis modules, and a HWM Dispensing Cell for an automatic dispensing module. The liquid chromatograph-mass spectrometric system is dedicated to precursors and radiopharmaceuticals analysis. The laboratory also includes a laminar shielded box with dose calibrator inside, cooled high-speed centrifuge, lyophilizer, ultra-pure water production and other laboratory equipment.

Nuclear Medicine Department, that allows the immediate PET diagnostic use or MicroPET-research use of even very short-lived tracers like ^{11}C, ^{13}N and ^{15}O, is rather unique within the EU are a component part of Biont.

Capacities and current activities of the Cyclotron and Nuclear Medicine Department of NRCAM

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The Cyclotron and Nuclear Medicine Department at the Nuclear Research Center for Agriculture and Medicine (NRCAM) has a cyclotron accelerator - model Cyclone 30 - which is a fixed frequency, dual beam extraction system with capabilities to produce proton beam in 15-30 MeV range of energy and deuteron beam in 7-15 MeV range of energy, with maximum output current of 500 μA and 150 μA, respectively. This Department consists of some laboratories and sections, such as: electro-chemical lab., inorganic-chemistry lab., labeling lab., radioisotopes production hot labs, quality control section, nuclear medicine section, etc.

According to these facilities, this Department is capable to produce various radioisotopes and radiopharmaceuticals, with high quality and quantity in accordance to the international standards, which are widely used in nuclear medicine, such as ^{201}Tl, ^{67}Ga,^{81m}Kr, ^{111}In, ^{18}FDG, ^{123}I, ^{103}Pd,^{57}Co. From these radioisotopes and radiopharmaceuticals the ^{201}Tl, ^{67}Ga,^{81m}Kr are routinely produced and sent weekly to about 35 hospitals and nuclear medicine centers around the country.

In order to achieve the knowledge of the routine production of ^{103}Pd and ^{57}Co radioisotopes, a technical cooperation (TC) project was defined under the support of the IAEA (IRA/04/032). There are also some new TC projects being proposed and to be submitted to the IAEA for support, e.g. proposed TC projects on “Production of ^{123}I through a new technique of bombarding the ^{123}Te isotope with proton”, and on “Achieving the knowledge of producing the seed form of ^{103}Pd for brachytherapy”.

With regard to international safety principles, radiation protection and environmental protection rules, the Department aims to choose the quality management standard system ISO 9001-2000 for its radiopharmaceutical production.

In this article we are going to introduce the Cyclotron and Nuclear Medicine Department at NRCAM, its facilities, current activities and future goals as well as demonstrate the indigenous capacity building of it for producing radiopharmaceuticals.

Session 6:

REACTOR BASED RADIONUCLIDES AND GENERATORS

Production of therapeutic radionuclides in medium flux research reactors

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Therapeutic use of radiation and radioisotopes, nearly all of which are artificially produced, is well established. Therapeutic effect in radiopharmaceuticals is due to high LET radiations such as α, β^-, e^- and most therapeutic radiopharmaceuticals employ β^- emitters, produced in nuclear reactors. The list of potential therapeutic radionuclides is large covering few α emitters (e.g. ^{211}At, $^{212/213}$Bi), mostly β^- emitters (e.g. ^{32}P, ^{131}I, ^{90}Y, ^{89}Sr, ^{166}Ho, $^{186/188}$Re, ^{153}Sm, ^{177}Lu, ^{175}Yb, ^{169}Er), and few Auger/Coster-Kronig/conversion electron emitters (e.g. ^{117m}Sn, ^{125}I, ^{77}Br). It is reported that in the world there are about 54 research reactors producing radioisotopes, of which 21 have $\phi<10^{14}$n/s/cm^2, 26 have $\phi\sim1\text{-}5\times10^{14}$n/s/cm^2 and 7 high flux reactors with $\phi>5\times10^{14}$n/s/cm^2 [1]. Although several potential therapeutic radionuclides are identified, unfavourable production logistics narrow the choice, and in some cases high flux reactors become essential. An over-view of the therapeutic radionuclides that can be produced in medium flux nuclear research reactors is attempted.

Therapeutic radiopharmaceuticals use radionuclides, mostly for treatment and palliation of cancers and to a lesser extent hyperthyroidism and synovitis apart from explored applicability for prevention of restenosis of blood vessels (endo-vascular radionuclide therapy-EVRT). The need in terms of quantities and specific activities would depend upon the end use. For example, in treatment of liver cancers or synovitis, high specific activity is not essential while to target receptors on cancer cells with radiolabeled peptides, high specific activity preparations are essential. Thus, ^{90}Y from ^{89}Y(n,γ) ^{90}Y can be used in former applications while ^{90}Y-lanreotide for treating somatostatin expressing cancers requires ^{90}Y from ^{90}Sr-^{90}Y generator [2]. The production feasibility of an isotope depends on the abundance of the target isotope, neutron absorption cross-section (σ), neutron flux (ϕ), irradiation duration and co-produced unwanted nuclides and their nuclear characteristics. While intrinsic factors like σ are not amenable to modification, epithermal / resonance absorptions have been used to advantage to attain higher yield and specific activity. We find that yields of ^{177}Lu and ^{153}Sm are always far higher than the calculated yields due to the contribution by the epithermal neutrons[3,4,5]. ϕ and isotopic enrichment of targets are modifiable and play a major role in the choice of the nuclide with production feasibility for therapy. Apart from these, the nuclear reaction itself also plays an important role. Direct neutron capture leads to low specific activity isotope of the target element and high specific activity can be achieved only in reactions with very high σ and high abundance of the target nuclide. But, reactions resulting in products of an element different from the target, such as (n,p), (n,γ, followed by fission/ β-/EC decay) could give “no-carrier added”(NCA) high specific activity products. Some examples from our experience: ^{177}Lu can be produced by irradiation of natural Lu (^{176}Lu~2.6%) by (n,γ) reaction ($\sigma\sim2100$b) with a specific activity of 5.6-6.7 MBq/μg when irradiated at $\phi\sim3\times10^{13}$n/s/cm2 for 7 days. Under the same conditions, 64% enriched ^{176}Lu (cost ~ 60 times nat. Lu target) yields 140-180 MBq/μg, which rises to >850 MBq/μg ($\geq$21atom%) on irradiation for 21 days at $\phi\sim9\times10^{13}$n/s/cm^2, quite suitable for receptor specific radiopharmaceuticals. Though NCA grade ^{177}Lu can be obtained by the reaction ^{176}Yb

$(n,\gamma)^{177}Yb(\beta^-)^{177}Lu$, the quantities are low [3,4]. Apart from specific activity, absence of chemical impurities is important for radiopharmaceutical applications.

Owing to their production routes, the following radionuclides are produced in medium flux reactors in large amounts in high specific activities: $^{32}S(n,p)^{32}P$; $^{130}Te(n,\gamma;\beta^-decay)^{131}I$.; $^{124}Xe(n,\gamma;EC)^{125}I$; $^{235}U(n,f)^{131}I$;^{90}Sr;^{137}Cs. Although enriched ^{235}U is irradiated specifically for production of nuclides by fission route, long lived nuclides such as ^{90}Sr ($T_{1/2}$28.8y), could be efficiently recovered from the waste from processed irradiated fuel also [2]. The role of epithermal and fast neutrons is significant in production of radionuclides, particularly in threshold reactions. e.g. ^{32}P yields from one of our reactors($\phi_{th}=1\times10^{13}$ n/s/cm^2;2% ϕ_{fast}) are more than twice those from another reactor($\phi_{th}=1\times10^{14}$ n/s/cm^2;1% ϕ_{fast}). Enriched targets enable use of moderate flux reactors for production of adequate quantities of radionuclides. For example, enriched ^{185}Re and ^{187}Re could be used for production of ^{186}Re and ^{188}Re. For nuclides such as ^{175}Yb, enriched target would additionally be desired to prevent/minimize concomitant production of unwanted radionuclides. $Yb_{nat.}$ yields ~2.2-2.6 MBq/μg ^{175}Yb with 2.6% ^{169}Yb+^{177}Lu, while $^{174}Yb_{99\%}$ yields ~ 7.4-9.3 MBq/μg ^{175}Yb of >99.9% RN purity [6].

Several therapeutic radionuclides can thus be produced in medium flux reactors in adequate quantities and of acceptable specific activity. Exploration to identify new therapeutic radionuclides continues owing to the need for varied uses. Few like $^{142/143}Pr$, ^{170}Tm, ^{141}Ce have potential for therapy and have been explored for production feasibility in medium flux reactors [7]. Additionally, mixed-radionuclide therapy such as in the case of ^{186}Re and ^{188}Re (by irradiation of nat. Re target), would merit a fresh look due to the ease of large scale production in many centres and could open more possibilities for production in medium flux reactors.

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The continuing important role of high flux research reactors for production of therapeutic radioisotopes

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Research reactors play a central role for the production of medical radioisotopes for both diagnostic and therapeutic applications. Many reactor-produced radioisotopes are neutron-rich and decay by beta particle emission. These radioisotopes and decay products are of interest for a variety of therapeutic applications in nuclear medicine and oncology. Although relative, for this discussion (Table I) the thermal flux (neutrons/cm^2-sec) is defined as "high" (> 10^{14}) and "very high" (> 10^{15}).

TABLE I. EXAMPLES OF HIGH/VERY HIGH FLUX RESEARCH REACTORS (IAEA Data 2005)

Reactor	Critical	Country	Max neutrons/cm^2-sec . x 10^{14}
"Very High" Flux Research Reactors			
SM3	1961	Dimitrovgrad, Russia	50
HFIR	1965	Oak Ridge, TN, US	25
BR2	1961	Mol, Belgium	10
"High" Flux Research Reactors			
MURR	1966	Columbia, Missouri, US	6
HANARO	1995	Taejeon, Korea	4
NRU	1957	Chalk River, Canada	4
HFR	1965	Petten, the Netherlands	2.7

In some cases - for example the fission production of 99Mo - high flux thermal flux is not required. In many other cases, however, the availability of high thermal flux allows production of higher specific activity (HSA) products and is also a benefit to produce other products which are inaccessible with sufficient SA from low or modest thermal flux reactors. HSA is often required to exploit specific biological cellular targeting mechanism, such as a limited receptor population. In addition, HSA can increase the radioisotope inventory shelf-life and can presumably reduce costs.

Reactor-produced radiolanthanide examples of current broad interest are lutetium-177 [1,2] and holmium-166, both of which can be produced by "direct" – Lu-176(n,γ)Lu-177 and Ho-165(n,γ)Ho-166 - and also via "indirect" production routes – Yb-176(n,γ)Yb-177(β-decay)Lu-177 and Dy-165(n,γ)Dy-166(β- decay)Ho-166. While reactor "indirect" production

routes can often provide HSA products, the disadvantages can include the modest production rates of the parent radioisotopes at lower flux (Yb-177), large target volumes may be required, and time requirements and costs. For lutetium-177, in particular, the opportunity to produce the very high multi-Curie levels at HSA (theoretical = 109 Ci/mg) at high flux would be expected to be required for preparation of HSA targeting agents (i.e. receptors) and for clinical trials and/or routine use. Production in very high flux reactors is expected to be preferred. Lower SA lutetium-177 produced in lower flux reactors is adequate for other applications, including bone pain palliation. The reactor production facilities required depend on SA requirements, the particular application and economics.

Another key example is tungsten-188, produced in even very high flux reactors with a low SA of only 4-5 Curies/mg W-186/Cycle partly because of the low cross sections for the double neutron capture process: W-186(n,γ)W-187(n,γ)W-188. The highest thermal flux available is thus required for production of tungsten-188, used for fabrication of the tungsten-188/rhenium-188 generators [3]. Other examples utilizing very high flux reactor indirect routes for production of radioisotopes for which the utility has not yet been fully established include platinum-195m and thorium-229. Platinum-195m is a potent Auger-emitting and can be produced by the Ir-193(n,γ)Ir-194(n,γ)Ir-195(β-decay)Pt-195m route [4]. Another example of broad current interest is thorium-229, the parent of actinium-225, used for the Ac-225/Bi-213 generator system. Thorium-229 is available in limited supply from decay of U-233, or Ac-225 can be accelerator-produced by the Ra-226(p,n)Ac-225. Because of the benefits of the long half life of Th-229 (7,340 y), another route which is being explored [4] is reactor production of Th-229 by the Ra-226(n, γ)Ra-227(n,γ)Ra-228 (n, γ)Ra-229(β-decay)Ac-229(β-decay)Th-229 route. The goal of this presentation will be to provide these and other key therapeutic radioisotope examples which require high and very high flux reactors for most effective production.

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Technological line for production of carrier-free ^{188}Re in the form of sterile, isotonic solution of sodium perrhenate (VII)

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Radiometric properties of ^{188}Re create convenient condition for medical application of this radionuclide. A big interest has arisen concerning the use of ^{188}Re for radioimmunotherapy, radionuclide synovectomy and bone pain palliation. This is due to the favorable characteristic of ^{188}Re ($T_{1/2}$ = 16,98 h), emission β^- particles with an average energy of 764 keV and emission of 155 eV gamma photon (15%, γ-rays), which permits the in vivo biodistribution evaluation of ^{188}Re-labeled ligands with gamma camera.

Radionuclide ^{188}Re is produced in results of ^{188}W decay. Tungsten-188 is obtained by neutron activation of ^{186}W according to nuclear reaction ^{186}W (n,γ) => ^{187}W (n,γ) => ^{188}W. At the Radioisotope Centre Polatom the technology for production of sterile and isotonic solution of ^{188}Re has been elaborated.

High specific activity ^{188}W is imported from RIAR, Russia with following specification; sodium tungstenate in sodium hydroxide solution, ^{188}W specific activity 195 GBq/g, ^{187}W to ^{188}W activity ratio 0,23%, total gamma emitters to ^{188}W activity ratio 0,3%, solvent concentration (sodium hydroxide) 0,24 mol/l and tungsten concentration 50 g/l.

The solution of sodium tungstenate is processing in technological line consisting of five lead-shielded chambers in which following operations are carried out.

1. Unloading of active material
2. Filling of ^{188}W solution on alumina column
3. Elution of ^{188}Re in form sodium perrhenate
4. Concentration of eluate
5. Proportioning of ^{188}Re solution to vials and its sterilization
6. Vials removal from technological line.

Alumina used for filling the generator columns was first activated using 0,9% NaCl in 0,001M HCl and 32% HCl to obtain final pH of about 3. Tungsten ^{188}W in the form of tungstenic acid was slowly loaded on the column (flow 0,1 ml/min). After ^{188}W deposition the alumina column was washed with 0,9% NaCl to remove the unbound ^{188}W. The adsorption capacity of alumina has been studied and the optimal conditions of ^{188}W adsorption have been selected. Three different eluents: 0.9% NaCl, 0,15M and 0,3M sodium acetate were used at the development phase for optimization of elution yield. For the eluates of ^{188}Re in 0,9%

NaCl the concentration system has been proposed consisting of anion and cation exchanging resins.

The obtained solution was purified and concentrated in the chromatographic system consisting of cation exchanger AG-50W-X12 Resin, 200-400 Mesh, hydrogen form and anionic column Accel Plus QMA Light, on which the perrhenate ions were concentrated. The Na^+ cations replace the H^+ ions on the cationite exchanger (Ag-50W-X12, Bio-Rad), then the H^+ bind to acetate ions and elute as acetic acid. The $^{188}ReO_4^-$ ions pass through the column and are stopped on the anionite column. Anionite column (Sep-Pak Plus QMA Light) first is washed with water to remove acetic acid and then $^{188}ReO_4^-$ ions are eluted in 1-2 ml 0,9% NaCl. The above described stationary generator system for preparation of sodium perrhenate-^{188}Re has been installed in the destined hot-cells, forming a complete production line. [1,2]

The quality of eluted ^{188}Re perrhenate solution was checked by means of paper chromatography using 0,9% NaCl as developing solution to evaluate its radiochemical purity. Chemical purity of the eluate was determined using ICP-Optical Emission spectrometer (Optima 33000XL, Perkin-Elmer) with a special consideration to the presence of W, Al and Zr. Radionuclidic purity of the eluates was checked by γ-spectroscopy. It covered the overall assessment of radionuclidic impurities related to the ^{188}Re activity and ^{188}W breakthrough.

Elaborated technology has given possibility for preparation of carrier-free ^{188}Re in form of sterile, isotonic solution of sodium perrhenate (VII) activity 79 GBq with radiochemical purity of 99.9% [3,4]. Obtained series of sodium perrhenate (VII) ^{188}Re are distributed to national nuclear medicine centres.

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Hydroxyapatite-based $^{99}Mo/^{99m}Tc$ and $^{188}W/^{188}Re$ generator systems

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Radioisotope generator systems have traditionally played a central role in nuclear medicine in providing short-lived radioisotopes for research and clinical applications. Techenitum-99m (half-life 6 h) is the most widely used radionuclide in diagnosis, and rhenium-188 (16.9 h) is a particularly attractive candidate for therapeutic applications. These daughter radioisotopes are provided by the $^{99}Mo/^{99m}Tc$ and $^{188}W/^{188}Re$ generator systems in which ^{99}Mo and ^{188}W are adsorbed onto an alumina chromatographic column and the less strongly bound $^{99m}TcO_4^-$ and $^{188}ReO_4^-$ are eluted with isotonic saline solutions.

This work proposes to use hydroxyapatite as the adsorbent material for both $^{99}Mo/^{99m}Tc$ and $^{188}W/^{188}Re$ generator systems [1]. Hydroxyapatite is an insoluble solid with anion exchange properties. A study of the adsorbent behaviour of ^{99}Mo, ^{99m}Tc, ^{188}W and ^{188}Re on hydroxyapatite in NaCl and $CaCl_2$ media was evaluated with batch experiments.

Our results demonstrated that while ^{99}Mo, ^{99m}Tc, ^{188}W and ^{188}Re are not adsorbed by the hydroxyapatite in NaCl solutions ($Kd<5$), use of $CaCl_2$ solutions results in strong adsorption of ^{99}Mo (see Fig. 1) and ^{188}W ($Kd>500$) whereas ^{99m}Tc and ^{188}Re are weakly adsorbed ($kd<5$).

Based on these measurements, hydroxyapatite $^{99}Mo/^{99m}Tc$ and $^{188}W/^{188}Re$ generator systems were then constructed and eluted in $CaCl_2$ solutions. The generator performances are presented and a method to modify the media of $^{99m}TcO_4^-$ and $^{188}ReO_4^-$ solutions is also proposed.

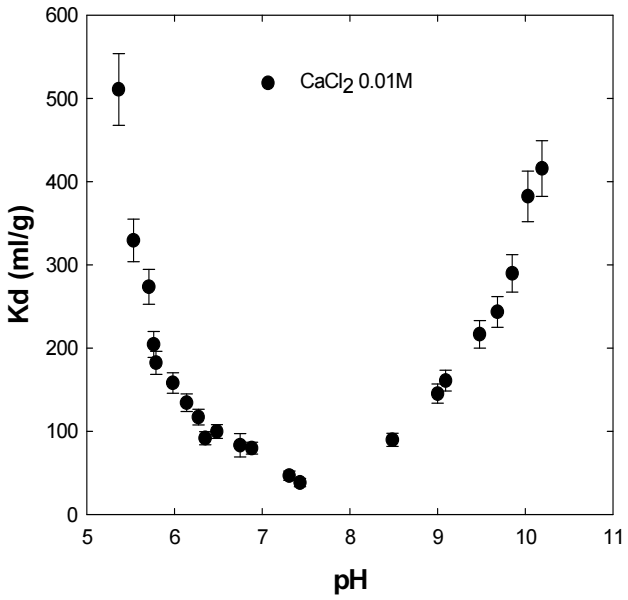

FIG. 1. Adsorption behaviour of ^{99}Mo on hydroxyapatite as a funtion of pH in 0.01 M $CaCl_2$ medium.

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Fostering development efforts towards (a) small scale local production of ^{99}Mo from LEU targets and (b) gel generator for ^{99m}Tc using (n,γ)^{99}Mo — Agency's role

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^{99m}Tc is the most widely used radioisotope in diagnostic nuclear medicine the world over with almost 40,000 procedures performed every day [1]. ^{99m}Tc is commonly availed by eluting alumina column loaded with ^{99}Mo of high specific activity obtained by thermal neutron fission of ^{235}U. Highly enriched uranium (HEU) targets of >95% ^{235}U are in use for this purpose [2]. Four commercial companies, namely, MDS Nordion, Canada, IRE, Belgium, Mallinckrodt, The Netherlands, and Nuclear Technology Products, South Africa, cater to most of the ^{99}Mo needs using HEU targets. ANSTO, Australia and CNEA, Argentina are among the other few producers. The former is at present using uranium dioxide pellets enriched to 2.2% ^{235}U, while the latter is using LEU targets, i.e. <20% ^{235}U content. The former is also slated to switch over to using LEU targets of <20% ^{235}U after the commissioning of the new reactor facility. In order to assure secured availability of ^{99}Mo, several research reactors (RR) are used for irradiating uranium targets. The need for far more assured RR irradiation services is being highlighted by industrial companies engaged in radioisotope production [2].

Measures advocated for reducing nuclear proliferation risks warrant that any materials of weapons potential be phased out of conventional commercial trade and HEU is high on the list. The Reduced Enrichment for Research and Test Reactors (RERTR) programme is a major initiative in this regard from the Department of Energy (DOE), USA, and is handled by the Argonne National Lab (ANL). The main activities are for supporting the conversion from HEU fuels to LEU fuels in research reactors and the return of fresh and spent HEU fuel to country of origin. Another related aim has been the development and deployment of LEU targets for production of fission produced molybdenum-99 (fission-moly). Accordingly, ANL, USA developed a technology for production of fission-moly using LEU targets and the process know-how called 'modified Cintichem process' is available for adaptation by interested Member States [3]. This method uses LEU foil target, while an alternate target is mini plate target, developed by CNEA, Argentina [3]. There are two routes of processing, the alkaline dissolution and the acid dissolution methods. Both methods involve multiple stages of ion-exchange chromatography for separation and subsequent purification of ^{99}Mo. The scope for separation of ^{131}I as a by-product has also been demonstrated.

Switching over to the use of LEU targets over a defined period of time by all the major industrial producers of ^{99}Mo would be necessary, but due to associated commercial aspects, such a discussion would be beyond the purview of this paper.

Amongst the alternate ^{99m}Tc delivery systems feasible using (n,γ)^{99}Mo of low/medium specific activity, gel generator based on zirconium molybdate-^{99}Mo (Zr^{99}Mo) is attractive in terms of user-friendliness, thanks to the convenience of column operation [4,5]. This would be a good substitute for the chromatographic alumina column generator, if there is ready

access to (n,γ)^{99}Mo of specific activity 0.5-1 Ci per g of Mo and process technology along with reliable process gadgets for gel preparation. Procedures for preparation as well as evaluation of clinical utility of gel generators are successfully established, but the process is demanding in terms of remote handling facilities for robust and safe operations [5–7].

The concept of post-elution concentration of perrhenate and pertechnetate mooted by the ORNL group and the growth of centralized radiopharmacy service providers have given a fillip to the prospects of using large gel bed (10–25g) generators loaded with (n,γ)^{99}Mo through the dual purpose use of the secondary trap column of alumina for ^{99}Mo necessary for Zr^{99}Mo gel generators [8,9]. The effective use of alumina trap column for purification-cum-concentration of pertechnetate after eluting the large gel bed with de-ionized water has widened the scope of utility of gel generators, even when faced with modest specific activities of ^{99}Mo. This fruitful outcome of post-elution concentration option augurs well for the wider adaptation of gel generator technology [9]. The need for large quantity of MoO_3 target for reactor irradiation, radionuclidic impurities in the irradiated material and their fate while preparing ^{99m}Tc gel generator systems have posed interesting challenges, but have been addressed satisfactorily [10].

In the Annual Meetings of the RERTR, several presentations have been made and lately through sessions dedicated to production of ^{99}Mo using LEU targets [3]. Following the RERTR Meeting in November 2004 held in IAEA, Vienna, a Consultancy Meeting (CM) was held by IAEA to review the various issues and consider formulating a Coordinated Research Project (CRP). The extensive work and good results reported in the case of the above two systems form the basis of the CRP launch recommended by the CM. Comparative assessment of production logistics and quality control testing aspects of ^{99}Mo from the HEU and LEU targets have been reported by ANL [3]. Small scale production of fission moly is envisaged locally in many participant centres under the CRP, with ANL, USA and CNEA, Argentina, providing the technology know-how for production of ^{99}Mo using LEU targets.

Thanks to the vast literature available on gel generators [4–7,9–10] and the strategy to exploit dual use of alumina trap column for post-elution concentration [9], Zr^{99}Mo gel generator system appears promising, despite technical complexities. This gel generator option to utilize local production of (n,γ)^{99}Mo will be pursued by a few other participants of the CRP, who have access to RR of suitable flux and assured operational features. Technical know-how for gel generator is expected to be available from India/Brazil.

In view of the need for securing reliable, diverse sources of ^{99}Mo and dissuade the use of HEU targets for fission moly production, all options for local production of ^{99}Mo and prospects of novel delivery systems for ^{99m}Tc merit attention not only in developing countries, but also in developed countries, and the IAEA will foster such efforts.

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Session 8:

NOVEL TECHNETIUM CHEMISTRY AND RADIOPHARMACEUTICALS - II

Novel technetium chemistry and radiopharmaceuticals: Tc(V), Tc(III) or Tc(I), which way to go for keeping Tc radiopharmaceuticals alive?

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Novel ^{99m}Tc based radiopharmaceuticals which have found market introduction are extraordinarily rare. Despite enormous research efforts over the past 10 years only a very few new compounds (^{99m}Tc or ^{188}Re) contribute to public health. New radiopharmaceuticals may belong to the class of perfusion or to targeting agents in which a complex is conjugated to a receptor binding molecule. Actual research focuses essentially on the 2nd class.

While other techniques are developing very rapidly, ^{99m}Tc based radiopharmacy risks to lose its leading role in nuclear medicine. The expression “working horse in diagnosis ” is likely to be taken over by PET or MRI. Certainly, radiotherapy can not easily be substituted and is still a unique possibility and driving force. Despite the author’s “dark” view for the future of ^{99m}Tc research, Re-186/188 and other therapeutic radionuclides will fuel research in group 7 chemistry. Of course, many scientific papers still can be produced (which is as important as finding a novel radiopharmaceutical) but the ultimate goal of contributing to public health should not get out of mind.

The question arises why the situation is as described and how it can be changed (rapidly).

The presentation aims at a critical review about limitations and requirements demanded for finding a novel radiopharmaceutical. The main focus here will be on the chemistry behind. Research in radiopharmaceutical chemistry should follow a “top-down” strategy, requirements from the market and production in clinics. The situation is inverse to normal pharmaceuticals where the second factor does not count at all. If an excellent radiopharmaceutical can not be synthesized on site, it is probably useless for routine application. Top-down in this respect means that companies and health authorities have to assign a target and to clarify eventual patent situations. Researchers will study targeting molecules, labelling and biological properties. The requirements from the user has to be considered from the beginning and is a prerequisite for labelling pathways. Synthons are required for this drug finding process. Currently 4 or 5 precursors are available, namely [Tc=O]3+, [TcO2]+ and [Tc≡N]2+ core from Tc being in the oxidation state +V, Tc(III) chemistry and [Tc(CO)3]+ chemistry in the +I valency [1].

The hynic approach represents another strategy but suffers from a yet undefined core. All these approaches are likely to be successful from a coordination chemistry point of view. The presentation will critically emphasis their weak and strong points with regard to different kinds of biomolecules.

Search for novel cores and strategies is not at its end, and a number of hypothetical cores and methodologies will be proposed to complement the available ones, underlining the importance of basic Tc-chemistry.

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New ^{99m}Tc-cytectrene piperidine compound as specific brain imaging agent

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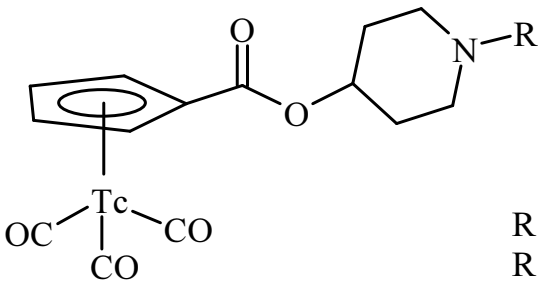
Radiopharmaceuticals that bind to the CNS receptors in vivo are useful for understanding the pathophysiology of several neuropsychiatric disorders.

For the diagnosis of these pathophysiological processes it is important to develop radioligands able to bind specifically to well defined CNS receptors in order to evaluate their density and distribution in the brain.

Due to the availability, low cost and optimal radiation properties of ^{99m}Tc, there is a considerable interest in the development of ^{99m}Tc radiopharmaceuticals for imaging CNS receptors using single photon emission tomography (SPET).

Biodistribution studies in rats of cyclopentadienyl technetium tricarbonyl conjugates of piperidine derivatives [1,2,3] showed that ^{99m}Tc cytectrenes, containing in their structure an N-methylpiperidine and an N-isopropyl piperidine, accumulated in the brain regions with different binding specificity.

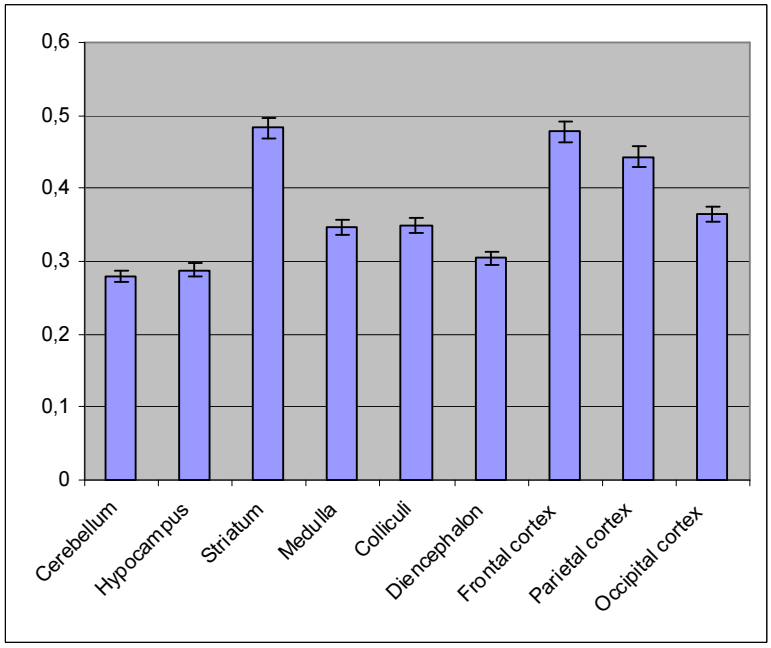
Therefore we attempt to study the biobehaviour of a new cytectrene in which the piperidine was not substituted.



R = Me (*cytectrene I*)
R = ^{i}Pr (*cytectrene II*)
R = H (cytectrene III)

Furthermore, in order to improve the reaction conditions for eventual routing use, we have carried out the radiochemical labelling following the tricarbonyl concept of Alberto, et al. [4] without the need for $Mn(CO)_5Br$.

The in vivo uptake of the ^{99m}Tc ligands in the whole rat brain and into brain regions was investigated. Samples of brain regions (cerebellum, colliculi, diencephalons, hypocampus, striatum, medulla, frontal cortex, parietal cortex and occipital cortex) were identified, removed, weighed and their radioactivity measured.



The highest uptake was observed in the striatum and in cortex regions. Receptor binding assays in rat brain homogenates and blocking studies are necessary to determine further data concerning, specificity, selectivity and non specific binding of this compound.

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Tricarbonyltechnetium (I) complexes with neutral bidentate ligands: N-methyl-2-pyridinecarboamide and N-methyl-2-pyridinecarbothioamide. Experimental and theoretical studies

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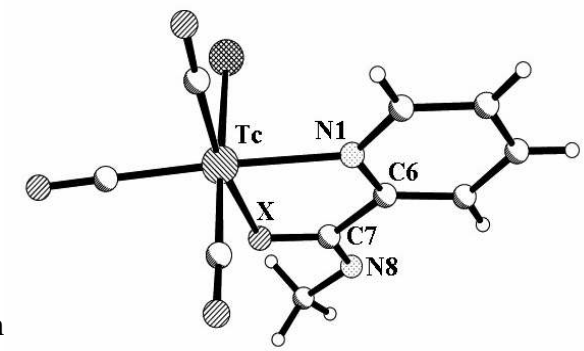
Chemistry of tricarbonyltechnetium(I) complexes, the derivatives of organometallic aqua-ion *fac*-$[Tc(CO)_3(H2O)_3]+$ (**1**), with a chelating ligand in the molecule, is being quickly developed for the last decade [1]. Due to the softness (HSAB concept) of the Tc(I) centre, chelators with soft donor atoms are preferred as the ligands. This work is aimed on finding ligands that form very stable tricarbonyltechnetium(I) complexes, and - after further functionalization - can be used for syntheses of ^{99}Tc radiopharmaceuticals of the 2nd generation.

Two $[Tc(CO)_3LNXB]$ complexes were obtained and studied, where: LNX is a neutral chelating ligand of N,S donor atoms: N-methyl-2-pyridinecarbothioamide, LNS, and its analog of N,O donor atoms: N-methyl-2-pyridinecarboamide, LNO; B is a monovalent anion or H_2O. The complexes with ^{99m}Tc at n.c.a. level (B = OH– or H_2O) and with ^{99}Tc in mg quantities (B = Cl–) were prepared according to the methods of Alberto, et al. [2]. The ligands coordinate the metal centre bidentatly *via* the pyridine nitrogen and the X atom (O or S), forming a five-membered chelate ring (Scheme). This conclusion results from: (1) the similarity of IR spectra of the technetium complexes studied and Formation of the $[^{99m}Tc(CO)_3LNXB]$ complexes was studied by HPLC. After 40 min. incubation at 75 ^{0}C $[^{99m}Tc(CO)_3LNSB]$, **3**, was obtained with nearly 100% (HPLC) yield, but two forms of the complex were observed: cationic ($B=H_2O$) and neutral (B=OH–). The equilibrium between these two forms depends on pH and shifts to over 90% of the neutral form at pH 7–10. The yield of the $[^{99m}Tc(CO)_3LNOB]$ complex, **4**, was lower, ca. 90%, but the cationic form of the complex predominated (over 60% at pH 7–10). The easier hydrolysis of the H_2O ligand in the complex with the LNS ligand, evidencing stronger coordination of the H2O molecule to the Tc atom in **3** than in **4**, was rather unexpected under assumption of stronger coordination of the LNS than LNO ligand, and seemingly inconsistent with the smaller positive charge on the Tc atom in **3** (0.14 e) than in **4** (0.31 e) [4].



from L to $Tc(CO)_3Cl$ in e) than for **4** (0.37 e), which means that the Tc-L_{NS} bond is more covalent than Tc-L_{NO} and the former ligand is

more strongly bound. However, the energy of complex formation, calculated as the differences between the total energies of the products and substrates in the gas-phase, is more negative for **3** (–201 kJ/mol) than for **4** (–186 kJ/mol). One of the reasons of this contradiction is a stronger hydration in aqueous solution, of L_{NO} than L_{NS} molecule, due to stronger H-bonds of water to the O than S atoms. The calculated difference in hydration energies, of ca. 16 kJ/mol, is insufficient, however, to explain the contradiction fully. The other reason can be the stronger deformation of the chelate ring in the optimized structure of **3** than in that of **4**. While the rings in the crystals of the analogous rhenium(I) complexes are planar, i.e the dihedral angle Θ(N1-C6-C7-X) = 0^0, it is not so in the optimized $[Tc(CO)_3LCl]$ molecules where $\Theta = 28.6^0$ in **3**, and 7.3^0 in **4**. Also the calculated Tc-S distance in **3** (266 pm) is much longer than the experimental Re-S distance (244 pm) [3]. The deformation of the quasi-aromatic chelate ring decreases the structure stabilization due to the expected conjugation of p orbitals perpendicular to the ring plane. If the real structures of the chelates in solution are less deformed than those calculated, a significant contribution to the stability of the chelate with the L_{NS} ligand would be observed. The other reason of the difference in the energies of formation of **3** and **4** can be different ground-state conformations of the free ligands in solution, due to rotation around the C6-C7 axis. The calculated energy differences between the gas-state *cis*- and *trans*- conformations of the free ligands studied exceed 50 kJ/mol.

The work is in progress. The DFT calculations may be considered a valuable tool for predicting the thermodynamic stability of metal complexes of interest for radiopharmaceutical chemistry.

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Modified bombesin analogue with technetium tricarbonyl precursor as prostatic radiodiagnostic agent

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Bombesin (BBN) and the molecularly related gastrin-releasing peptide (GRP) act as neurotransmitters and endocrine cancer cell-growth factors on normal tissues as well as on neoplastic cells of various origins, that includes prostatic carcinomas and many breast carcinomas. Modifications of the molecule BBS (7-14) have been attempted in order to obtain derivatives that can increase plasma stability and provide easy labeling with radionuclides. The synthesis of Tc-carbonyl synthon has opened the door for ^{99m}Tc(I) peptide chemistry.

The aim of the study was the evaluation of the labeling and biodistribution of modified BBN analogue with Tc-carbonyl core as a prostatic tumour diagnostic agent.

BBS (7-14) was synthetized by substituting methionine (14) by norleucine and coupling the (NαHis)Ac ligand for the application of the Tc-carbonyl labeling technique. Preparation of the Tc-carbonyl precursor was done according to published procedures. To 50 μg of BBS it was added 450 μl of ^{99m}Tc-carbonyl. The mixture was heated for one hour at 75 °C and later cooled in ice bath. Radiochemical evaluation was done in Whatman n.1, TLC-Al with solvent mixture MeOH/HCl 6M (99.5/0.5) and HPLC The product was purified before biological studies with C18 SepPack cartridge. The impurities were eluted with water and ^{99m}Tc$(CO)_3$-BBS with ethanol Biodistribution studies were performed in normal swiss mice at 1.5, 4 and 24 h post-injection and in nude mice bearing prostate cancer cells PC-3, 1.5 h post-injection. Scintigraphic images were documented in these last animals.

Synthesis and labeling of the peptide was succesful. Yield of the tricarbonyl intermediate was greater than 90%. Radiochemical purity for the radiolabeled BBS was 86.3 ± 1.2%, with a Rt = 19.1.

Biodistribution study results suggest that ^{99m}Tc $(CO)_3$-BBS was mainly excreted by the hepato-biliar system and had large intestine uptake. The tumour uptake was 1.15±0.05%ID/g with tumour/blood and tumour/muscle ratios of 2.67 and 3.19, respectively. Activity in the pancreas was used as a measure of receptor binding. At 1.5h post-injection the activity was only 1.31±0.04%ID/g. Scintigraphic imaging in nude mice bearing PC-3 cells showed a very low uptake by the tumour.

Labeling conditions permitted a good yield. Substitution of the aminoacid in the position 14 by Nle in the molecule had the advantage that no oxidation took place during synthesis and labeling, rendering it easier to work with this compound. Nevertheless, the radiopharmaceutical didn't show improved uptake by prostatic cell tumour, in comparison with findings observed without this modification.

Synthesis and characterisation of enantiomerically pure bipyridinyl-MTO as the ^{99m}Tc-tricarbonyl-conjugate - ^{99m}Tc-metomidate

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Metomidate (MTO) is a potent inhibitor of steroid 11β-hydroxylation in the adrenal cortex [1,2]. For labelling with technetium-99m, two nucleophilic centres were introduced by replacing the phenyl ring of MTO by the bipyridinyl moiety for bidentate binding with the cis-tricarbonyl-technetium complex [3,4]. Complex formation proceeded with high yield, followed by conjugate formation with the BiPy-precursor. For characterization of the ^{99m}Tc-tricarbonyl-BiPy-MTO-conjugate the analogous Re-tricarbonyl-BiPy-MTO-conjugate was produced, showing identical retention times. (R)-configuration was demonstrated to be essential for high-affinity binding to adrenocortical tissue when tested by radioligand displacement [5].

Synthesis of MTO derivatives: The BiPy-MTO-precursor 1-(2,2'-bipyridinyl-5-ethyl)-imidazole-5-carboxylic acid methyl ester was obtained by several reaction steps, finally chiral HPLC-separation of enantiomers was performed. In addition, several derivatives have been synthesized to evaluate the functionality of the chiral C-atom, and also the biochemical acceptance of replacing the phenyl ring. Compounds synthesized included demethyl-MTO (no chiral C-atom), ethyl-MTO (ethyl instead of methyl), and (S)-MTO. As a replacement of the phenyl ring, pyridine and bipyridine were chosen, producing pyridinyl-MTO (Py) and bipyridinyl-MTO (BiPy), both as the demethylated achiral derivatives and as the biologically active (R)-enantiomers. Structural verification was obtained by ^{1}H- and ^{13}C-NMR spectroscopy.

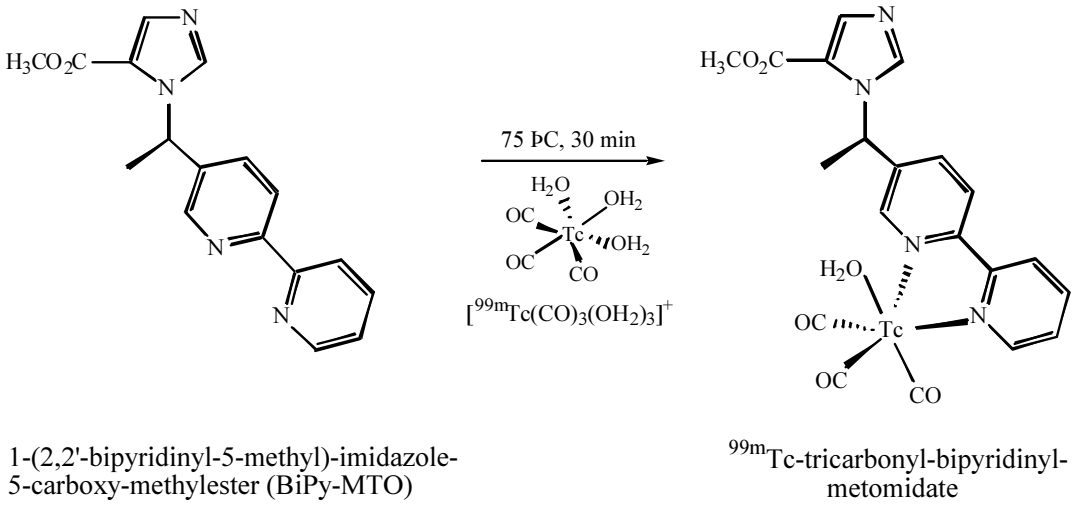
Radiolabelling: First, the ^{99m}Tc- tricarbonyl-complex was prepared using a kit formulation (Isolink™, Mallinckrodt). After cooling and pH-adjustment (pH 7), the BiPy-MTO-precursor was added and the reaction mixture heated for conjugate formation. Fig. 1 shows the reaction conditions for synthesis of the hypothetical ^{99m}Tc-tricarbonyl-BiPy-MTO-conjugate. Purification by analytical HPLC: The ^{99m}Tc-tricarbonyl-BiPy-MTO-conjugate was eluted with a retention time (t_R) of 19.5-20.2 min. The ^{99m}Tc-tricarbonyl-complex and ^{99m}Tc-pertechnetate would be eluted at 5.0 and 10 min, respectively.

For characterization of the ^{99m}Tc-tricarbonyl-BiPy-MTO-conjugate the analogous Re-tricarbonyl-BiPy-MTO-conjugate was produced. BiPy-MTO was reacted in methanol with bis-tetraethyl-ammonium-tribromo-tricarbonyl-rhenate(I) to produce the rhenium conjugate. Compounds were characterized by means of NMR and IR spectroscopy and elemental analysis.

Radioligand displacement: Membranes prepared from rat adrenals were incubated with 20.000-40.000 cpm of ^{131}I-IMTO together with 2 nM carrier (resulting in a specific activity of 330-660 GBq/mmol) at 23°C for 20-30 minutes. Bound radioligand was isolated by filtration through glass fiber filters. For displacement studies test compounds were added at 0.1–100 nM. Non-specific binding was determined with ETO (10 μM). IC_{50}-values were evaluated by non-linear, least squares regression analysis.

Derivatives were chemically characterized as inhibitors of specific ^{131}I-IMTO binding. The replacement of the phenyl ring as (R)-pyridinyl-MTO (Py) showed a moderate decrease in the binding affinity corresponding to an IC_{50}-value of 20.7 nM, this effect is pronounced with the bipyridinyl-derivative (BiPy) (IC_{50} = 170 nM). If the methyl substituent of MTO (no chiral C-atom) is removed, the demethyl-analogue (dme) had an IC_{50} value of 28.8 nM. In case of Py, removal of chirality produced a considerable loss of affinity corresponding to an IC_{50} of 870 nM. In case of BiPy, affinity lies in the micromolar range.

Formation of the ^{99m}Tc-tricarbonyl-complex using a commercial kit was quantitative, coupling with the BiPy-precusor proceeded with high yield. HPLC separation produced a pure ^{99m}Tc-tricarbonyl-BiPy-MTO conjugate. Structural verification was obtained by analysis of the analogous Re-tricarbonyl-BiPy-MTO-conjugate. Preliminary results have shown high adrenal accumulation in a rat. SPECT studies in two animal species will follow.



1-(2,2'-bipyridinyl-5-methyl)-imidazole-5-carboxy-methylester (BiPy-MTO)

^{99m}Tc-tricarbonyl-bipyridinyl-metomidate

FIG. 1. Labelling of (R)-BiPy-MTO with the ^{99m}Tc-tricarbonyl-complex.

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Direct labelling of lipiodol with [^{188}Re(CO)$_3$]-chelates

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Hepatocellular carcinoma is one of the 10 most common tumours in the world [1] and the most frequent malignant tumour in large areas of Asia and Africa [2.3]. Internal radiation therapy (IRT) has been used extensively in the management of HCC which has been treated with ^{131}I iodized poppy seed oil (Lipiodol^{TM}), ^{90}Y labeled glass microspheres or ^{188}Re-HDD/Lipiodol.

Radioactive $^{186/188}$Rhenium is gaining prominence and significance as the main therapeutic radionuclide by virtue of its distinctive physical properties. Currently, interest in low-valent organometallic complexes has emerged, as these are stable *in vivo* due to the high inertness of the d^6 electronic configuration [4]. Alberto et al. presented the first synthesis of the water and air stable organometallic aqua complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ directly from $[^{99m}TcO_4]^-$ in saline under 1 atm of CO [5]. This complex has been proved to be a versatile synthon that leads to a rich aqueous chemistry. Recently, Schibili et al. presented two preparation kits for $[^{188}Re(OH_2)_3(CO)_3]^+$ in high yield and high specific activities [6].

The radiographic contrast medium available under trade name of Lipiodol™ (GUERBET) is derived from natural poppy seed oil. Lipiodol, when injected through hepatic artery, accumulates in liver cancer cells. This makes arterially directed treatment of liver especially attractive, since the tumour can be made either ischemic or infused with cytotoxic agents while uninvolved liver is spared [7]. Various scientists have developed methods to use Lipiodol as a drug delivery carrier, combined with different β^- emitting radioisotopes or chemotherapeutic agents. Currently almost all of the applications were based on dissolution of either of these into Lipiodol.

We have synthesized bi- and tridentate ligand systems featuring long alkyl chain as Lipiodol surrogates and their $[Re(CO)_3]$ complexes were synthesized by various labelling approaches; pre-labelling, post labelling and 2+1 mixed-ligand approach. In this synopsis, direct labelling of the natural product Lipiodol with $[Re(CO)_3]$ centre will be high-lighted.

The first and only attempt to covalently label ^{188}Re with Lipiodol was reported by Wang et al. by using the linker EDTB (EDTB = N,N,N',N'-terakis(2-benzymidazoylmethyl)-1,2-ethanediamine) affording EDTB conjugated Lipiodol [8]. Unfortunately the exact structure of the labelled Lipiodol could not be verified and the toxicity of EDTB is unknown and therefore is an obstacle for further clinical trials.

Ligand entity, that can connect Lipiodol to the metal centre chemically, can be visualized through derivatization of Lipiodol such that it has the functionality to coordinate to the metal (M= Tc or Re). Alternatively, the ligand on the metal (M= Tc or Re) can be functionalized,

such that it chemically condenses with Lipiodol or its derivative. We have covalent bonded Lipiodol with $[Re(CO)_3]$-complexes by the following method (Scheme 1).

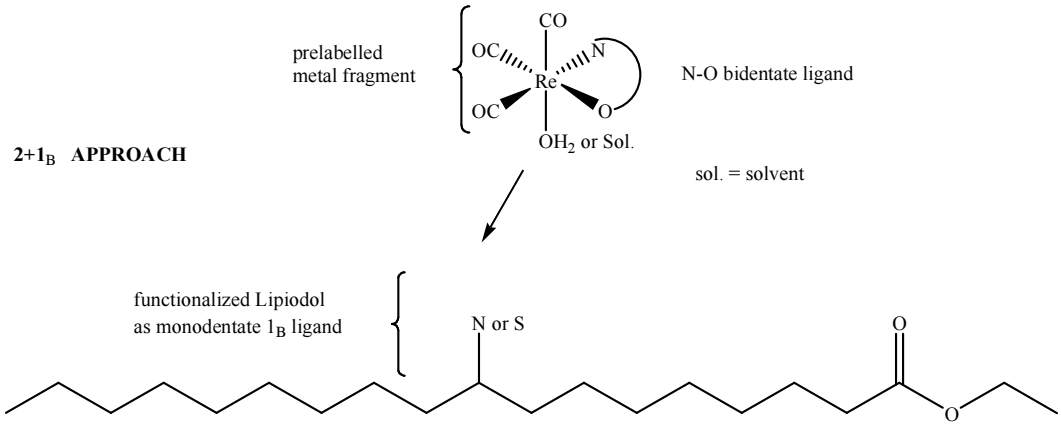

Fig 1: **Scheme 1. Covalent bonding of Lipidol with Re(I) complexes:**

ESI/MS was chosen as the sole analytical tool for studying direct labeling experiments as it is well known for efficient method for characterization of metal complexes in solution in general. [9] We have studied derivatized-Lipiodol with monodentate bifunctional linkers (BFC); 4-mercaptopyridine and imidazole (Scheme 1). Our ESI/MS study of incorporation of $[Re(CO)_3]$-metal fragments with derivatized Lipiodol clearly showed that direct labeling of Lipiodol with $[Re(CO)_3]$-core is feasible, but further characterization is very challenging. To overcome this difficulty, we have synthesized a Lipiodol mimetic and it's $[Re(CO)_3]$-metal complex **1** by the direct labeling method mentioned above (Fig. 2).

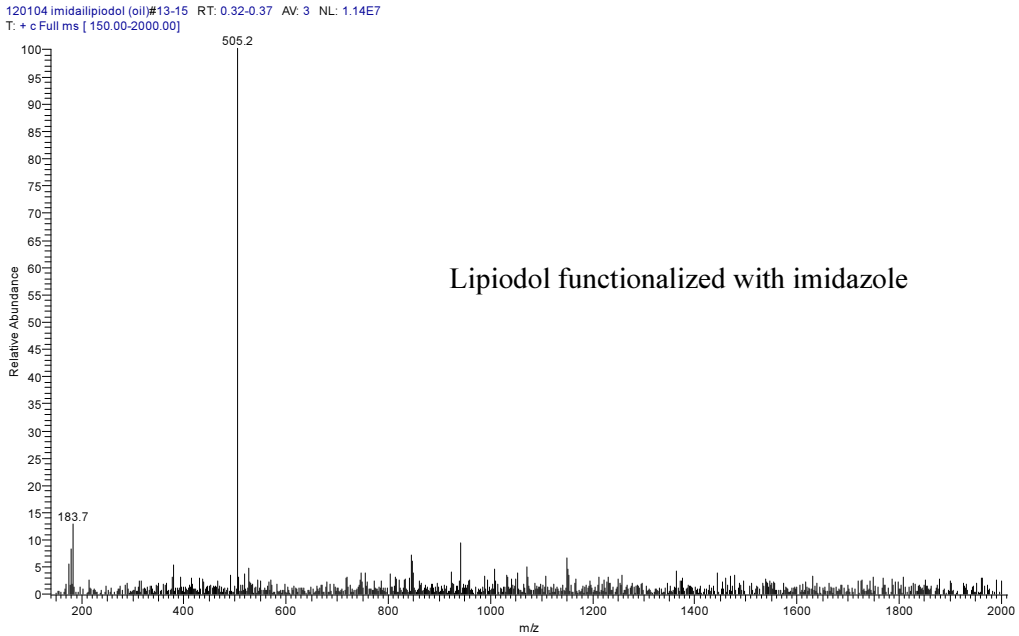

FIG. 2. ESI/MS spectrum of Lipiodol functionalized with imidazole.

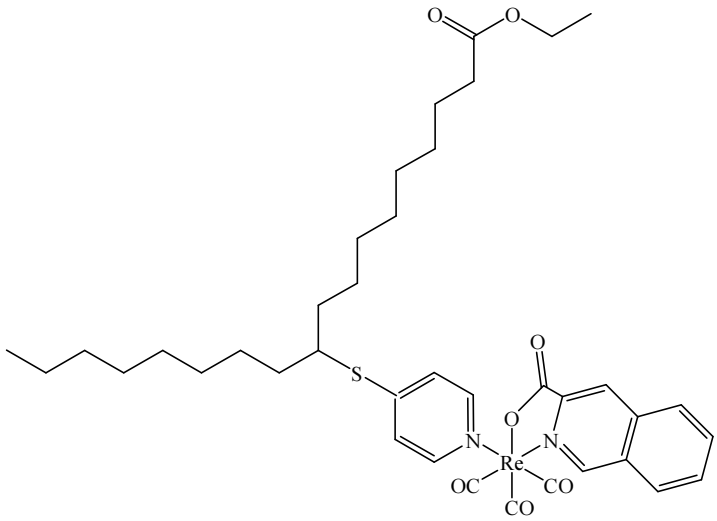

FIG. 3. Structure of a Lipiodol mimetic directly labeled with [Re(CO)$_3$]-metal fragment

As the final part of this project, we have studied biodistribution of imidazole-derivatized Lipiodol **2** directly labeled with [^{99m}Tc(CO)$_3$]$^+$. Biodistribution study of [^{99m}Tc(isoquinoline-1-carboxylate)(**2**)(CO)$_3$] showed that the complex is localized mainly in the liver with some excretion into the intestine.

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Session 9:

PHARMACOLOGY AND THERAPEUTIC PHARMACEUTICALS

Biological and chemical evaluation of various radiocolloids used for clinical radiosynovectomy

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Synovectomy by intraarticular application of β-emitting radiocolloids (radiation synovectomy: RSV) was introduced in 1952 by Fellinger, et. al. [1] for treatment of an inflamed synovial membrane. Since that time number of colloid radiopharmaceuticals are used to treat patients suffering from inflammatory-rheumatoid and degenerative joint diseases [2,3,4].

TABLE I. RADIOPHARMACEUTICALS USED FOR INTRARTICULAR THERAPY

^{32}P-colloid	^{198}Au-colloid	^{165}Dy-FAMA
^{90}Y-citrate colloid-silicate	^{166}Ho-hydroxyapatite,-phytate colloid	
^{153}Sm-hydroxy MA	^{169}Er-citrate colloid	^{186}Re-colloid,-rhenium sulfide
^{188}Re-tin colloid, -sulfur colloid, -microsphere		

In our study three approved radiopharmaceutical products (^{90}Y-citrate colloid, ^{90}Y-silicate and ^{169}Er-citrate colloid) and two experimental products (^{188}Re-tin-colloid and ^{166}Ho-phytate colloid) were evaluated and compared. Rate of colloid bound activity, stability in synovial liquid and particle size range of colloids were always determined. In vivo studies to determine extra-articular leakage of varoius radiocolloids to liver, blood pool, kidneys and inquinal lymph nodes were determined in rabbits. Injection technique developed and practiced were controlled by X ray, US and scintigraphy allowed to study personnel to control any leakage relating to the non correctly positioned needle in the knee joint cavity [5].

TLC studies showed that more than 99% activity in colloid form and results of sample incubated in synovial fluid (rabbit) also proved to be stabil (>99%). The mean particle size of colloids are the followings:

^{90}Y-citrate colloid: 3,1 μm; ^{90}Y-silicate: 0,9 μm

^{167}Er-citrate-colloid: 1,1 μm; ^{166}Ho-phytate: 0,66 μm; ^{188}Re-tin colloid: 0,6 μm

The distribution of injected activity in various organ (liver, spleen, kidneys, blood, lymph node, skeleton) outside knee ranged up to 10% even after 14 days of injection.

Activity values retained in knee ranged 95 to 87% of I.D. The activity recovery during animal studies were also high at early time point (90-95%) and after 2 week of injection dropped slightly below 90% only.

Based on the favourable intraarticular retention and very low leakage value in all cases the calculated absorbed dose showed high value in target synovial surface (~40Gy), low effective dose (5.3 mGy) and also low whole body absorbed dose (1.9 mGy) values.

According to preclinical data obtained during this study authors concluded that all the colloid radiopharmaceuticals tested showed leakaged activity value up to 2 weeks is around 10% of I.D. and organs cumulate insignficant amount of radioactivity only and this lead to very favourable dosimetric calculation. An overall summary of the beneficts of colloid radiopharmaceuticals used for radiosynovectomy are the following:
Approxymetely 75% of the patients have a significat improvement in the quality of life. The method is cost-effective compared to surgical synovectomy, essentially no side effect noted and has a possibility of combined clinical application [6].

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Targeted radiotherapy with alpha particle emitting radionuclides

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Targeted radiotherapy involves the use of a molecular carrier such as a receptor-avid compound or an antibody to deliver a radionuclide to malignant cell populations. This emerging therapeutic strategy has a number of potential advantages that compensate for its relatively complex nature. Compared with conventional external beam radiation treatment, targeted radionuclide therapy offers the prospect of more selectively delivering lethal radiation doses to tumour cells while leaving neighboring normal cells intact. And unlike conceptually similar targeted therapeutics employing toxins or chemotherapeutics as the cytotoxic agent, radionuclides do not require intracellular localization to be effective. Indeed, one of the attractions of radionuclide therapy is the existence of radiation with quite different dimensions of effectiveness, ranging from sub-cellular (Auger electrons) to hundreds of cell diameters (β-particles). One of the attractive features of α-particles for targeted radiotherapy is their intermediate tissue range equivalent to only a few cell diameters.

An important consequence of the relatively short range of α-particles is that this characteristic, in combination with their high energy (4-9 MeV for the radionuclides of interest for radionuclide therapy), impart a high linear energy transfer (LET) quality to this radiation. Yttrium-90 emits high energy β-particles that have a mean LET of about 0.2 keV/μm; in comparison, the LET of clinically relevant α-particle emitters is several orders of magnitude higher, about 100 keV/μm. The radiobiological implications of high LET radiation are perhaps the most compelling rationale for pursuing α-particle emitters for therapy. Of primary importance is the fact that the relative biological effectiveness of high LET radiation is considerably higher than β-particles or standard external beam radiation, and this has been validated experimentally with a variety of radionuclides, carrier molecules and human cancer cell lines. In addition, the conditions under which high-LET radiation is maximally effective are relatively wide-ranging, not being compromised by a lack of oxygen, cell cycle stage or dose rate.

Although the potential advantages of α-particle emitters for targeted radiotherapy have been appreciated for many years, translation of this concept into the clinical domain has been slow. Many of the reasons for this are in the realm of radiopharmaceutical chemistry. One problem has been the poor availability of α-particle emitters with real potential for cancer treatment. Another is the need for labeling methods that provide sufficient stability in the *in vivo* environment to be suitable for patient studies. An important consideration for therapeutic radiopharmaceutical chemistry that is particularly pertinent for α-particle emitters is the potential deleterious effects of radiolysis on labeling chemistry and product stability.

Even though more than 100 α-particle emitting radionuclides exist, to date, less than 10 of them have received serious attention for targeted radiotherapy applications. This can be attributed in part to the fact that most α-particle emitters are part of natural decay chains with multiple daughter radionuclides, necessitating the development of strategies that can compensate for the often divergent chemical behaviour of the radioactive parent and daughter. To date, the α-particle emitters that have been utilized for clinical investigations include 45.6-min ^{213}Bi, 7.2-h ^{211}At, and 11.4-d ^{223}Ra, while 61-min ^{212}Bi, 4.2-h ^{149}Tb and 10-d ^{225}Ac have

been explored in cell culture and animal models of human cancer. A wide variety of molecular carriers have been investigated including monoclonal antibodies, peptides, bone-seeking complexes as well as receptor- and transporter-avid molecules.

In this review, the current status of targeted α-particle radiotherapy will be summarized. Among the topics to be presented will be radiolytic effects on labeling chemistry and heterogeneous dose delivery, two problems that must be solved if targeted radiotherapy with α-particle emitting radionuclides is to become a practical approach for cancer therapy.

DOTA-Tyr3-Octreotate labelled with ^{177}Lu and ^{131}I – comparative evaluation

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Our goal in this work was to study, select and optimize the radiolabelling parameters of DOTA-Tyr3-Octreotate with ^{131}I and ^{177}Lu, for obtaining DOTA-^{131}I-Tyr3-Octreotate, ^{177}Lu-DOTA-Tyr3-Octreotate and ^{177}Lu-DOTA-^{131}I-Tyr3-Octreotate and to determine their binding affinity to the specific membrane surface receptors.

Radioiodinated DOTA-TATE was synthesized using the Chloramine-T (Cl-T) method. The radiolabelling method of DOTA-TATE with ^{177}Lu was optimized. For both radiolabelling procedures the optimal values for beta-emitters to peptide molar ratios, pH, temperature and incubation time were established, taking into account the radioactive and biological therapeutic doses. $^{177}LuCl_3$ in 0.05N HCl, 285 GBq/mg ^{177}Lu (Polatom) and 1660 GBq/mg ^{177}Lu (Nordion) respectively Na^{131}I, 18 500 MBq/mL, carrier free, were used in the experiments.

The stability of DOTA-TATE labelled with different radionuclides was performed by incubation in 0.9% NaCl and human serum. The competitive binding and the saturation binding assays were performed using rat brain cortex membrane.

^{177}Lu-DOTA-TATE with high specific activity and radiochemical purity higher than 95% was obtained. The radiochemical purity of DOTA-^{131}I-Tyr3-Octreotate was increased to 96% after purification step. The ^{131}I-DOTA-TATE stability in 0.9% NaCl is higher than that of ^{177}Lu-DOTA-TATE, while in human serum ^{177}Lu-DOTA-TATE proved to be more stable than ^{131}I-DOTA-TATE.

The experimental data were analysed using PRISM-2 Program. The IC_{50} value determined for ^{nat}Lu-DOTA-TATE is 4.74 nM while the value of K_d is 142.8 pM. The Kd parameter value for DOTA-^{nat}I-Tyr3-Octreotate obtained by Scatchard plot is Kd = 158.6 pM and the IC50 value for DOTA-^{131}I-Tyr3-Octreotate was determined to be 1.28 nM. These parameteres are shown in the figures.

The *in vivo* studies were performed using normal as well as hepatoma HRS1 tumour bearing rats, which overexpress somatostatin receptors. The results show a high and specific uptake of the luthetium radiolabelled somatostatin, starting at 24 h post injection, up to 168 h, followed by renal elimination.

As conclusion, the DOTA-TATE labelling processes with ^{131}I and ^{177}Lu are controlled by the molar ratio of the biomolecule to beta-emitter, pH, temperature and incubation time. The in vitro stability show that the DOTA is a stable chelator for ^{177}Lu and Tyr3 is a ligand with high chemical affinity for electrophylic iodine. The IC50 and Kd values for DOTA-^{131}I-Tyr3-TATE show a high binding affinity of radioligand for somatostatin receptors. The biodistribution confirms the *in vivo* stability of the ^{177}Lu-DOTA-TATE and its selective affinity for HRS1 tumours.

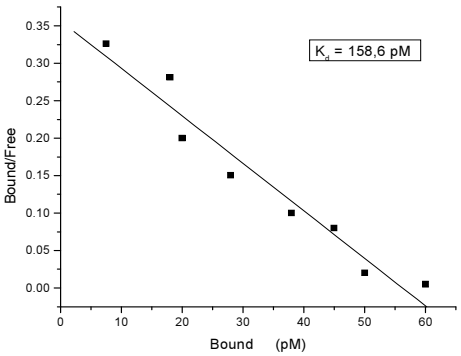

FIG 1. The saturation binding curve of DOTA-^{131}I-Tyr^{3}-Octreotate.

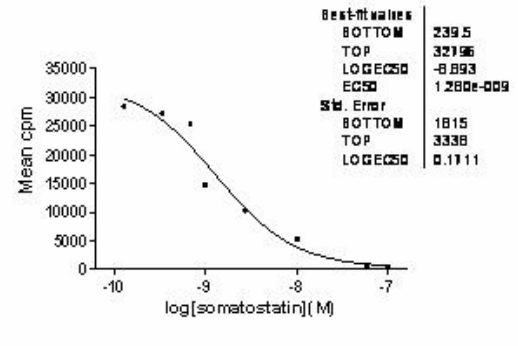

FIG 2. The competitive binding curve of DOTA-^{nat}I-Tyr^{3}-Octreotate.

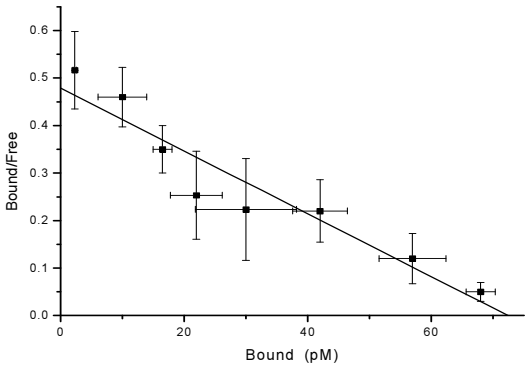

FIG. 3. Saturation binding curve of DOTA-TATE.

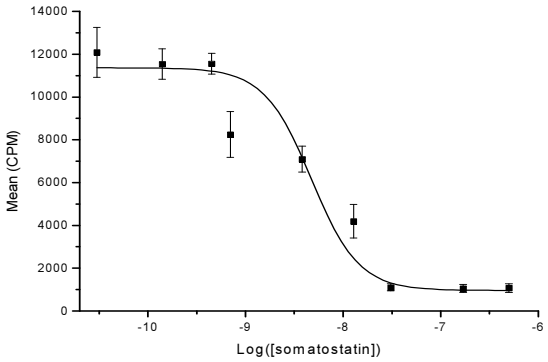

FIG. 4. Competitive binding curve of ^{177}Lu-^{nat}Lu-DOTA-TATE.

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^{177}Lu labelled nitroimidazoles and nitrotrifazoles for possible use in targeted therapy of hypoxic tumours

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In our attempt towards the development of a potential therapeutic agent for targeting hypoxic tumours, ^{177}Lu possessing the desirable nuclear characteristics [$E_{\beta(max)}$ = 497 keV, E_γ = 208 keV (11%), 113 keV (6.4%), $T_{1/2}$ = 6.73 d] has been chosen as the radionuclide. The representatitive molecules chosen as the tumour targeting agents for radiolabeling with ^{177}Lu are metronidazole, a 5-nitroimadazole and sanazole, a nitrotriazolesF earlier documented as potent tumour-avid substrates. As direct incorporation of the ^{177}Lu in either of the aforementioned nitroimidazole or triazole moiety is not feasible, indirect incorporation of ^{177}Lu through a suitable bifunctional chelating agent (BFCA) is envisaged. For the present study, metronidazole was coupled with *p*-amino-benzyl-DOTA following a two-step reaction wherein metronidazole was first oxidized to the corresponding carboxylic acid derivative viz. 2-[N-(2'-methyl-5'nitro)imidazolyl]ethanoic acid using alkaline $KMnO_4$ and then the oxidized product was coupled with the BFCA. The other conjugate viz. sanazole-BFCA was prepared via coupling of the carboxyl group present in the sanazole derivative with the amino group available in *p*-amino-DOTA-anilide, the BFCA chosen for effecting the desired derivatization. Both the conjugates were characterized by employing normal spectroscopic techniques, such as, FT-IR and ^{1}H-NMR spectroscopy.

^{177}Lu, which is presently being considered as a pivotal radionuclide for targeted tumour therapy was produced by thermal neutron bombardment on isotopically enriched (60.6% ^{176}Lu) Lu_2O_3 target at a neutron flux of 3×10^{13} n/cm^2/s for 14 d. The irradiated target was dissolved in 1 N aqueous HCl, evaporated to near-dryness and reconstituted in deionized water. About 5×10^3 Ci/g (~185 TBq/g) of ^{177}Lu activity was obtained after radiochemical processing at 6 h post end of bombardment. The radionuclidic purity of ^{177}Lu produced was ~99.985% as determined by analyzing gamma ray spectrum. The sole radionuclidic impurity burden in the form of ^{177m}Lu was found to be 150 nCi of ^{177m}Lu / mCi of ^{177}Lu (5.5 kBq of ^{177m}Lu / 37 MBq of ^{177}Lu). This has been ascertained by recording the gamma ray spectrum of the irradiated target allowing complete decay of ^{177}Lu (45-65 days, ~8 to 10 half-lives of ^{177}Lu).

In a typical radiolabeling procedure, 100 µg of the conjugate was dissolved in 180 µL of 0.1 M NH_4Ac buffer (pH 5) and 20 µL of $^{177}LuCl_3$ (~50 MBq of ^{177}Lu, 0.2 µg of Lu) was added. The resulting solution was incubated at 50°C for 1 h. Various reaction parameters, such as, ligand concentration, pH, incubation time and temperature were varied in order to obtain maximum complexation and in both the cases >95% complexation were achieved at the optimized conditions. The radiolabeled conjugates were characterized by paper chromatography using 50% aqueous acetonitrile and thin layer chromatography using 10% ammonium acetate : methanol (1:1, v/v) as the eluting solvents. The radiolabeled conjugates were further purified by high performance liquid chromatography using water (A)-acetonitrile

(B) with 0.1% trifluoroacetic acid as the mobile phase employing a gradient elution technique [0-4 min: 95% A, 4-6 min: 95% A to 80% A, 6-9 min: 80% A to 40% A, 9-12 min: 40% A, 12-18 min: 5% A]. The radiochemical purity of the labeled conjugates as determined using standard quality control techniques were found to remain constant for 7 days at room temperature indicating the high stability of the preparations.

The in vivo biological behaviours of the ^{177}Lu labeled conjugates were studied by injecting the radiochemical preparations in Swiss mice bearing fibrosarcoma tumours. (1.0-1.3)% of the injected activity (ID) was observed to be accumulated in per gram of tumour for both the radiolabeled conjugates within 1 h post-injection (p.i.). >90% of the ID was observed to be cleared at this time point via the renal pathway with no significant uptake of activity in any of the vital organs and tissues. The activity accumulated in the tumour was observed to be reduced with progress of time. However, as the activity cleared out rapidly from all the non-target organs, the tumour/blood ratio (4.0-5.2 at 1 h p.i. and 18.0–22.5 at 24 h p.i.) and tumour/muscle ratio (4.6-12.2 at 1 h p.i. and 15.0–18.0 at 24 h p.i.) were observed to be quite high throughout the period of study (1 h to 24 h). The promising potential of the present agents are demonstrated adequately in a comparative study with other established agents available for hypoxia imaging wherein the developed agents exhibit superior tumour/blood and tumour/muscle ratios.

Labelling and biological valuation of anti-CD-20 for treatment of non-Hodgkin's lymphoma

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Anti-CD20 monoclonal chimeric humanized murine antibodies (Rituximab), have been successfully applied for the treatment of Non Hodgkin's Lymphoma. However, upon labelling of the mab-CD20 with ß-emitters as ^{90}Y, the therapeutic efficacy has significantly increased due to radiological effects of ionizing radiation [1].

Our objective was to develop reliable and efficient methods for labelling anti-CD20 with ß-emitters of therapeutic interest and simple and rugged quality control methods to evaluate radiochemical purity, biological performance and immunoreactivity assessment. ^{131}I and ^{188}Re have been used for the labelling of anti-CD20 as two attractive alternatives due to decay properties and availability (^{188}Re: $E_ß$max: 2.2MeV, Eγ 0,155MeV, T=17h, generator produced; ^{131}I: $E_ß$max: 0,63MeV, Eγ 0,364MeV, T=8d). Labelling of anti-CD20 was optimized following the oxidation procedure of chloramine-T in the case of ^{131}I [2] and the synthesis of ^{188}Re(IV) complex with the previously reduced monoclonal antibody [3]. Quality control of the species obtained were done by physicochemical methods, including ITLC-SG and HPLC, non specifc protein precipitation, biological distribution in normal mice and immunoreactivity studies with membrane antigens extracted from isolated leucocytes.

^{131}I- (more than 3.7 GBq/mL) was introduced on tyrosyl residues of the protein chain by adding 28 MBq to 20µg of anti CD20 (Mab Thera, 10mg/mL) at pH 7.4 and 1.3µg of Choramine-T. Purification was done by gel-permeation with sephadex G-25 (PD-10, Pharmacia). For labelling with ^{188}Re, anti CD20 was first reduced by incubation with 2-mercaptoethanol and purified over a PD10 column. Fractions of reduced antibody were pooled and formulated as kit for instant labelling. Each kit contained 1mg anti-CD20; 82.8mg of sodium tartrate; 1.67 mg of stannous fluoride and 0.25 mg gentisic acid. For the labelling, sodium perrhenate (1.5-1.9 GBq) was acidified, added to the kit and then incubated for 1 hour at room temperature. Radiochemical purity of ^{188}Re-anti-CD20 was evaluated by ITLC-SG using MEK and Saline, and by saturated ITLC-SG strips (BSA 5%) using EtOH-NH_4OH-H2O (2:1:5) as solvent. It was also evaluated by HPLC using an SW300 protein pak column and eluting with phosphate buffer 0.01M pH 7.4 at 0.5mL/min.

For immunoreactivity assays the preparation of isolated CD-20 antigen membranes was developed. A pool of blood bank leucocyte concentrate was initially centrifuged at 500g for 10 min at 4°C. Supernatant containing extracted cells was homogenized. Serial centrifugations were then performed to obtain free leucocyte CD 20 antigen membranes. Final protein concentration was determined by Lowry. Immunoreactiviy was evaluated by specific binding of tracer to membrane preparations ranging from 0.25mg/mL to 30mg/mL, using an excess of $1.5x10^4$ and $1x10^5$ times of unlabelled monoclonal anti-CD 20 as non specific binding. Inhibition studies were conducted by incubating a fixed membrane concentration of 1 and 4 mg/mL with increasing concentrations of unlabelled anti-CD20 (0.3 to 3 mg/mL and 0.18 to 1.8mg/mL) and 2500 Bq of tracer. Biodistributions studies at 4 and 24 h post

injection, were carried out in CD1 normal mice by intravenous administration of 9.3-55.5MBq of ^{188}Re-anti-CD20.

Radioiodination was dependent of the amount of monoclonal present and concentration of activity of the radionuclide. Best results were obtained when excess monoclonal and high concentrations of activity were present. Yields ranged from 43% to 82% and specific activity was over 30μCi/μg. The labelling with ^{188}Re gave a radiochemical purity higher than 95% for up to 3 h elapsed time and a specific activity was 40-50μCi/μg. Specific binding of 131I-anti-CD20 to membrane antigens reached 20.2 ± 0.5% for a total protein content of 16.5mg/mL. Inhibition of binding was 67.2 ± 1.2% when 290μg of unlabelled anti-CD20 was added. Specific binding of ^{188}Re-antiCD20 to membranes reached 46 ± 1% for a total protein content of 33 mg/mL. Inhibition of binding was 65.9 ± 5% when 700 μg of unlabelled anti-CD20 was added. The biological distribution showed high urinary elimination at 24 h (59%) while gastrointestinal excretion was 10%. Negligible uptake by thyroid and stomach (less than 0.7% and 1.9%, respectively) was observed.

The labelling of anti-CD20 with ß-emitters of therapeutic interest, in our case ^{131}I and ^{188}Re, gave reliable results by simple and efficient methodologies yielding products compatible with clinical radioimmunotherapy. Quality control methods for evaluation of radiochemical purity showed good reproducibility with short benchtime. Immunoreactivity studies showed binding dependency on membrane antigen concentration and good specificity of binding demonstrated by inhibition with unlabelled anti-CD20. Use of membranes, stable at -80ºC for more than 6 months instead of concentrated short lived leucocytes, has shown an excellent reproducibility and therefore they are convenient at production centres not near blood banks.

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Session 10

THERAPEUTIC RADIOPHARMACEUTICAL APPLICATIONS

^{177}Lu-DOTA-antiPSMA: Toxicity, dosimetry and clinical efficacy

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Prostate carcinoma expresses prostate-specific prostate antigen [PSMA] which is virtually restricted to prostate tissue and the expression of which is increased in prostate carcinoma. The antigen is a type II membrane glucoprotein with an extracellular, transmembrane and intracellular portion. Antibodies to PSMA have been developed and these antibodies have been radiolabeled. As ^{111}In capromab pendetide, an antibody to the intracellular portion of the epitope, it is available in the United States as a diagnostic agent. For the past several years, we have been engaged in the development and evaluation of a radiolabeled antibody to the external portion of the PSMA complex [J591] labeled with a β-emitting radionuclide for use as a therapeutic agent in metastatic prostate carcinoma. After demonstrating that the antibody is internalized following binding, radiometals such as ^{90}Y and ^{177} Lu were selected for further evaluation since once internalized, these tracers remain localized in tumour foci (as well as other tissues including non-specific hepatic uptake) compared to ^{131}I labeled proteins. Metastatic prostate carcinoma, in particular, is an ideal target for radioimmunotherapy because of the restricted expression of PSMA, the pattern of metastatic involvement often involving micrometastases in lymph nodes and bone marrow and the general lack of suitable chemotherapeutic or other medical therapy.

In human trials, ^{177}Lu-DOTA-J591 (a de-immunized monoclonal antibody-DOTA construct labeled with Lutetium-177, a radiometal with a low energy β emission as well as a γ photon emission that makes it convenient to demonstrate localization and quantify biodistribution and turnover externally) was evaluated. All human studies were approved by the institutional IRB and were performed as phase 1 IND studies.

Initially, small doses of ^{111}In-DOTA-J591 were used to assess the influence of total protein content on biodistribution. From these studies, it was determined that 10 mg/m^2 total protein per patient was sufficient to reduce non-specific organ uptake, potentially optimizing tumour access to labeled antibody. Dose limiting hematologic toxicity was observed at 75 mCi/m^2, with a MTD of 70 mCi/m^2. Patients also tolerated multiple doses of 30 mCi/m^2. In 35 patients, the appearance and severity of myelotoxicity correlated with the calculated bone marrow radiation absorbed dose. Responses determined by significant decreases in PSA and/or bone flare phenomenon followed by improvement in bone scan findings have been observed. A phase II trial is underway and a multi-dose trial is planned.

Radiolabelled somatostatin analogs for radionuclide therapy of tumours

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Introduction

Molecular imaging and therapy are rapidly developing fields and will become important topics in medicine in the 21st century. Nuclear medicine has applied molecular imaging and therapy since the 1940s by imaging and treating thyroid disorders with radioactive iodine transported into the target cells via the sodium iodide pump. We transferred the idea of radionuclides for molecular imaging and cancer treatment to peptides because peptide receptors are known to be over expressed on certain cancers and started using radiolabelled somatostatin derivatives to image and treat tumours [1].

Targeting peptide receptors

Radiolabelled peptides that bind to receptors form an important class of radiopharmaceuticals for tumour diagnosis and therapy. The specific receptor binding property of the peptide can be exploited by labelling with a radionuclide and using the radiolabelled peptide as a vehicle to guide the radioactivity to tumours expressing a particular receptor. The high affinity of the peptide for the receptor plus the internalization of the receptor-peptide complex facilitates high uptake of the radiolabel in receptor-expressing tumours, while its relatively small size facilitates rapid clearance from the blood, resulting in low background radioactivity. The use of radiolabelled peptides is growing rapidly due to these favourable characteristics, their low antigenicity and ease of production.

Receptor-binding peptides labelled with gamma radiation emitters or positron emitters enable non-invasive, whole-body visualization. This process is referred to as peptide-receptor scintigraphy (PRS) and is being used to detect, stage and plan the therapy of receptor-expressing tumours, and also to follow tumours after therapy. In addition, labelled with therapeutic beta-emitter these peptide molecules have potential to destroy receptor-expressing tumours: an approach referred to as peptide receptor radionuclide therapy (PRRT).

Somatostatin Receptor Imaging

The first peptide evaluated was somatostatin. To date five somatostatin receptor subtypes (sst1-sst5) have been identified and cloned [2]. The stabilized somatostatin analogue octreotide binds with high affinity to subtypes sst2 and sst5. The diagnostic accuracy of In-111-labeled octreotide to visualize tumour lesions after intravenous injection has been determined in large series of patients with sst2-positive, mostly neuroendocrine tumours [3]. Most interesting is the recent application of somatostatin analogues in PET, after labelling with positron emitters.

As soon as the success of peptide receptor scintigraphy for tumour visualization became clear, the next logical step was to try to label these peptides with therapeutic radionuclides and to treat receptor-positive tumours with peptide receptor radionuclides.

Somatostatin Receptor Radionuclide Therapy

So far, Y-90, emitting beta-particles with a high maximum energy (2.27 MeV), and Lu-177, emitting beta-particles with a lower maximum energy (0.5 MeV) and gamma radiation suitable for scintigraphy, are the most frequently used radionuclides in PRRT. The second generation somatostatin analogue DOTA-Tyr3-octreotide can form a stable complex with Y-90. In rats with subcutaneous CA20948 pancreatic tumours, Y-90-DOTA-Tyr3-octreotide effectively controlled tumour growth [4].

Studies to determine the therapeutic efficacy of Y-90-DOTA-Tyr3-octreotide in cancer patients are ongoing at various institutions in Basel, Milan, Brussels and Rotterdam [1, 5, 6] and show most promising rates of complete plus partial remissions.

The new somatostatin analogue Tyr3-octreotate has an increased affinity for sst2 compared to octreotide and Tyr3-octreotide [7]. Lu-177-DOTA-Tyr3-octreotate has proved very successful in achieving tumour regression in animal models [8]. Patients with gastro-entero-pancreatic neuroendocrine tumours, known to be resistant to external beam radiation, were treated with 3–4 administrations of 200 mCi (7.4 GBq) Lu-177-DOTA-Tyr3-octreotate in our hospital. Three months after the final treatment, complete or partial remission was achieved in an impressive 30% of the patients, minor remission in 21%, and a further 26% of patients with progressive disease at the start of PRRT showed stabilization [9].

The kidney is the dose-limiting organ in these peptide receptor radionuclide therapy studies, as the peptides are reabsorbed and retained in the proximal tubule cells after glomerular filtration. The uptake of radiolabelled peptides can be reduced by blocking the negative charges on the tubule cells with positively charged molecules (e.g. lysine and arginine). Therefore, during PRRT an infusion containing the positively charged amino acids L-lysine and L-arginine is given (25 g lysine and 25 g arginine are infused over 4 h) in order to reduce the renal uptake [6, 10] and to allow further dose escalation in PRRT.

Conclusion

With the development of new somatostatin analogues that bind with high affinity to receptors on tumours, the available tools for radionuclide imaging and therapy of these tumours have increased significantly.

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Iodine whole body scan, thyroglobulin levels, Technetium-99m MIBI scan and computed tomography: results in patients with lung metastasis from differentiated thyroid cancer

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The level of thyroglobulin (Tg), ^{131}I Whole body (^{131}I WB) scan, ^{99m}Tc sestamibi (Tc MIBI) scan and computed tomography (CT) are important parameters in the evaluation of lung metastasis in differentiated thyroid cancer (DTC). We have examined the correlation of ^{131}I WB and Tc MIBI scans, CT and the value of routine follow up for ^{131}I WB scan and Tg levels in the assesment of the treatment.

Pulmonary metastasis were detected in 32 patients out of 583 patients with DTC who have been admitted to our clinic betwen 1985 and 2004. Pulmonary metastasis diagnosed by positive ^{131}I WB scans, increased Tg levels and/or other positive radiological findings. Histopathologically papillary in 15/32, follicular in 13/32 and mixed type in 4/32 patients were classified. Ages of the 32 patients were ranged from 22 to 79 years (mean; 58 $\pm$ 19 years ; 15 F and 17 M). A total of 100-1450 mCi (3,7-53,65 GBq) ^{131}I activity was given to each patient. All the patients were evaluated with neck palpation, lung X ray, Tg and thyroid stimulation hormone (TSH) levels before they have received their radioactive iodine treatment. ^{131}I WB scans, Tg levels and CT were used in the assesment of diagnosis and follow up of patients with lung metastasis. Tc-MIBI scan was also performed on 19 patients who were chosen randomly among the patients.

19/32 of the patients had lung metastasis before they have received the first ^{131}I treatment. Pulmonary metastasis were detected after the first theraphy, during the course of follow up with ^{131}I WB scans, Tg levels and/or CT in 13 patients. Pulmonary metastasis were observed in the first ^{131}I WB images of all the patients except one; whereas no pulmonary metastasis were detected in CT in 3/32 patients. Metastasis were detected in 12/19 patients who went through Tc MIBI scan, 18/19 showed metastasis in ^{131}I WB scan and 17/19 in CT. Of the seven patients witout the sign of metastasis in Tc MIBI scintigraphy 1 was negative in terms of metastasis in ^{131}I WB scan and 1 in CT. The last ^{131}I WB scans became normal in 13/32. Tg levels diminished in 21/32 patients and elevated in 3/32. ^{131}I WB scans continued to be abnormal in 2 out of 3 patients with increased Tg levels but became normal in 1 patient whose CT stil demonstrated macronodullary lesions. Tg levels did not change significantly in 8/32. ^{131}I WB scans became normal in 5/8 patients and 4/5 showed macronodules in their CT's. Fibrosis was observed in 2/32 patients in CT. One patient developed dediferentiation decided by negative ^{131}I WB and positive CT.

The ^{131}I WB scans and Tg levels are the most important parameters in the evaluation of lung metastasis in differentiated thyroid cancer. CT is an addition effect to ^{131}I WB scan and Tg level, on the other hands MIBI imaging alone may not be enough to detect these metastasis.

^{99m}Tc-MIBI and ^{131}I scintigraphy in the follow-up of differentiated thyroid carcinoma (DTC) patients after surgery

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MIBI scan has been reported to be a highly sensitive imaging technique for detection of differentiated thyroid carcinoma (DTC) metastases that have lost the capability to uptake ^{131}I.

The **purpose** of the present study was to evaluate retrospectively the value of the ^{99m}Tc-MIBI scan and ^{131}I whole body scintigraphy using thyroglobulin (Tg) levels as a basis to comparison.

Eighty-four patients (63F/21M) with an age of 17-74 yr (mean 43.5 yr) with DTC (47 cases with papillary, 18 cases with follicular and 19 cases with papillary-follicular) were assessed. All of them had undergone total or near-total thyroidectomy and received radioiodine treatment for ablation of post surgical residual thyroid tissue. They were examined after L-thyroxin withdrawal oven 4-5 weeks in the follow-up of DTC. Planar and whole body images were acquired at 15 min and 180 min after i.v. application of ^{99m}Tc-MIBI (555-740 MBq) and at 48 h after p.o. administration of ^{131}I (111-185 MBq) on Toshiba GCA gamma camera. Serum Tg assays were performed to clarify the presence of residual recurrent malignancy.

All scintigraphic findings were compared to US, CT or MRT data.

^{131}I scan was positive in 55 patients showing thyroid remnants in 31 cases, lymph node metastases in 24 cases (17 in the neck, 7 to neck/mediastinum), pulmonary metastases – in 6 cases and bone lesions – in 2 cases.

In 18 patients ^{131}I scan was negative, Tg was undetectable, so patients considered tumour-free.

In 11 patients ^{131}I scan was negative while serum Tg was increased. These false negative results were observed predominantly in cases with less differentiated metastatic cells, especially after several courses of high-dose ^{131}I therapy. ^{99m}Tc-MIBI scan revealed the presence of lymph node and/or lung metastases (non-functioning metastases) in 9 of them, false negative results were obtained in 2 cases.

Serum Tg was increased in all patients with local lymph node and distant metastases, visualized by ^{131}I or by ^{99m}Tc-MIBI, but also in 18 patients with thyroid remnants only.

Considering ^{131}I scan as the most specific standard procedure we may conclude that the combined ^{99m}Tc-MIBI scintigraphy and serum Tg assay appear to be an alternative substitute of radioiodine diagnostic imaging to demonstrate the extent of the disease in cases with DTC and elevated Tg.

Results of knee radiosynoviorthesis in hemophilic and rheumatoid arthritic patients with ^{32}P colloid of local production

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The objective of this study was to assess the effects of radioactive treatment in large joints, using a colloidal suspension of ^{32}P, a pure beta emitter, developed in our country. We selected knee joints with refractory synovitic inflammations in hemophilic patients. Results were then compared with those from chemical synovectomy injections. A smaller population of rheumatoid arthritis (RA) patients was also treated. Comparison in this case was against intra-articular steroids and systemic drugs (aspirins, steroids, antimetabolites, penicillamine, among others) [1]. The amelioration of synovial inflammation with radiosinovectomies (RS) was evaluated against the alternative treatments in the studied pathologies, also taking into account the cost-benefit ratio to justify the present option.

Imported colloidal radiopharmaceuticals as ^{90}Y, ^{186}Re or ^{169}Er are expensive in our country, whereas ^{166}Ho macroaggregates, even though we have the technology to prepare it, is not yet allowed by our National Regulatory Authorities to be used in patients. In Europe intra-articular injection of beta-colloid particles is approved for the local treatment of different painful arthropaties. Both nuclear medicine specialists and orthopaedic surgeon should be trained in the management and waste handling of unsealed sources, have appropriate radiation safety equipment, and take adequate care of the patients´ condition.

The radiopharmaceuticals were applied in children and young patients, since the benefit of treatment was likely to outweigh the potential hazards. We avoided intra-articular joint injections when instability or bone destruction was present.

RS were performed using ^{32}P colloid in 58 male hemophilic patients, aged 4–52 years. Nine of them had re-treatments (67 procedures), either in the same or in the contralateral knee. The doses used were found to be safe and are based on results of several trials. Adults were injected with 37–74 MBq; children of 2–6 years of age received 1/3 the activity of the adult; 6–10 year olds received one-half the activity for an adult, whereas 10–16 years olds were injected with ¾ activity used in adults. When children had an overweight of more than 20% from normal (for age and height), ¼ more activity from the adult dose was added. Informed consent from adults or from the parents of children was obtained [2]. Patients were included in this study only if several knee episodes had occurred exclusion criteria: large Bakers cyst, grades I and IV arthropaties, infections in the skin of the joint area and bleeding at the time of the RS. Documentation of patients' hemophilic history, AHF therapy and clinical examination

were registered as well as a pre 3-Phase MDP scan. Patients were followed-up with the 3-phase bone scans through 1, 3, 6, 9 and 12 months. If required, joint aspiration was carried out. The puncture sites for intra-articular therapy for either the RS ^{32}P in 44 patients or the antibiotic Rifampicin-^{99m}Tc macroaggregates (4 MBq) in 14 patients were monitored in the gamma camera. Saline flushing was done before the needle was withdrawn. ^{32}P Bremsstrahlung emission was used in the gamma camera for early and late 24–48-h imaging to confirm the absence of leakage. After the procedures, immobilization and relative rest for 72 h followed.

Twelve RA patients were studied: six received ^{32}P therapy and the other six intra-articular corticoids. Clinical, blind evaluation (state of joint involvement, pain, motility, requirements of antihemophilic factors-AHF-, corticoids or analgesics) was registered in follow-up charts. For intra-articular chemical or corticoid injections therapy, 4 MBq of ^{99m}Tc MAA was added in order to obtain gamma camera images. Immobilization for 48–72-h followed the procedures.

For the haemophilic patients, there were neither local or systemic effects, nor leakage during ^{32}P treatment. Intra-articular Rifampicin procedure required frequent injections. Comparison of Rois in treated knees during soft tissue scintigraphies in pre and post third MDP control showed knee improvement. The follow-up evaluation demonstrated an increase in joint motion, diminished volume and less requirement and frequency of the use of Antihemophilic Factors (AHF) in 80% of the radiosynovectomies (54/67 procedures), thus lowering health costs dramatically. Outcomes in RA lasted 2–3 months, and were not so promising.

One intra-articular knee RS in haemophilic patients provides between 3–6 month relief of symptoms after treatment with locally produced ^{32}P. In RA a maximum of a three month pain palliative effect was documented. RA is predominantly a pathology of small joints, but the high beta radiation of ^{32}P colloid allowed only the knees to receive this radiotherapeutic alternative, being its use, thus less beneficial. RS turned out to be a safe, cost-effective alternative therapy in emerging nations, and could be considered as an initial procedure for hemophilic hemartrosis where availability of AHF is difficult and expensive.

This study is part of an IAEA CRP and we acknowledge its support.

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Session 11:

PET RADIOPHARMACEUTICALS

Alternative methods for making [^{11}C]amides — Application to the preparation of 5-HT1A receptor radioligands

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The amide group is widely found in drug-like ligands for neurotransmitter receptors from which radioligands are developed for imaging these receptors in brain with positron emission tomography (PET), either in clinical research or drug development. An important consideration for radioligand development is the position at which a positron-emitting radioistope, frequently carbon-11 ($t_{1/2}$ = 20.4 min), should be introduced into the ligand in order to avoid radioactive metabolites that may enter brain with the undesirable possibility to confound PET measurements of radioligand binding to the target receptor. Amides are often metabolized by simple hydrolysis in the liver to produce the parent amine and carboxylic acid in the blood. Generally, simple carboxylic acids, because they are ionized at physiological pH do not enter brain to a great extent, whereas the brain penetration of amines is subject to many factors. Hence, for a potential PET radioligand containing an amide linkage, introduction of a carbon-11 label on the carbonyl side of the amide bond, and even in the carbonyl entity, may be preferred. This is so for imaging brain 5-HT_{1A} receptors with ^{11}C-labeled WAY-100635 (**1**). Such considerations create a need for effective methods for labeling amides in the carbonyl group with cyclotron-produced carbon-11. Various methods have been developed during the last two decades, and these methods will be surveyed here together with their previous and ongoing application to the preparation of antagonist- and agonist-type PET radioligands for 5-HT_{1A} receptors.

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F-18 and carbon-11 PET tracers for research and routine

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The development of new tracers for positron emission tomography (PET) is a prerequisite for the success of this technique as a tool for routine diagnosis. Routine PET is mainly used for oncological, cardiological and neurological/psychiatric questions. Since the quality of the radiotracers – such as specificity and selectivity – for the target organ or functional unit, a strong focus has to be put on research in this field. Necessary for the improvement of the status of existing radiotracers is a wide understanding of the mechanisms involved in the uptake precess. There is a significant difference in the research access, whether there is a saturable process or not or if there are enzymes or receptors involved as targets. Radiopharmacological aspects such as metabolic stability and the behaviour of these potential radioactive metabolites should also pay a pivotal role in the design of new radiotracers.

Additionally the feasibility of the preparation method should be considered for the further implementation of the process for routine demands. There are few methods for the introduction of F-18 into molecules ranging from electrophilic substitution over nucleophilic substitution until the method of simple isotopic exchange or fluorinated synthons. In the case of C-11 the range of widely used methods is even punier: methylation on a heteroatom or a reaction of C-11 carbondioxide with a Grignard-reagent.

The preparations and formulations play a pivotal role in the selection of new radiotracers. Due to the short halflives of F-18 (~109min) and C-11 (~20min). only fast and reproducible methods can be used for these radiosyntheses. Additionally the preparation method can significantly influence the stability of the radiotracer in vivo. For example two different preparation methods for [^{18}F]-FFMZ (a F-18 fluorinated analogue of the benzodiazepine-antagonist flumazenil) yield in different biodistribution patterns in rodents. On the other hand different formulations can achieve different stabilities of the radiotracers in vitro.

The lecture will adress aspects on the preparation and application of newly developed radiopharmaceuticals and discuss differences and improvements such as drawbacks as compared to routine tracers. The content is understood to be a bridge between nature science as radiochemistry and applied clinical sciences as nuclear medicine. Emphasis in this lecture will be put on tracers for the dopamine transporter, for the GABA receptor, the serotonin receptor (5HT1A) and μ-opioid receptor. Also new attempts for the imaging of adrenocortical pathologies ([^{11}C]-MTO, [^{18}F]-FETO) will be discussed as examples for innovative oncological tracers.

[Methyl-^{3}H]-choline tumour incorporation

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[Methyl-^{11}C]-choline is proving to be a useful positron emission tomography imaging tracer for several tumour types such as breast and prostate cancer, but the identity of metabolites giving rise to the ^{11}C signal in tumour tissue is currently unknown. Evidence suggests that choline metabolism is related to cellular proliferation.

Methods: Tumour cells were incubated with [Methyl-^{3}H]-choline for 10 minutes then in a non-radioactive medium to simulate the rapid blood clearance of [Methyl-^{11}C]-choline. Cells were then extracted with organic and aqueous solvents to determine the intracellular distribution of the tracer. Aqueous extracts were subject to thin layer chromatography (TLC), ion exchange chromatography, and a choline extraction procedure to identify ^{3}H-containing metabolites. Enzymatic assays were carried out to determine choline kinase (ChoK) and phospholipase D (PLD) activity. In addition, kinetics of choline transport were determined. Flow cytometry and [Methyl-^{3}H]-thymidine incorporation into DNA were used as measures of cell proliferation. Procedures were carried out on fast and slow growing populations of MCF-7 cells to determine the relationship of choline incorporation with proliferation.

Results: Effluxed activity increased for the first few minutes then plateaued at about 25% of intracellular activity after about 10 minutes. Only about 5% of [Methyl-^{3}H]-choline was present as phospholipid. [Methyl-^{3}H]-choline incorporation was found to be related to S-phase fraction. In another experiment [Methyl-^{14}C]-choline incorporation was found to correlate with [Methyl-^{3}H]-thymidine incorporation. The activity of ChoK as well as PLD were significantly higher in the exponentially growing cell population, showing increased phosphocholine (PCho) production, approximately 60% of the aqueous fraction of the cell, in the exponentially growing cells compared to the confluent cells. Moreover, TLC results indicate that betaine was about 20% of the aqueous fraction of the cell. The affinity (K_m) for choline transport in the exponentially growing cells was significantly lower (26 μM) than in growth-inhibited cells (68 μM), whilst the maximum velocity of the reaction (V_{max}) for choline transport for exponentially growing cells (15.20 nmol/μg protein/10 min) was higher than for growth-inhibited cells (3.97 nmol/μg protein/10 min).

Conclusion: Choline incorporation into tumour cells under conditions that simulate rapid blood clearance of [Methyl-^{11}C]-choline correlates with proliferation. Most of the activity (about 95%) was in the non-lipid fraction of the cell.

Production, formulation, module design and quality control of [^{13}N]NH3 for future PET studies in Iran

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Improvements in positron emission tomography (PET) have engaged many research groups to prepare different radiopharmaceticals following administration to humans. [^{13}N]NH_3 is probably the only clinically used nitrogen-13 radiotracer (positron energy=0.64 MeV, tissue range=2.4 mm, half-life=109.7 min)[1-4]. Its short half life can be an advantage. We have been interested in the preparation and quality control of PET radiopharmaceuticals in the country for ultimate use in clinics [5-7]. In this study, one of the most simplest routes of production, *i.e.* irradiation of natural high-purity water by protons followed by catalytic reduction, has been targetted as well as manufacture of a prototype [^{13}N]NH_3 production module.

EXPERIMENTAL - Selection of the production parameters -In this research, $^{16}O(p,\alpha)^{13}N$ was selected as the best nuclear reaction for the production of ^{13}N, using natural water as the target material, due to the small amount of O-18 resulting in fluorine-18 that could be easily separated by physical methods and also for cost-effectiveness.

Preparation of [^{13}N]-Nitrate/nitrite anions: Nitroxy anions were prepared by 18 MeV proton bombardment of a DDH_2O sample (1.7 ml). The sample was irradiated for 15-20 min by 18MeV protons in an all-silver target in a 30 MeV cyclotron at NRCAM. Cooling was performed using a pressurized flow of He gass.

Radiochemical purity of [^{13}N]NH_3-Radio thin layer chromatography was performed using a mixture of acetone-propionic acid-staurated brine (1:2:4) (Fig. 1 and 2). NO_3 and NO_2 anions eluted at R_f of 0.45. Thus, the radiochemical yields (more than 98% in each case, n=9) were determined by comparison of NO_3 and NO_2 and the major radio peak at R_f= 0.80 for ^{13}N-NH_3.

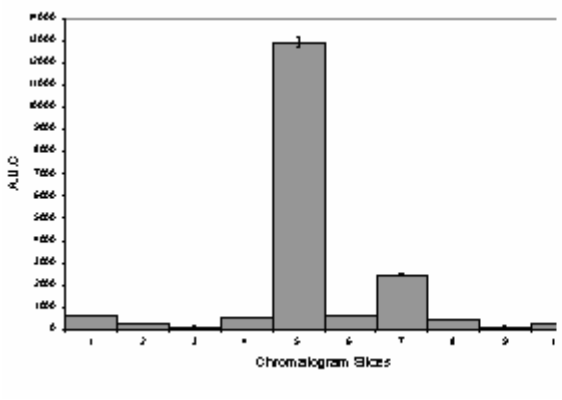

FIG. 1. target liquid before catysis.

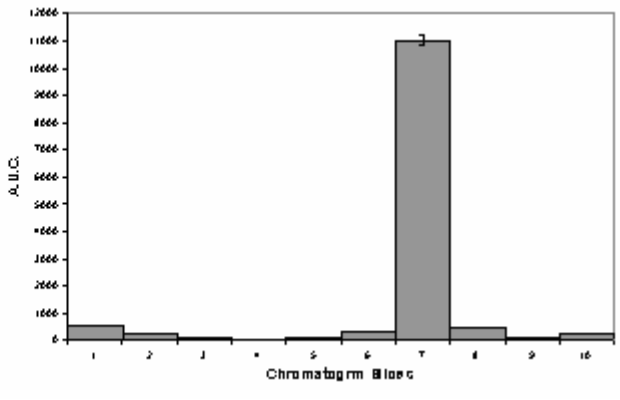

FIG. 2. target liquid after catysis.

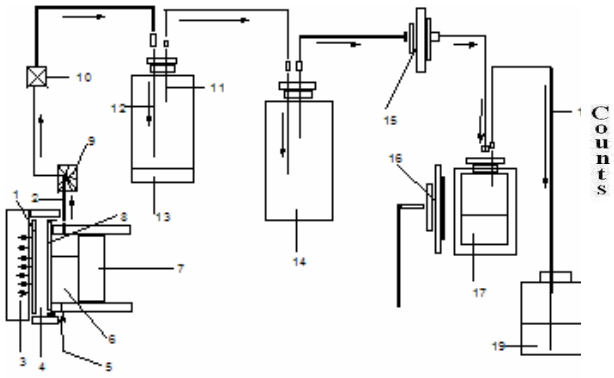

FIG. 3. scheme to the production module.

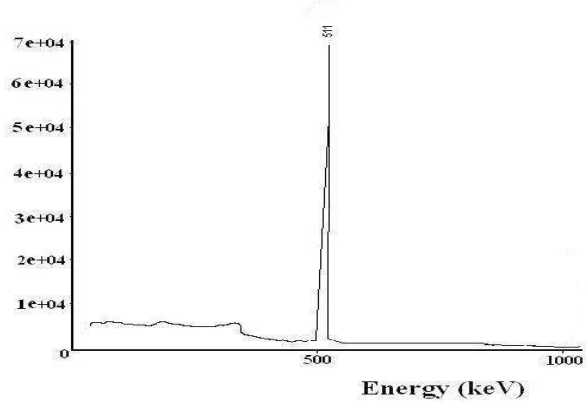

FIG. 4. Gamma spectrum of the final sample in a HPGe detector.

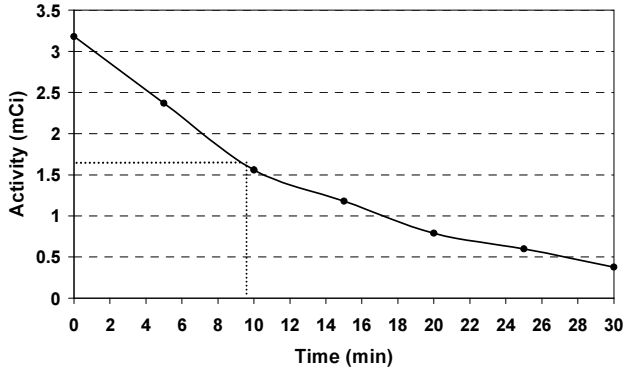

FIG. 5. half-life determination of the final sample.

Conclusion

[^{13}N]NH_3 was prepared by 18 MeV proton bombardment of the ^{nat}O target. The target was bombarded with a current intensity of 8μA for 20 minutes (13.5 ±0.5 μAh). The chemical con version of NOx anions into NH4+ cation was performed using an in-house made DeVarda's catalyst at our center. The possibilty of the formation of various radionuclidic impurities was considered using nuclear codes. The chemical purity was checked using colorimetric assay. The chemical separation process was based on a distilation method. The resulting activity of

[^{13}N]NH3 was 10-20 mCi at the end of bombardment (E.O.B.) and the production yield was 5mCi/μAh.

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Session 12:

RADIOIODINE RADIOPHARMACEUTICALS

Production of radioiodines with medical PET cyclotrons

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In this paper we wish to discuss the to-days possibility of producing radio-iodine with mass-numbers 120,123, and 124 via proton reactions on enriched TeO_2 targets with medical PET cyclotrons under three aspects:

- yield and impurity considerations
- technical feasibility and
- aspects of radiopharmaceutical chemistry.

The thick target yield for the ^{XXX}TeO2 (p,n)-^{XXX}I process is of the order of 2.5×10^9 atoms relatively independent on the mass number (XXX), which is reasonable high. Consequently, the (p,n) process could be a suitable alternative to the well known ^{124}Xe (p,2n) – large scale production technology for ^{123}I.

Earlier discussions on disturbing radionuclide impurities (iodine with mass numbers 124, 125, 126, 128, 130 and 131) does not play a practical role any longer, since today the target material with an enrichment close to 100 % is available without increased costs. Consequently, the radionuclide purity of ^{123}I preparations produced along the ^{123}Te(p.n)^{123}I process is comparable with the product obtained in the ^{124}Xe technology.

The standard medical PET cyclotrons and a standardised separation technique can be used for the production of all three isotopes: ^{120}I, ^{123}I and ^{124}I in high purity via the (p,n)-reaction. The enriched sensitive target material TeO_2 (melting point 735^{o}C, about 100 Pa vapour pressure at this temperature) has to be irradiated. A suitable target consists of a platinum disc carrying about 200 - 300 mg of the expensive target material. A dedicated **CO**mpact **S**olid **T**arget **I**rradiation **S**ystem (COSTIS) has been developed, that is now commercially available from IBA. Irradiation conditions are: 13 – 15 MeV protons, 20 $\mu A/cm^2$ beam intensity. Thus, 10 GBq or 1.5 GBq batches of ^{123}I or ^{124}I, respectively, are practically available using a PET cyclotron.

The radio-iodine is separated from irradiated TeO_2 targets using a thermochromatographic process. The procedure can be performed in a module like facility, remotely and safe, leading to a final volume of 0.5 to max. 1.0 ml of iodide solution. The TeO_2 targets cycled in this way can be reused immediately for the next production run without further treatment. A corresponding GMP-conform separation module, that performs this separation remotely and automatically has now been developed (TERIMO = **TE**llurium based **R**adio-**I**odine production **MO**dule) and is commercially available from HWM and ELEX. The losses of

target material for one cycle is negligible (<0.2 mg/cycle). The technique is applied today at Eastern Isotopes (IBA) in the US for ^{124}I production based on the GE PETTRACE cyclotron.

A local in-house production of radio-iodine (A = 123 and 124) has the advantage of using an irradiated TeO_2 target directly to obtain an iodinated radiopharmaceutical compound avoiding the intermediate step of isolating the radio-iodine in alkaline solution. Preliminary examples along this line will be presented.

The technology described is identical for all three mentioned iodine isotopes with the mass-numbers 120, 123 and 124.

New development in radio-iodinated radiopharmaceuticals for SPECT and radionuclide therapy

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Most nuclear medicine departments possess one or more imaging devices for single-photon emission tomography (SPECT). Nuclear medicine became Molecular Imaging. The use of "smart" radiopharmaceuticals for the imaging of the biochemical changes that come with any disease has allowed a world-wide recognition of the usefulness of scintigraphic imaging in medicine and biology.

The latter requires molecules of biological interest, especially for functional imaging of metabolism, tumours and neurotransmission functions using SPECT (single photon emission computed tomography). Often labelling with ^{123}I is preferred as the introduc- tion of ^{99m}Tc (I, III or V) by bi-, tri- and tetradentate- complexing groups into small molecules can change dramatically the biological activity. An effective labelling of compounds of biological interest is required for the preparation of these radiopharmaceuticals and therefore the radiolabelling methodology is considered as one of the pillars on which nuclear medicine rests.

Iodine-^{123}I ($t_{1/2}$ = 13.2 h, γ emission 159 keV) cyclotron produced is best suited for nuclear medicine imaging agents used in diagnosis by SPECT while iodine-^{131}I [$t_{1/2}$ = 8 d, γ emission 364 keV (83%) and β^- emission 606 keV (90%)] reactor produced is rather used for human therapy BUT in some countries still for diagnosis. ^{123}I can be produced via a ^{124}Xe(p,x)^{123}I reaction**,** formed indirectly via the decay of ^{123}Xe. The precursor ^{123}Xe itself is formed both directly via the (p,pn) reaction and via the decay of ^{123}Cs which is produced by the (p,2n) reaction with a "30 MeV cyclotron". An alternative method coupled to a smaller (cheaper) cyclotron is the ^{123}Te(p,n)^{123}I reaction**.** This is a low energy route but demands the use of highly enriched $^{123}TeO_2$ as target material. The suitable energy range for production is E_p = 14.5 $\rightarrow$ 11.0 MeV.

Many methods have been described but it appears that only a few chemical techniques allow radiopharmaceutical preparation with a good labelling yield and a high specific radioactivity. Based on the reaction types used, chemical radioiodination processes can be divided into nucleophilic and electrophilic substitution reactions. The choice of the labelling site is determined by biological, chemical and structural considerations while a particular attention must be paid to the stability of the carbon-iodine bond. Moreover, the high costs of radioiodine isotopes make region-selective and site-specific labelling reactions with high yields obligatory.

Daily routine preparations require one pot Kit-synthesis as simple as the routine ^{99m}Tc kits (mix and heat) or an automated system with a fast mini-column purification system. For the first the Cu^{1+} assisted nucleophilic exchange in reducing aqueous conditions is well suited while for the second the eletrophilic substitution with the lipophilic tri-Butyl-Sn as leaving group offers a good alternative.

Radioiodination by nucleophilic exchange was used for a large variety of tracers: *meta*-iodobenzylguanidine MIBG (cardiology and tumours), rose Bengal (tumours), iodohippuric

acids (renal clearance), quinoline derivatives IQMB (CNS benzodiazapin receptors), iodobenzyl quaternary ammonium compounds (cardiology), *N*-isopropyl-*p*-iodoamphetamine (IMP) and iodobenzylpropanediamine derivatives (HIPDM) (stroke) , 2-iodospiperone (CNS dopamine D_2 receptors), vesamicol derivatives (cholinergic), iodo-PK 11195 (benzodiazepin receptors, antgonist), deltorphin and dermorphin analogues (CNS opioid receptors), 2α-carbomethoxy-3β- (4'-iodophenyl)tropane (*β*-CIT) (serotonin transport), L-6-iododopa (brain tumour), and benzamide – analogues (brain receptors [D and S] and typical tumours) .

The electrophilic destannylation reaction requires often much lower amounts of substrate than does the nucleophilic substitution. This is the favourite method for the preparation of brain receptor tracers with high specific activity coupled to small column HPLC or mini-column separation.

The recent applications of ^{123}I-labelled compounds can be divided into geographic regions and anatomic regions. Geographic: Japan uses many ^{123}I-radiopharmaceuticals while in the US and Europe the clinical use of ^{123}I compounds is rather limited due to the competition of the ^{99m}Tc-kits, for the rest of the world ^{123}I is still a to expensive isotope. Anatomic: apart from one commercial company in the UK the application to brain receptors is limited to research and for quantification PET is the concurrent technology, there is an increasing interest in tumour specific SPECT tracers for follow up of tumours after surgery and irradiation or /and chemotherapy (^{18}FDG not specific enough). Moreover when the compound is highly retained in the tumour the ^{131}I analogues are an alternative for coupled radionuclide therapy.

^{123}I-MIBG is used as well as a receptor based (adrenergic receptors) radiopharmaceutical in cardiology as a tracer or even therapeutic for neuroblastoma and other tumours. Radioiodinated benzamides have been / are used for both brain receptors (dopamine and sigma receptors) and tumour diagnosis. Different ^{123}I labelled tropane analogues with exotic names like FISCH, TISCH,... and IBF were developped and succesfully applied for reseach on dopamine receptors and the coupled diseases. The serotonin system and its pathologies were brought into image by compounds like ^{123}I-beta-CIT, ^{123}I-ADAM, MADAM etc linked to the serotonin transporter and ^{123}I-5-I-R91150 a post- synaptic antagonist. For metabolic uses, for example, ^{123}I-iodofatty acids have been applied to study beta-oxidation of myocardial cells. ^{123}I-α-methyl-iodotyrosine (^{123}IMT) and newer amino acid analogues like L-2-I-Tyrosine and L and D 2-I-Phenylalanine are amino acids for tumour diagnosis linked to an increased amount of amino acid transporters in different types of tumour cells. The latter amino acid analogues where shown to be more tumour specific than ^{18}FDG as their uptake in inflamed tissue was almost not significant and did not show renal accumulation as do ^{123}IMT.

Some cancer cells show over-expression of G-coupled peptide receptors such as somatostatin, neurotensin and bombesin. Within those series until now only ^{123}I-[Tyr^3]-octeotride has been used with success for endocrine-related tumours such as menengiomas, breast tumours, astrocytomas, oat cell carcinoma of the lung etc.

^{123}I-vasoactive intestinal peptide (VIP) was used for scanning of adenocarcinomas and endocrine tumours of the gastrointestinal tract. Other peptides than somatostatine and VIP are under development such as Minigastrin, GLP-1, Substance P, etc. Another approach of cancer detection is apoptosis where ^{123}I-rh-annexin V is in a clinical phase.

Presently, many successful examples of several types of ^{123}I labeled compounds can be found in the literature. Although only a few of them are commercially available at a reasonable price all over the world.

Comparison of [^{131}I]-Tyr3-octreotate and [^{131}I]DOTA-Tyr3-octreotate: the effect of DOTA on the pharmacokinetics and stability

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The introduction of radiolabeled somatostatin analogs for peptide receptor imaging and therapy of neuroendocrine cancer have become a primary focus of interest in nuclear medicine [1]. In this work we studied the possibility of preparing radioiodinated octreotate derivatives, with high stability and favourable kinetic *in vivo*, because ^{131}I-iodide is most frequently used in therapeutic nuclear medicine and aviable. We studied molar peptide to radionuclide ratio in order to obtain the mono-iodinated peptide (di-iodinated no longer binds to the somatostatin receptor [2]). Like other radioiodinated proteins labeled on constituent tyrosine residues, it was important to study the possibility of dehalogenation *in vivo*. Despite DOTA chelating group was not necessary to the radioiodination procedure, we intended to evaluate the influence of the chelating group on the pharmacokinetic and the *in vivo* stability of the labeled peptide.

^{131}I radiolabeling of Tyr^3-octreotate and DOTA-Tyr^3-octreotate was performed using Chloramine T method. A solution of 10 μg of peptide/40 μL of PBS (0.05M phosphate-buffered saline pH 7.5) was transferred to an Eppendorf cap. After addition of 10 μL (74 MBq) radioiodine and 5 μL of chloramine T solution (1mg/mL PBS), the cap was carefully stirred and the labeling reaction was allowed to proceed for 3 minutes at room temperature; 5 μL of sodium methabisulfite solution (2mg/mL PBS) was introduced as reducing agent.

Radiochemical purity was determined by HPLC (Waters) using a RP C18 column (4.2 x 50 mm, 5 μm, Waters) with UV (230 nm) and radioactivity (Packard Canberra) detection, flow rate of 0.5 mL/minute with a linear gradient of 40-80% (v/v) methanol in 50 mM sodium acetate buffer (pH 5.5) for 20 minutes, maintained for another 25 minutes. Free radioiodine was also determined by horizontal zone electrophoresis (Amershan Pharmacia) on Whatman 1 paper, 0.05M barbital buffer, pH 8.6, 300V, 40 minutes. The stability of the compounds were evaluated after incubation in human plasma at 37^{o}C. Biodistribution of ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate were studied on normal *Swiss* mices and *Nude* mouse bearing AR42J rat pancreatic tumours. About 540kBq of the radiopharmaceutical/0.1 mL was injected intravenously into the tail vein. The animals were sacrificed at different time points post injection, and the organs of interest were dissected. Tissue samples were weighted and the radioactivity was measured using a gamma counter. Data was expressed in%ID/g tissue.

The compounds were obtained in radiochemical purity superior than 97% as determined by electrophoresis method. HPLC profile of the ^{131}I-DOTA-Tyr^3-octreotate obtained when using molar peptide to radionuclide ratio of 2.73 (7.4 MBq/μg) evidenced the formation of one radiochemical form, probably the mono-iodinated compound . When using the molar peptide to radionuclide ratio of 0.54 (37 MBq/μg) , a second radiochemical form can be observed in HPLC profile, probably related to the di-iodinated form of the peptide.

Biological distribution studies developed with radiolabeled peptides obtained in a molar peptide to radionuclide ratio of 2.73 and with radiochemical purity superior than 97%, showed higher uptake of ^{131}I-Tyr^3-octreotate in liver and intestines, when compared to ^{131}I-DOTA-Tyr^3-octreotate in normal *Swiss* mice (Tables 1). The compounds presented similar uptake in tumour and in organs with high density of sst receptors as adrenals and pancreas (Table II).

TABLE I. BIODISTRIBUTION OF ^{131}I-TYR^3-OCTREOTATE AND ^{131}I-DOTA-TYR^3-OCTREOTATE IN NORMAL SWISS MICE

Tissue	% Dose/g ^{131}I-Tyr^3-octreotate		% Dose/g ^{131}I-DOTA-Tyr^3-octreotate	
	1 h	4 h	1 h	4 h
Total Blood	3.17±0.32	0.81±0.12	1.09±0.12	0.43±0.11
Liver	2.28±0.78	0.42±0.06	0.70±0.05	0.36±0.05
Spleen	0.76±0.25	0.37±0.11	0.57±0.06	0.27±0.04
Stomach	3.51±1.67	1.94±1.02	3.32±0.45	2.02±0.59
Intestine (small)	12.30±4.36	1.33±0.96	1.48±0.13	0.57±0.12
Intestine (large)	0.96±0.58	14.85±0.83	0.44±0.08	2.18±0.25
Kidneys	12.54±0.51	9.77±3.46	12.18±0.86	9.86±1.00
Muscle	0.47±0.24	0.13±0.06	0.26 ±0.02	0.13± 0.03
Brain	0.08±0.01	0.024±0.003	0.08± 0.03	0.04±0.010
Heart	0.66±0.11	0.20±0.08	0.50 ±0.10	0.20±0.05
Lung	1.59±0.40	0.45±0.14	0.80± 0.42	0.49±0.22
Thyroid *	0.49±0.07	1.04±0.20	0.55 ± 0.10	1.23± 0.17

* -% dose organ ; Values are mean±SD (N=6)

TABLE II. BIODISTRIBUTION OF ^{131}I-TYR^3-OCTREOTATE AND ^{131}I-DOTA-TYR^3-OCTREOTATE IN NUDE MICES BEARING AR42J RAT PANCREATIC TUMOURS

Tissue	% Dose/g ^{131}I-Tyr^3-octreotate		% Dose/g ^{131}I-DOTA-Tyr^3-octreotate	
	1 h	24 h	1 h	24 h
Adrenals*	0.021±0.010	0.002±0.001	0.018±0.004	0.003±0.001
Pancreas	0.781±0.048	0.038±0.001	1.150±0.286	0.047±0.013
tumour	1.097±0.450	0.175±0.079	1.733±0.011	0.131±0.006

* -% dose organ; Values are mean±SD (N=3)

Despite DOTA was not necessary to the radioiodination procedure, the chelating group seems to decrease the liphofilicity as evidenced by low uptake in liver and intestines of ^{131}I-DOTA-Tyr^3-octreotate. The fast blood clearance and renal uptake suggest the predominant urinary elimination of compound. The tumour uptake of both compounds are similar and ^{131}I-DOTA-Tyr^3-octreotate can be considered as an ^{131}I- labeled somatostatin derivative with better pharmacokinetic profile,to be applied in therapy of neuroendocrine tumours.

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Iodine labelled diethylstilbestrol (DES) of high specific activity: A potential radiopharmaceutical for therapy of estrogen receptor-positive tumours and their metastases?

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After binding to the estrogen receptor (ER) estrogens are localized very close to the DNA. Therefore, radioactively labelled estrogens, especially in connection with Auger-electron emitters which have only a very short range (1 – 10 nm) of radiation, are excellent candidates to achieve high specific cytotoxicity, in combination with a low degree of side effects.

Diethylstilbestrol (3,4-Di-(4'-hydroxyphenyl)-hexene-3) is a well-known, synthetic, non-steroidal estrogen, which has a higher affinity to the estrogen receptor than the natural hormone estradiol itself. The idea to use iodine labelled DES for imaging of ER positive tumours is not new. Several working groups successfully tried to label DES with different methods and investigated the diagnostic usefulness of the product. Most attempts for labelling with radioiodine have been made with a water soluble derivate of DES, the tetra-sodium salt of diethylstilbestrol-diphosphate (DES-2P). Former labelling methods had some disadvantages: low yield (20-30%), low specific activity (0.7 - 2.9 GBq/mmol), bad reproducibility, and time consuming procedures. Presumably, the low specific activity was the reason for the unsatisfying biodistribution data observed with this labelled compounds.

In the present work a fast and simple labelling and purification method for *I-DES was used and its binding affinity for ER was determined. For testing its cytotoxic effects on MCF-7 mamma carcinoma cells different iodine isotopes bound to DES or in form of iodide were compared with regard to apoptosis, necrosis, and viability. Last but not least first animal experiments with tumour bearing mice were carried out.

DES was iodinated by Chloramine T in methanolic solution. Purification and quality control was carried out with reversed phase HPLC (column: Column: Hypersil ODS, 250 x 4 mm, 10 µm, Eluent A: Methanol G, Eluent B: Water G, Gradient: 20 % A to 70 % A within 5 min, Flow: 1 ml/min, UV-Detection: 254 nm).

The dissociation constants for the ^{125}I-DES- and ^{125}I-Estradiol-ER-complex were determined by DCC (dextran coated charcoal) saturation analysis. The determination of the dissociation constants was done by Scatchard plot.

MCF-7 tumour cells (DKFZ, Heidelberg, Germany) were cultivated in Dulbecco's Modified Eagle Medium (DMEM) from GIBCO (Grand Island, New York) , as well as Dulbecco's Medium without Phenol Red. The cells' estrogen receptor status was evaluated with the ER-ICA test from ABBOTT.

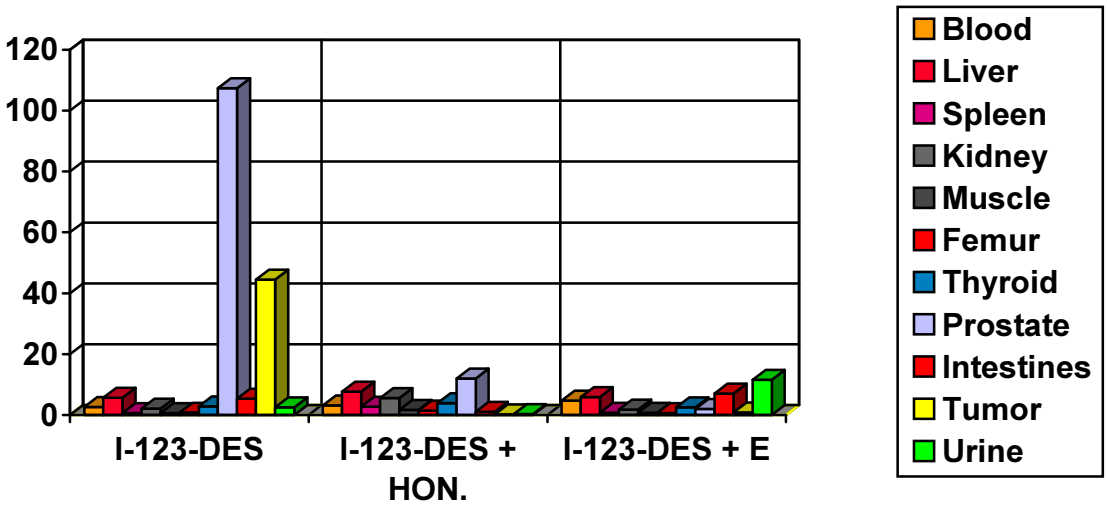
For investigation of cell viability the "Cell Proliferation Reagent WST-1" and for determination of apoptosis or necrosis the "Cell Death Detection ELISA" both from Roche Diagnostics were used, according to the manufacturers' recommendations.

Animal experiments were carried out with male tumour bearing DBA/2 mice (Charles River). Three groups of animals (N=5 each) were injected a) 1.5 MBq ^{123}I-DES, b) 7 µmol DES-2-P + 2 MBq ^{123}I-

DES, c) 7 µmol Estradiol + 2 MBq ^{123}I-DES. Three hours after i.v. injection the animals were sacrificed and organs, tissues, and blood were measured in a well counter.

With this modified Chloramine T method DES could be radioiodinated with good yields (50-74%), with high radiochemical purity (>98%), and – contrary to former attempts – a very high specific activity (^{123}I: 8800 TBq/mmol, ^{125}I: 80 TBq/mmol, ^{131}I: 870 TBq/mmol). The complex dissociation constant for ^{125}I-DES with ER was determined by Scatchard plot and is comparable to that of ^{125}I-estradiol (^{125}I-DES: $K_D = (2.67 \pm 1.02) \cdot 10^{-9}$ mol/L, ^{125}I-estradiol: $K_D = (3.92 \pm 2.27) \cdot 10^{-9}$ mol/L) under the same conditions. In the case, when *I-DES labelled with the Auger-electron emitters is used, the viability of MCF-7 cells decreases very rapidly according to increasing radioactivity concentration (^{123}I: 50% viability of the control group at 0.8 MBq/ml, ^{125}I: 50% at 0.5 MBq/ml). A little less effective is the β^--emitter ^{131}I (50% at 1.7 MBq/ml) and the highest concentrations are needed for comparable effects with the nuclides applied in form of iodide (50% at >1 MBq/ml for ^{123}I and ^{125}I, > 4 MBq/ml for ^{131}I).

%ID/g



Apoptosis increased with rising activity concentrations up to 35fold for ^{123}I-DES, 10fold for ^{125}I-DES and 3 fold for ^{131}I-DES compared with a control group. Necrosis increased parallel to apoptosis. Presumably this was not really necrosis, but a late phase of apoptosis.

Animal experiments showed high specific uptake in prostate and tumour, which could be blocked by estrogen or Honvan (= DES-2-P).

Radioactive labelled DES would be an promising compound for therapy of ER-positive mamma carcinomas and their metastases. With the present work, a fast and simple labelling method has become available to produce *I-DES of high specific activity. This compound showed in vitro a high cytotoxic potential to destroy ER-positive tumour cells very specifically. Biodistribution experiments were also very encouraging.

Session 13:

F-18 RADIOPHARMACEUTICALS AND AUTOMATION OF SYNTHESIS

Advances in F-18 radiopharmaceutical chemistry

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Concomitant with the increasing variety of biochemical principles applied for molecular imaging and hence with the growing number of compound classes used, there is a permanent demand for new or adapted methods of labelling. Besides carbon-11 ($T_{1/2}$ = 20.4 min), fluorine-18 ($T_{1/2}$ = 109.7 min) is still the most commonly employed positron-emitter in radiopharmaceuticals for PET.

Not as much for tracers of metabolic substrates but as, e.g., for ligands of neurotransmission or for enzyme inhibitors a high specific activity is crucial and demanding. Also for application in small animal PET-devices significant activity levels have to be attained in minimum volumes without pharmacological mass interferences. In spite of recent attempts to achieve this goal for electrophilic ^{18}F-fluorination by means of electrochemistry or via radiation induced chemistry, a proper realization was hampered by low radiochemical yields.

Still today, nucleophilic substitution with n.c.a. [^{18}F]fluoride appears to be the only practical way to achieve high specific activity products. While this is generally easy to attain with aliphatic or aromatic molecules activated for nucleophilic exchange of suitable leaving groups, it is still a problem with electron-rich arenes, especially if they are of complex structure. Enhancement of the ^{18}F-labelling possibilities was obtained by the use of activated aromatic heterocylic compounds such as pyridines and recently 1,3-thiazoles as well as by the introduction of aryliodonium moieties as nucleofugic groups. Especially the thienyl-iodonium leaving group offers possibilities for direct n.c.a. radiofluorination of electron rich arenes.

With complex molecules to be labelled, build-up reactions cannot be avoided. But again the recent development of ^{18}F-labelled synthons like fluorophenol, fluoroaniline or fluoro-bromo(iodo)benzene, in part prepared by the above mentioned methods, facilitates their preparation on a n.c.a. scale. Especially fluorohalobenzenes offer versatile possibilities via Grignard-reactions and lithium intermediates or in Pd-mediated coupling reactions. Similarly, new or improved prosthetic groups meet the fast-growing interest in ^{18}F-labelling of peptides and oligomers.

Further technical improvements in preparation methodologies help to fulfil the demands of increased clinical study frequencies. These newer radiofluorination procedures will be exemplified with relevant radiotracers for various areas of application.

F-18 based radiopharmaceutical and automation of synthesis: New F-18 radiopharmaceuticals

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Among various positron emitting isotopes, fluorine-18 has gained wider popularity. This is largely because of its favorable physical half life (110 min), allowing multi-step synthesis and ability to transport labeled radiotracer to sites remote from the synthesis laboratory. From the initial 0.3 mL low pressure silver body target design to next generation 3 mL high pressure tantalum body target, several target configurations are explored to increase ^{18}F fluoride production yields to meet the increased demand of ^{18}F radiopharmaceuticals. In our laboratory, we use a 3mL tantalum body high pressure target and produce ~4000 mCi of ^{18}F fluoride after a 180 min bombardment at 48 μA current. Handling of such large quantities of radioisotopes mandates the automation in delivery of isotope as well as for the radiochemical synthesis.

Despite a large inventory of radiopharmaceuticals for positron emission tomography (PET) imaging, ^{18}F Fluoro-deoxy glucose (FDG) remains the leading radiopharmaceutical for the oncology, cardiac, and neurological applications. The synthesis of FDG is well described and several automated synthesizers are available in the market. We routinely produce ~4000 mCi of ^{18}F FDG per synthesis batch. For laboratories with smaller FDG consumption, a 100–300 mCi production yields are common.

The expanded role of PET imaging largely depends on the availability of multiple radiopharmaceuticals. There has been a flurry of activity to develop new and novel PET radiopharmaceuticals over the last two decades. Although, majority of neuroreceptor ligands are C-11 labeled compounds, a number of ^{18}F compounds have been developed for neuroimaging, oncological, and cardiac application.

Prostate cancer represents a significant health problem throughout the world. Early diagnosis and detection of this disease will aid in selecting appropriate treatment regimen for better therapeutic outcome. FDG is ineffective in the detection or localization of this disease. Recently, ^{18}F -Choline(1), ^{18}F -FDHT (2), and ^{18}F -FMDHT [3] have been developed to image prostate tumours. In mice bearing prostate tumour xenografts, the tumour uptake for ^{18}F choline was similar to that for the FDG. Whereas, in patients with prostate cancer, ^{18}F-choline detected more lesions and showed higher SUVs than the FDG. Use of ^{18}F -FDHT in prostate cancer patients showed an avid uptake in tumour areas on PET images. Nonetheless, a rapid metabolic degradation of this compound was seen in humans. We recently developed a metabolically stable ^{18}F androgen (FMDHT) that exhibit a high affinity to tumours expressing androgen receptors [3]. In our preliminary studies, we observed a selective uptake and distribution pattern of this tracer in mice and rats.

The other area of significance for PET imaging is to diagnose breast cancer and to follow therapy outcome in those patients. Although FDG has been successful in imaging breast tumours and its recurrence, development of receptor based ligand viz ^{18}F fluoroestradiol (FES) would also provide information on hormonal status of the tumours. Thus development

of this tracer offers advantages over FDG that includes localizing breast cancer sites, predicting the response to tumour therapy, and assessing estrogen blocking effect of hormonal therapy *viz* tamoxifen and aromatase inhibitors. Initial clinical studies performed using this tracer show encouraging results].

Several ^{18}F labeled amino acids have been developed as PET imaging probes that utilizes active amino acid transport mechanism for their accumulation in target sites. One of the amino acids, ^{18}F fluoro methyl tyrosine (FMT) exhibited higher SUV in brain tumours than that seen with the FDG [6].

A number of radiopharmaceuticals have been developed over the years that utilize active transporter mechanism for their delivery and retention in target sites. Meta-iodobenzylguanidine (MIBG) was developed as a nor-epinephrine mimic to detect and treat neuroendocrine tumours. Successful application of MIBG in the detection of tumours of neural crest origin and cardiac sympathetic innervations lead us to develop ^{18}F labeled analog *viz* ^{18}F *p*-fluorobenzylguanidine (PFBG) for PET imaging. In small animals, PFBG showed higher specificity and avidity for the target tissues as compared to that with MIBG. Encouraged from those studies, we further explored the potential of PFBG utilizing myocardial infarct model in dogs. In these experiments, PFBG was successful in the detection and quantification of myocardial innervations [7,8]. Besides its role in cardiac imaging, PFBG show potential for oncology application as well. When tested in dogs with cancer, this tracer showed a rapid and high uptake in tumour sites with peak uptake within minutes post injection. PET images of dogs with spontaneous pheochromocytomas, showed an SUV of 20-45 utilizing PFBG as the radiotracer [9]. These PET images reveal the tumour mass in the adrenal gland area and extending into myocardial wall. These experiments and PET images demonstrate that PFBG would be a useful PET tracer to image neuroendocrine tumours and to image cardiac abnormalities including myocardial ischemia, infarction, cardiomyopathy etc.

Because of relatively short half life of ^{18}F, its role in utilizing large molecules viz. monoclonal antibodies (MAb) and other bioactive molecular probes has been ignored. Continuing our work on developing novel radiohalogenation methods to label MAbs, we explored the possibility of labeling MAbs with ^{18}F [11]. Our newly developed labeling technique has been successful in providing good yields of labeled MAbs with in two hour (time frame compatible with short half life isotope) while retaining the immunoreactivity of the labeled Mab [10.11]. In tumour bearing mice, ^{18}F labeled MAb showed significant tumour uptake with in 2 - 4 hour post injection [12]. We further assessed the utility of ^{18}F labeled MAb for radioimmunodiagnosis in dogs. PET scan using ^{18}F labeled TP-3 Fab antibody fragment was successful in the detection of spontaneous osteosarcoma in dogs [10,13].

A large collection of ^{18}F labeled radiotracers exist for the neuro-receptor imaging. Recently developed tracers include ^{18}F ACF and ^{18}F AFM for the SERT, ^{18}F reboxitine analog to image NET (14) and ^{18}F Fallypride for D2 Dopamine receptor imaging.

While ^{18}F compounds and molecular imaging probes are being developed at an increased pace, their handling and usage in radiochemical synthesis requires particular care. In general, for same amount of radioactivity (say 1 mCi), a positron emitting isotope imparts 5-10 fold higher radiation dose to personnel than that deposited from routine SPECT isotope. In addition, a shorter physical half life PET isotopes require handling of larger quantities of PET isotope during their incorporation in to drug molecules. Therefore, it is important to develop an automated or remote handling procedure for the synthesis of PET radiopharmaceuticals. Although several manufacturers now have developed automated synthesis boxes for certain radiotracers, most ^{18}F compounds are still being developed that would require automation. We

have adapted several of our synthesis strategies to the existing automated systems or improvized in house developed automation.

It is important to develop newer and novel radiotracers to advance the field of radiopharmaceutical chemistry. The path is to develop novel radiotracers while the goal is to develop novel radiotracers with clinical relevance. To achieve that goal, one would need to combine expertise from medicinal chemistry, organic chemistry, radiochemistry, radiation safety and a good understanding of clinical issues. It is important to adapt ourselves to bridge the gap between preclinical testing of the newly developed radiotracers and carefully and cautiously testing and using them in humans.

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Rapid method for radiofluorination of pyridine derivatives: Prosthetic groups for radiolabelling bioactive molecules

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One of the factors influencing the tissue localization of radiolabeled molecules is their lipophilicity. The replacement of a benzene ring with pyridine has been reported to decrease significantly the lipophilicity of the resultant molecular entity. Fluorobenzoates have been used extensively as prosthetic groups for labeling bioactive molecules. Very few attempts have been made to develop pyridine derivatives for the same application. We have therefore, embarked on the development of fluorinated pyridine derivatives as potential prosthetic groups for fluorination of protein and peptides. We report here an efficient synthesis of 6-fluoronicotinic and 2-fluroisonicotinic acid and their N-succinimidyl esters. In addition, N-succinimidyl activated ester of the 2-[^{18}F] fluoronicotinic acid was used to label several peptide analogues.

The radiochemical synthesis of ethyl 6-[^{18}F]fluoronicotinate and ethyl 2-[^{18}F]fluoroisonicotinate were accomplished by catalyzed nucleophlic no-carrier-added fluorination. Treatment of the 6-N,N,N-trimethylammonium ethylnicotinate and 2-N,N,N-trimethylammonium ethylisonicotinate triflate precursors with radiofluoride and Kryptofix 222 in anhydrous acetonitrile at 100^{o}C gave ethyl 6-[^{18}F]fluoronicotinate and 2-[^{18}F]fluoroisonicotinate intermediates in greater than 90% radiochemical yield within two minutes reaction time. The fluorination reactions were consistently higher than 90% when studied over a time range of 2-15 minutes and deferent amount of triflates. These intermediates were converted to the corresponding acids followed by the reaction with O-(N-succinimidyl) N,N,N,'N'-tetramethyluronium tetrafluoroborate (TSTU) in acetonitrile for 10 minutes at 100^{o}C. The resulted activated esters of the N-succinimidyl 6-^{18}F-nicotinate and 2-^{18}F-isonicotinate were purified using silica Sep-Pak cartridge. These purified activated esters have been successfully used to label chemotactic peptide and other bioactive molecules through their amine moieties and their biological evaluation are in progress. The overall radiochemical yields ranged between 60-70% (decay corrected) with preparation time of about 50 minutes.

This method in comparison with the replacement of halogen or nitro groups by fluoride procedure appear to be advantageous in the synthesis of high radiochemical yield fluorine-18 labeled compound in shorter time. Hence, this technique may be applied to obtain high specific activity products, a prerequisite for studying low capacity and saturable sites.

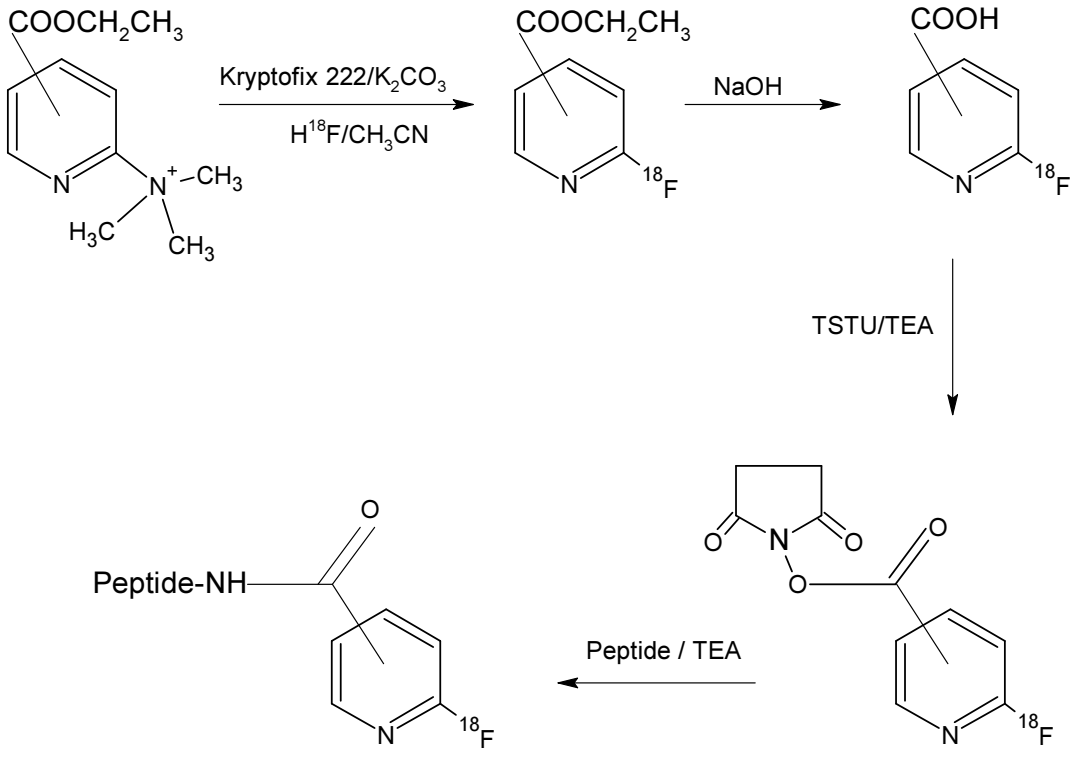

FIG. 1. Radiofluorination of [^{18}F]-fluoropyridine derivatives and bioactive molecules.

1-[^{18}F]Fluoroethyleneglycol-2-nitroimidazoles, a novel class of potential hypoxia PET markers

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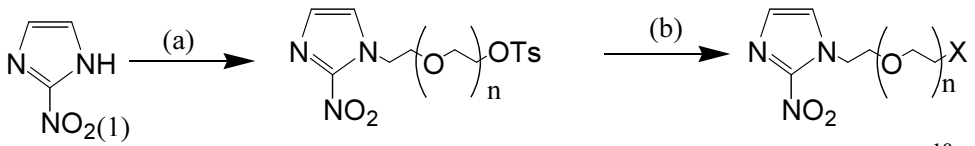
Several hypoxia markers contain a nitroimidazole moiety as the reactive chemical species, e.g., ^{18}FNIM [1], ^{18}FETNIM [2], ^{18}FMISO [3] or ^{18}FAZA [4]. It is believed that these imaging agents primarily enter tissue by diffusion and are trapped due to radical formation and subsequent intracellular reaction in case of decreased oxygen concentration [5]. In particular, the blood brain barrier (BBB) permeability is thought to play a crucial role in the cerebal uptake of the marker within hypoxia tissues [6].

Recently, new hypoxia PET markers [7] have been developed with higher lipophilicity compared to ^{18}FMISO to enhance the BBB permeability while avoiding excess nonspecific biodistribution. However, none of the developed markers offered improved biological properties over ^{18}FMISO, a well accepted hypoxic agent. This unsuccesful enhancement of trapping may be due to the lack of an oxygen atom in β-position to the 2-nitroimidazole ring in these markers [6].

In an attempt to develop new hypoxia markers exhibiting rapid localization in hypoxic tissue, we present herein the synthesis of new hypoxia PET markers with higher lipohpilicity than ^{18}FMISO and the required β-oxygen atom.

Scheme 1



2a n = 1
b n = 2
c n = 3

3a n = 1 **4a** n = 1
b n = 2 **b** n = 2
c n = 3 **c** n = 3

a) TsO-$(CH_2CH_2O)_{n=2,3,4}$-Ts, Et_3N, DMF, rt, 4 d; b) (i) CsF, [bmim][BF_4], CH_3CN, 100 °C, 1h or (ii) *n*-Bu_4NF, THF, rt, 5d; ^{18}F-radiolabeling: (iii) K^{18}F/Kryptofix 2.2.2., CH_3CN, 80 °C, 10 min.

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Radiosynthesis and in vivo evaluation in melanoma-bearing mice of O-(2-[^{18}F]Fluoroethyl)-L-tyrosine as tumour tracer

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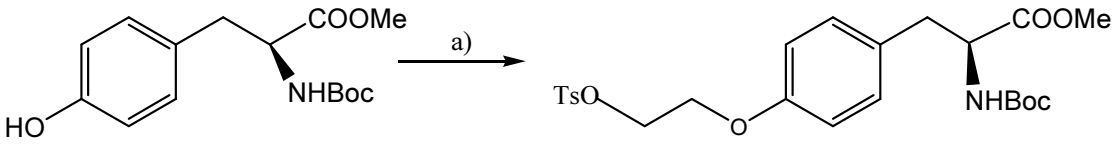
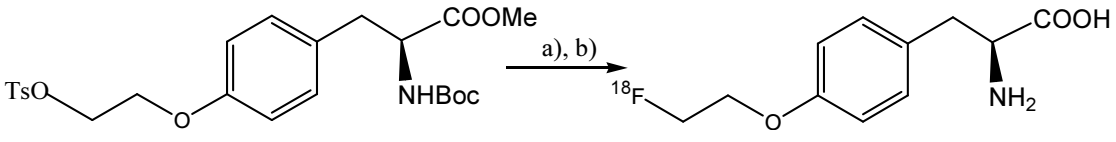
Objectives: ^{18}F-labeled amino acid O-(2-[^{18}F]Fluoroethyl)-L-tyrosine ([^{18}F]FET) , a very promising tumour tracer[1-4], was synthesized by a different chemical route and evaluated in melanoma-bearing mice with the way of biodistribution and atuoradiography.

Methods: the precursor *N*-tert-butyloxycarbonyl-*O*-(2-tosyloxyethyl)-L-tyrosine methyl ester was easily obtained from the reaction of commercially available *N*-(tert-butoxycarbonyl)-L-tyrosine methyl ester and 1, 2-bis(tosyloxy)ethane in anhydrous acetonitrile under the presence of potassium carbonate at 90℃ for 3~4h (scheme 1).



Scheme 1: a) $TsOCH_2CH_2OsT$, CH_3CN, K_2CO_3, 90 ℃, 3~4h

Radiosynthesis (scheme 2) of [^{18}F]FET was started from the direct radiofluorination of the precursor with the activated complex $^{18}F^-/K_{2.2.2}/K_2CO_3$ in acetonitrile at 130 °C for 30min. Then the reaction mixture was treated with trifluoroacetic acid and sodium hydroxide to realize the hydrolysis in less 10min. The separation was carried out by solid phase extraction on Sep-pak plus silica cartridge and eluted with acetonitrile and phosphate buffer saline (PBS) in turn. Thus the final formation of [^{18}F]FET was obtained as PBS solution and used for the following animal experiments after dilution.



Scheme 2: a) $^{18}F^-/K_{2.2.2}/K_2CO_3$, CH_3CN, 130□, 30min; b) TFA, RT, 3min; $NaOH/H_2O$, 90 ℃, 5min.

The animal experiments were investigated in B16 melanoma-bearing C_{57}BL/6 mice and performed 10d after subcutaneous inoculation of the tumour cells in the right axillas when the tumours weight 50~300mg. 1.5~2.3MBq [^{18}F]FET in about 100μL of PBS solution was injected into tail vein. The animals for biodistribution were killed by eye dislocation at

various times post injection, and the organs of interest were rapidly dissected. The tracer uptakes in tissues were determined by gamma counter. The results were expressed as percentage of the injected dose per gram tissue (ID% /g). The animals for whole-body autoradiography were killed by the above same protocol without dissection, and then directly exposed to multisensitive imaging plates. Imaging plates were scanned by phosphor storage imaging system.

Results: The radiochemical yield was with an average of 45% and radiochemical purity was more than 95%. The total synthesis time was less than 60min from EOB.

[^{18}F]FET showed very fast accumulation into the whole body with peak occurring within 10min after i.v. administration (table 1). The highest radioactivity ratios of tumour-to-brain and tumour-to-skin reached 3.4 - 5.1 and 4.1 - 2.7 at 30 – 90min p.i.. Visual evaluation from autoradiography showed evident difference between tumour and other tissues (Fig 1).

Conclusion: An efficient one-pot synthesis method of [^{18}F]FET was established in good radiochemical yield and radiochemical purify. Higher ratios of tumour-to-non-tumour was found in our experiments which promise a high sensitivity for the detection of cerebral and peripheral tumour.

Key Word: [^{18}F]FET, L-tyrosine, Radiosynthesis, Biodistribution, autoradiography.

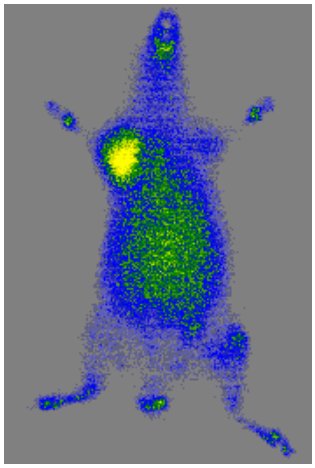


FIG 1. Autoradiography of the whole body of B16 melanoma-bearing mice 30min post injection of O-2-[^{18}F]Fluoroethyl)- L-tyrosine into tail vein. Tumour was visualized clearly.

TABLE I. TISSUE DISTRIBUTION OF RADIOACTIVITY AFTER INTRAVENOUS INJECTION OF O-(2-[^{18}F]FLUOROETHYL)-L-TYROSINE INTO B16 MELANOMA-BEARING MICE

	Uptake(ID%/g)							
	5 min	10 min	30 min	60 min	90 min	120 min	150 min	180 min
tumour	12.7±6.6	19.4±5.6	16.3±5.7	14.6±0.7	12.9±2.0	8.2±3.8	2.4±0.1	2.0±0.1
skin	5.0±3.6	7.5±1.8	4.8±0.2	3.4±1.4	2.5±1.2	1.9±0.0	1.0±0.1	1.3±0.2
brain	2.4±1.3	4.3±0.7	3.9±0.6	4.0±0.0	3.5 ±0.3	3.4±1.8	2.5±0.3	2.0±0.1
liver	11.3±4.4	14.2±1.5	7.5±0.5	4.8±0.4	4.2±0.3	2.9±0.6	1.8±0.7	1.5±0.7
heart	12.5±4.5	14.3±2.6	8.1±0.6	5.3±0.4	4.7±0.5	3.0±0.8	2.4 ±0.4	1.8±0.5
lung	10.9±3.9	14.4±2.6	8.6±0.6	5.6±0.6	4.7±0.6	3.3±1.0	2.7 ±0.2	1.8±0.7
spleen	9.3±5.1	15.0±1.7	8.4±0.8	6.0±0.5	5.8±0.6	3.5±0.9	3.4 ±0.9	2.0±0.7
kidney	15.4±6.5	14.9±1.8	9.3±0.1	6.6±0.6	5.7±0.9	3.7±0.7	3.3±0.7	2.1±0.2
Intestine	9.7±4.3	12.8±1.6	7.3±0.8	5.0±1.0	5.0±0.6	5.0±0.3	2.5±0.6	2.0±0.2
pancreas	27.5±3.7	59.8±9.1	41.1±8.1	32.9±8.6	26.4±9.2	21.1±7.0	15.8±7.8	11.1±1.5
muscle	9.5±4.9	12.2±2.4	8.1±0.6	5.1±0.5	4.6±0.5	3.0±0.6	2.8±0.4	1.9±0.0
bone	10.0±4.1	17.7±1.7	14.6±3.3	11.9±1.1	11.3±1.0	10.9±3.8	9.6±0.8	8.2±1.2
stomach	10.3±5.8	10.7±1.3	6.0±1.2	5.9 ±0.8	5.8±1.8	4.4±1.3	3.3±0.5	2.5±0.2
blood	12.0±3.8	12.6±3.8	8.0±0.6	5.2±0.2	5.0±1.4	2.8±0.6	2.7±0.3	2.0±0.3

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Session 16:

CYCLOTRON BASED RADIONUCLIDES AND GENERATORS

Production of radionuclides with a cyclotron

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The production of radioisotopes for use in biomedical procedures such as diagnostic imaging and/or therapeutic treatments may be achieved through nuclear reactions from charged particle bombardment using an accelerator. The goal is to get the target material into the beam, keep it there during the irradiation and the remove the product radionuclide from the target material efficiently and quickly. The specific design of the cyclotron target is what allows one to achieve this goal. For every radionuclide, there are usually nearly as many target designs as there are people producing the isotope. The design and use of cyclotron targets can be a very complex problem and involves the use of physics, chemistry and engineering in order to produce a target which is reliable and efficient.

This presentation will address how basic principles of physics, engineering and chemistry apply to radionuclide production with an accelerator. The concept of power density applied to cyclotron targets and various means of heat removal from the target. The efficient extraction and separation of the desired product radionuclide from both the target material and the other radioisotopes present will be discussed.

Perspectives for the large-scale production of radiolanthanides with medical potential

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The new developments in systemic radionuclide therapy based on chelated peptides now strongly call for metallic radioisotope preparations with new characteristics. Especially important for the new applications is high specific activity of the radiotracer in therapeutic amounts. The rare earth elements play a dominant role in this concern, because radionuclides of this group of chemically very similar elements provides the whole diversity of radiation properties and half-life's one would wish.

The presently used methods in production of the corresponding isotopes have reached their technical limitations and the progress in the systemic radionuclide therapy is limited by the availability of radio nuclides with the wanted high quality characteristics. These radionuclides are either only or best produced in high-energy spallation and fragmentation reactions. An opportunity for industrial scale production of such new radioisotopes for future medical use in a fully parasitic or prime user mode is described.

The target and ion-source techniques developed for making the high purity mass-separated radioactive ion-beams (RIB) at CERN ISOLDE is now a well-documented new type of rapid, efficient, continuous and automatic radiochemical separations. Its key element the electromagnetic Isotope Separator On Line (ISOL) allows producing efficiently very pure samples of almost all radioactive isotopes with the highest possible specific activity, i.e. the carrier free form. Until now these techniques have exclusively been used to make RIB of short-lived species for scientific purposes. It is now generally recognized that simplified variants of these techniques also will allow harvesting samples of longer-lived, high purity and carrier free radio nuclides as a byproduct from the spent target material, spent beam absorbers or eventually from dedicated on-line target stations. At present they permits producing "exotic" radioisotopes that hitherto were not available on the market for medical use but only in amounts that has supplied the R&D work and validation of the methods.

We believe that the time has come to prepare for more rational and large-scale industrial radioisotope production methods using the ISOL target techniques in particular in conjunction with electromagnetic mass separation.

The future production sites should be put in synergy with either the planned upgrade of the CERN ISOLDE and/or one of the major new physics-research facilities planned for using GeV proton beams of MW power like EURISOL, neutrino factories or spallation neutron sources where the production rates will be orders of magnitude higher than at present facilities.

Production of ^{123}I MIBG at IPEN-CNEN/SP

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Sodium [^{123}I]-iodide is obtained in Cyclone 30 (IBA) at IPEN-CNEN/SP. The production process uses the following nuclear reaction: ^{124}Xe (p, 2n)→^{123}Cs→^{123}Xe→^{123}I. The ^{124}Xe gas is highly enriched (>99.8%) which results in ultra pure ^{123}I end product. The water-cooled target (75 mL of internal volume) is machined from aluminum alloy. In front of the target there are two helium cooled molybdenum windows and an alignment system consisting of a pair of four sectors collimators. The irradiation is performed with protons of 30 MeV of energy and effective beam current on target of 60–70 μA. The ^{124}Xe transference from the storage bottle to the target and the recovery of the gas after irradiation to the bottle is made cryogenically with liquid nitrogen, through stainless steel pipes. The [^{123}I] activity on the wall of the target is rinsed with sterile water and the [^{123}I] active solution (60–70 mL) is transferred to the hot-cell. With a ^{124}Xe gas pressure (without proton beam) of 2 bars it was obtained about 220MBq/μA of ^{123}I at EOB (end of bombardment).

The labeling process is based in the cooper (I)-assisted exchange radiodination methods using MIBG-sulphate, $(NH_4)_2SO_4$ and $CuSO_4$ at 165–170ºC during 30 min. After reaction time the vial is cooled at room temperature and sterile saline-benzyl alcohol 1% solution is added. The volume is completed until a desirable radioactive concentration and the active solution sterilized through a 0.22 μm Millipore filter. After quality control aproval the final product is delivery to Nuclear Medicine Centres. [1–2].

The radiochemical and radionuclide tests of ^{123}I are determined in Whatmann 3MM paper (1.5×12cm) in 85% MeOH (R_f *I^- = 0.75 and R_f *IO_3^- = 0.40) and by γ-ray spectroscopy using hiper-pure Ge-detector, respectively, before labeling procedure. The radiochemical impurity of ^{123}I-MIBG is evaluated in a fast paper chromatographic system: Whatman 3MM (1×8 cm), in n-butanol: acetic acid: water (5:2:1) as a solvent. The values are: R_f ^{123}I-MIBG = 1.0 and free [^{123}I]-iodide = 0.0 [3–4]

The retention of ^{123}I-solution in a strong anionic resin is greater than 99.00% and the recovery of ^{123}I-Na is more than 97% of total activity in 3 mL of 0.02N NaOH. The radionuclide purity of ^{123}I-Na and the radiochemical purity of ^{123}I-MIBG are >98% and >97%, respectively, in 90% of all routine production without any purification step. The SD is less than 1.0% (n=3). The microbiological analysis is determined in different culture medium incubated at room temperature and at (33 ± 2)º C. The apirogenicity is evaluated using the “in vitro” Limulus test (LAL). The method was developed, validated and simplified to extend it to large-scale productions at Radiopharmacy Centre of IPEN-CNEN/SP.

During 2004, 129.5 GBq of ^{123}I-Na and 48 GBq of ^{123}I-MIBG in 37 batches, respectively, were distributed to approximately 28 Hospitals and Nuclear Medicine Centres in Brazil.

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New strontium-82/rubidium-82 generator

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Due to similarity of rubidium and potassium cations the radionuclide ^{82}Rb, a positron emitter, has been used in nuclear medicine to characterize myocardial perfusion with high sensitivity and specificity [1,2,3]. The advantage of ^{82}Rb positron emission tomography (PET) versus classical SPECT with ^{201}Tl is the short half-life of ^{82}Rb ($T_{1/2}$= 75s) which allows one to scanning of patients every 10 min and to reduce the exposure of patient to radiation. Additionally, ^{82}Rb is generator produced from the longer lived parent radionuclide ^{82}Sr ($T_{1/2}$= 25.55 days), which permits clinical PET studies also in hospitals which do not have expensive on-site cyclotrons. Numerous methods for manufacture of ^{82}Sr/^{82}Rb generator have been already described [4,5]. All these procedures, however, suffer from various limitations e.g. complicated multi-step separation of ^{82}Sr from rubidium target, insignificant radiation resistant of the organic extractans and ion exchange resins. To avoid these disadvantages we used an inorganic ion exchanger - cryptomelane MnO_2. The cryptomelane MnO_2 has a tunnel-framed structure with exchangeable alkali or alkali earth cations. The average tunnel diameter is 280 pm, therefore the sorbent is selective for the cations with crystal ionic radii of 130-150 pm, e.g. K^+, Rb^+, Ba^{2+} and Ra^{2+}. To find the optimum conditions for Rb^+-Sr^{2+} separations, the distribution coefficients (K_d) of Rb^+ and Sr^{2+} on cryptomelane MnO_2 were determined as a function of HNO_3 concentration. The influence of the HNO_3 concentration of on the K_d for Sr^{2+} and Rb^+ on cryptomelane MnO_2 is shown in Fig. 1. It can be seen that K_d for Rb^+ even at 1 M HNO_3 is very high. This confirms high affinity of cryptomelane MnO_2 for cations with ionic radii close to 150 pm. For Sr^{2+} whose ionic radius is lower (118 pm), K_d decreases with increasing concentration of H^+ ions. For the efficient separation of the Rb^+-Sr^{2+} pair 0.5 mol dm^{-3} HNO_3 was chosen as optimal solution, wherein the K_d for Rb^+ on cryptomelane MnO_2 is greater than 10^4, while for Sr^{2+} it is close to 1. This allows one to perform simple and quantitative separation of ^{82}Sr from the irradiated rubidium target.

The isotope ^{82}Sr was produced on the AIC-144 cyclotron located in the Institute of Nuclear Physics, Cracow. In the pilot experiment a target of 0.133 g RbCl of natural isotopic abundance (72.17% ^{85}Rb, 27.83% ^{87}Rb) was irradiated with the internal proton beam of 48 MeV, 0.5 μ A, during 4 h. At this energy, proton activation of the natural rubidium target leads to direct or indirect formation of ^{82,83,85}Sr and ^{82,83,84,86}Rb isotopes. The radionuclides detected by gamma spectrometry in the irradiated target is presented in Table I. After the 8 days waiting period which is enough for decay of ^{83}Sr, the RbCl target was dissolved in 0.5 M HNO_3 solution. Next, the solution was passed trough the cryptomelane MnO_2 column bed. The inactive rubidium (target material) and ^{83,84,86}Rb were quantitatively adsorbed on the cryptomelane MnO_2. The effluent from the column was alkalized by 1 M NaOH to pH=6-8. Afterward, the strontium radionuclides from the neutralized solution were loaded on top of the SnO_2 aq. bed. The inorganic ion exchanger - tin oxide was prepared by acidification of sodium stannate solution according to the procedure described in [6]. The ^{82}Rb formed from decay of ^{82}Sr was eluted from the column by 0,9% NaCl (physiological saline). The elution was performed every 10 min. The radionuclide purity of the effluent was measured by gamma

spectroscopy after the decay of ^{82}Rb. Additionally, we measured also the decay curves of the effluent fractions (Fig. 2). The ^{82}Sr and ^{85}Sr breakthroughs measured by gamma spectroscopy were lower than the established limits. After passing 1 liter of 0.9% NaCl through the column, no significant breakthrough was observed either by gamma spectrometry or by analyzing the decay curves. The half life of the eluted ^{82}Rb determined from the decay curve measured for more than 6 expected half-lives, is identical with the literature value.

TABLE I. RADIONUCLIDES DETECTED IN THE ^{nat}RBCL TARGET AFTER IRRADIATION WITH 48 MeV PROTON BEAM

Radionuclide	$T_{1/2}$ (days)	Activity (MBq)	Nuclear reaction
^{82}Sr	25.5	6.49	^{85}Rb(p,4n) ^{82}Sr
^{83}Sr	1.35	4.45	^{85}Rb(p,3n) ^{83}Sr
^{85}Sr	64.8	8.55	^{85}Rb(p,n) ^{85}Sr ^{87}Rb(p,3n) ^{85}Sr
^{83}Rb	86.2	18.60	^{83}Sr(EC, β^+)$\rightarrow$ ^{83}Rb
^{84}Rb	32.9	14.29	^{85}Rb(p,pn) ^{84}Rb
^{86}Rb	18.7	18.66	^{85}Rb(n,γ) ^{86}Rb

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Cyclotron production of ^{103}Pd via proton-induced reactions on ^{103}Rh target

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Prostate cancer is a common malignancy in men in the Western world. In recent years, improvement in biochemical diagnostic methods and the availability of a wide range of treatment options have increased the number of patients surviving free of disease. It has been noted that the proportion of patients treated by permanent brachytherapy is rapidly increasing (more than 40,000 in 1998 in the USA) and is expected to reach 50% in 2006 [1]. The favorable results of permanent implants may however not be reproducible if strict treatment procedures and patient selection guidelines are not followed. For this reason regular updates on recommendations are published by the American Brachytherapy Society (ABS) [1], where also the large number of clinical studies and dosimetery problems are discussed (see for instance [2–12]). Essentially two radionuclides, namely ^{125}I and ^{103}Pd, are used for this technique. As early as 1958, ^{103}Pd was proposed by Harper, et al. [13] for interstitial implantation. It was not until 1987 that encapsulated ^{103}Pd sources became commercially available in the USA, where a company now operates more than 10 dedicated accelerators to produce this nuclide [14]. Recently a manufacturer in Europe also brought his patented type of ^{103}Pd seed implants to the world market.

The accelerator production method for ^{103}Pd used nowadays is based on the irradiation of rhodium metal with rather low energy protons via the reaction ^{103}Rh (p,n)^{103}Pd followed by a default chemical separation of the radionuclide from the expensive target material. Harper, et al. [16] and Lagunas-Solar, et al. [17] described some early procedures.

An alternative production and purification route for ^{103}Pd employing silver targets has been proposed by Fassbender, et al. [18].

Irradiated rhodium metal targets (plated layers, foils and wires) have been frequently dissolved by sodium bisulphate fusion (time-consuming, complex medium), by gold tetrachloroaurate oxidation (very expensive, time consuming) and by alternating current electrodissolution in hydrochloric acid. Up to now the latter method has been recommended for the solubility of foils (not applicable for rhodium powder, wires or fragments) [19-20]. A new high current-density electrodissolution technique resulting in quantitative solubility of target material in acid has been developed.

Since Rh is a precious metal, it is therefore, essential to be recovered from the processed solution of the radiochemical separation and re-use the obtained Rh for preparation of the electrodeposition bath. The electrodeposition of Rh using Rhodex baths gives quite acceptable quality for the irradiation purposes. However, after electrodissolution and radiochemical separation , rhodium presents as chloride complexes in about 6 N HCl solutions which can not

be converted to such a solution that contains chemical components as in the Rhodex bath. The investigations were, therefore, conducted to the cycle of recovery/electrodissolution/ electrodeposition for routine production of ^{103}Pd.

Rh target preparation from sulfate plating baths

To prepare the sulfate baths, the hydrated rhodium oxide (Rh_2O_3.aq) was used which in term had been recovered from hydrochloride acid solution containing rhodium chloride complexes. Therefore, the procedure included two parts:

a) Recovery of Rh as rhodium oxide from chloride solution

The hydrochloric acid solution containing Rh was primarily obtained from an electrodissolution of the irradiated rhodium target on which a radiochemical separation of ^{103}Pd had been performed. This solution was passed through a 0.45 μm filter (HVLP, Millipore), the filtrate evaporated to near dryness, 300 ml water added to the residue, and the pH of the solution adjusted to 10-10.5 by 10 N NaOH until a yellow, colloidal solution of Rh_2O_3.aq was formed.

To improve the filterability of the yellow Rh_2O_3.aq precipitate, the solution was allowed to digest for 24 h at 50°C under gentle stirring then passed through a Bleu Band filter paper (Schleich & Scheull 589). The precipitate on filter was washed several times with water to remove most of the adsorbed Cl^- ions. Since some of the yellow precipitate had gone through the filter paper, the filtrate was passed through a second 0.45 μm filter. The Rh_2O_3.aq on the filters was left in air for 24 h to be dried, followed by grinding into fine powder, then dried for 48 h under vacuum at the room temperature and finally weighed.

b) Electrodeposition of Rh on the Cu backings

To prepare the plating solution, a 5.7 g of the recovered hydrated rhodium oxide, for 4 targets with thickness of about 48 μm, was transferred into a 100 ml beaker with a magnetic stirring bar. 10 ml of 95% sulphuric acid was then carefully introduced into the beaker. The beaker was covered with a watch-glass. The solution was heated up to 350°C under gentle stirring until SO_3 fumes evolved and thereafter heating continued for 15 minutes. A brownish yellow solution was then obtained. The solution was added to 300 ml of water in a 600 ml beaker, passed through a 0.45 μm filter paper and the pH adjusted to 1.0–2.0 by 10 N NaOH. 5 g sulfamic acid was then added to the solution and diluted to 450 ml by water. The electrolyte solution was transferred into the plating vessel and a DC current applied to the electrodes. The electrodeposition was carried out at 60°C under a bi-directional stirring (1000 rpm, 8s/8s) for 24 h using a current density of 8.55 $mAcm^{-2}$.

The electrodissolution system consists of a cylindrical upper body (diameter 70 mm, height 120 mm) and a conical lower part (height 30 mm) ending in a 12 mm diameter circular window allowing the attachment of the filter combination. The latter consists of a classical G-4 glass frit fitted with an end glass tube allowing removal and recirculation of the solution by means of a high flow-rate (1 l/min) peristaltic pump. This loop contains cooling water (glycol) allowing removal of heat produced during the electrochemical dissolution of the Rh. The G-4 supports a home-made graphite fibre (diameter 100 mm, 360 holes of 0.5 mm) clamped in a ring of a copper (to ensure electrical contact) and Perspex ring. Above the filter combination, a supply compartment allows introduction of reagents that is also done by means of peristaltic pumps. The upper graphite ring electrode (thickness 2 mm, external diameter 100 mm, window diameter 20 mm) is also mounted in copper/Perspex ring

combination at 30 mm above the graphite filter. Sealing of the system is obtained by means of O-rings and clamping different parts. The upper window of unit is closed by means of perspex cover fitted with nipples allowing escape of nitrogen oxide and excess of chlorine gases. The latter are adsorbed in sodium hydroxide solution to avoid corrosion of the environment.

Dissolution time of the Cu carrier was controlled by flow-rate controlled introduction of nitric acid into the vessel holding the vertically mounted target. The resulting Cu $(N0_3)_2/HN0_3$ solution was removed by filtration through a glass/graphite filters combination whereby the Rh fragments are collected on the filter and the vessel walls, Rh Fragments were washed with water and then Hydrochloric acid and chlorine gas was introduced. Electrochemical dissolution of the Rh was done by applying a high ac current density (2.4 $A.cm^{-2}$) between the electro-graphite filter and a perforated circular upper graphite electrode mounted at an appropriate distance from the carbon filter.

Anion exchange involves the dissolution in 0.03N HCl and the Cu/Rh/Pd separation is achieved using a Dowex1X8 (Cl-)/100–200 mesh column (1.5×10 cm). Copper is eluted with 0.03M HCl, rhodium with 6M HCl and palladium with a 1:1 mixture of 0.5M NH3 /NH4Cl.

FIG. 1. Electrodissolution set up.

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The status and potential of new radionuclide generators providing positron emitters to synthesise new targeting vectors for PET

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Radionuclide generator systems continue to play a key role in providing both diagnostic and therapeutic radionuclides for various applications in nuclear medicine, oncology and interventional cardiology. Key advantages for the use of radionuclide generators include reasonable costs, the convenience of obtaining the desired daughter radionuclide on demand, and availability of the daughter radionuclide in high specific activity, no-carrier added form. Although many parent/daughter pairs have been evaluated as radionuclide generator systems, in particular for the application of labeled PET radiopharmaceuticals, there is a relatively small number of generators which are currently in routine clinical and research use.

Those generators can be categorized according to the half-life of the daughter nuclide. The short-lived daughters cover half-lives of a few minutes. As the short half-lives do not allow radiochemical synthesis, these systems are relevant for perfusion imaging exclusively.

The longer-lived daughter nuclides, on the other hand, provide a potential for the development of labeled radiopharmaceuticals. Recently, the ^{68}Ge/Ga and ^{72}Se/As systems have found impressive application, but also the ^{44}Ti/Sc generator represents a promising system.

The ^{68}Ge/Ga generator (^{68}Ge, T½ = 270.8 d) provides a cyclotron-independent source of positron-emitting ^{68}Ga (T½ = 68 min, β^+ branching = 89%), which can be used for coordinative labelling. Recently, tumour imaging using ^{68}Ga-labelled DOTA-conjugated peptides became one of the most exciting approaches to diagnose neuroendocrine and other tumours and metastases because (i) octreotide derivatives with high affinity and selectivity to somatostatin receptor expressing tumour cells are available, (ii) syntheses of DOTA-conjugated targeting vectors are straight forward due to the kit-type labelling, and (iii) PET/CT scanners perfectly correlate morphological and functional parameters. However, for labelling of biomolecules via bifunctional chelators, ^{68}Ga(III) as eluted initially need to be pre-concentrated and purified from ^{68}Ge(IV), Zn(II), Ti(IV) and Fe(III). We describe a system for simple and efficient handling of the ^{68}Ge/^{68}Ga generator eluates with a micro-chromatography column filled with about 50 mg of a cation exchange resin (Bio-Rad AG 50W-X8) as the main component. Chemical purification and volume concentration of ^{68}Ga are carried out in a 80% acetone/0.15 M HCl solution. Finally, more than 97% of ^{68}Ga are obtained in 400 µl of a 97.6% acetone/0.05 M HCl solution. The initial ^{68}Ge contamination of the eluate was reduced by a factor 1000. Contents of Zn(II), Fe(III) and Ti(IV) were reduced significantly. Consequently, the processed fraction can be used directly for the syntheses of radiopharmaceuticals. For labelling with ^{68}Ga(III), DOTA-octreotides (DOTATOC, DOTANOC) and Desferrioxamine-B-succinyl-octreotide (DFOOC) were used. Within 25 min, an injectable radiopharmaceutical, e. g. ^{68}Ga-DOTATOC can be prepared with specific activities of up to 450 MBq/nmol. The developed system represents a simple and efficient way for labelling of DOTA-conjugated biomolecules with generator-produced ^{68}Ga(III). [^{68}Ga]DOTATOC and [^{68}Ga]DOTANOC were successfully used in a series of human somatostatin receptor-expressing tumours diagnosis with PET/CT. Moreover, a variety of other ^{68}Ga labelled compounds might be synthesized for many other applications.

The ^{44}Ti ($T_{½}$ = 47 a) / ^{44}Sc ($T_{½}$ = 3.927 h) radionuclide generator provides a very similar chemical system. The longer-living daughter ^{44}Sc, however, presents a physical half-life adequate to biological investigations, where the 68 min half-life of ^{68}Ga appears to be too short.

In addition, in particular if targeting vectors such as monoclonal antibodies are used instead of small peptides, we recently proposed the radioarsenic isotopes ^{72}As ($T_{½}$ = 26 h, 88% β^+ branching) and ^{74}As ($T_{½}$ = 17.78 d, 29% β^+ branching). As a proof of principle, we successfully tested the hypothesis that a new chimeric IgG_3 monoclonal antibody ch3G4 (Tarvacin®), directed against anionic phospholipids and labeled with radioactive arsenic isotopes, can be used for the vascular targeting and molecular imaging of solid tumours in rats *in vivo*. For generators using no-carrier-added ^{72}Se, we described the distillation of $AsCl_3$, while Se remains in non-volatile compounds in the residue, and developed a solid phase extraction system with ^{72}Se fixed as metallic Se. Systematic chemical investigations on the labeling chemistry of no-carrier-added radioarsenic are currently developed prior to the application of ^{72}As labeled compounds.

Despite of high-resolution PET imaging, the ^{68}Ge/Ga and ^{44}Ti/Sc generators might also be used in combination with therapeutic approaches. Both ^{68}Ga, but eventually even better ^{44}Sc might be labeled to chemically similar targeting vectors such as DTPA- or DOTA-conjugated peptides, antibodies or antibody fragments. This might allow for better pre-therapeutic diagnoses in terms of tumour detection and radiation dosimetry, prior to the application of analogue radiotherapeuticals labeled with ^{90}Y, ^{177}Lu or other particle emitting trivalent metals radionuclides.

Due to the long half-life and the low cross sections, in particular for the parent nuclides ^{44}Ti and ^{68}Ga, the production rates are relatively low and require long high-current irradiations. Although this results in rather high cost per generator, the number of PET scans achievable (up to several hundreds per single ^{68}Ge/Ga generator for example) definitely lowers the costs per individual patient investigation.

Finally, the ^{68}Ge/Ga and ^{44}Ti/Sc generators offer a kit-type synthesis of PET radiopharmaceuticals. This might become a significant advantage when compared to ^{11}C or ^{18}F labelled PET tracers, in particular in centres without a cyclotron and / or without a sophisticated organic synthesis infrastructure and experience. With new kit type compounds to be developed for coordinating generator derived ^{68}Ga or ^{44}Sc, there will be a fascinating perspective for several systems, which partly might introduce PET in directions, today (still) covered by similar ^{99}Mo/^{99m}Tc generator systems and SPECT.

Session 17:
RADIOPHARMACY

Nuclear pharmacy practices in the USA

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The practice of radiopharmacy in the USA has been directed towards the Centralized Radiopharmacies (CRPh) in the recent years. CRPh(s) provide single patient unit doses in shielded containers. The main advantages of this system to nuclear medicine practitioners are to minimize radiation exposure, simplify the requirements of regulatory agencies including the paperwork, and to reduce the risk to the staff, patients and the public. In general dose preparation, quality assurance and waste retrieval services are provided by CRPh, in both an efficient and cost effective manner. On the other hand, in a high volume nuclear medicine facility, a Hospital Radiopharmacy (HRPh) may be a highly cost effective endeavor compared to purchasing unit doses.

Currently, there are more than 450 radiopharmacies in the U.S.A. CRPh(s) represent about 80% of these, operated by four major CRPh companies. Cardinal Health, formerly Syncor, has the largest network with over 150 CRPh(s). The others are Tyco Healthcare/Mallinckrodt, GE Healthcare, formerly Amersham and Geodax Imaging. There are two radiopharmacy companies specializing in positron emission tomography (PET) radiopharmaceuticals; CTI/PETNET and Eastern Isotopes. There are also several independent CRPh facilities in the U.S.A. Institutional HRPh(s), representing 20% of the radiopharmacies in the U.S.A., provide multidose preparations for larger nuclear medicine departments located in university or hospital settings.

All commercial CRPh(s) are licensed by a state board of pharmacy and operated under the supervision of an authorized nuclear pharmacist. The possession, handling, dispensing and waste management of radiopharmaceuticals are regulated by the Nuclear Regulatory Commission (NRC) or an agreement–state regulatory body such as the State Board of Radiation Protection. The NRC requires that all nuclear pharmacists complete a certificate program, consisting of 200 h of didactic and 500 h of experimental training, working with radiopharmaceuticals. Some universities and commercial radiopharmacy companies offer certificate programs by on-site and remote education. The first graduate radiopharmacy program was established between 1968 and 1986 at the University of Southern California. Purdue University program has been in operation since 1972. University of New Mexico and University of Arkansas have jointly developed a remote learning program on the internet.

The NRC requirements for radiopharmacy facilities are well-established which are based on radiation safety principles. Additionally requirements for aseptic compounding and dispensing of radiopharmaceuticals have recently been enforced by the United States Pharmacopoeia USP <797> regulations. This seminar will provide an update and a discussion regarding the impact and arguments on USP <797> within CRPh and HRPh settings.

Regulatory aspects of hospital radiopharmacy and clinical trials

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Number of clinical research studies is increasing as the scientists concentrate their efforts on diagnosing,treating and preventing diseases by increasing knowledge about human health. A clinical trial may be defined as “Any investigation in human subjects intended to discover or verify the clinical, pharmacological or pharmacodynamic effects of a drug, identify any adverse reactions related to a drug,study the absorption, distribution, metabolism and excretion of the drug with the object of ascertaining its safety and/or efficacy”.

Clinical trials may be commercial or non-commercial . Clinical studies set up for commercial goals are usually run by industrial companies for the purpose of drug development and registration . It takes years for a new drug to be put on the market and only results obtained by authorized clinical trials can be used for marketing authorization of an Investigational Medicinal Product (IMP) . Non-commercial clinical trials are generally conducted by academia and use existing medicinal products to optimize treatment regimes or establish new techniques with the same products. An individual who participates in a clinical trial as either a recepient of the IMP or as a control is the “Subject “of this trial. The subject can be normal volunteers or patients.

All clinical research studies involving human beings must be designed according to a protocol which is a document that describes the objectives,design,methodology, statistical considerations and organisation of the trial.

A clinical trial must be carried under the responsibility of a physician using material prepared at licensed sites by Good Manufacturing Practices (GMP) and must be conducted according to the principles of Good Clinical Practices (GCP) that are designed to ensure the protection of the rights, safety and well-being of clinical trial subjects and other persons.

Declaration of Helsinki adopted in 1964 and its revised forms should constitute the basis for all medical research on human beings with a set of recommendations guiding the responsible physicians all over the world. Special caution should also be given to the welfare of animals used for research and to the environment in which the trial is conducted . The European Directive on Good Clinical Practice in Clinical Trials - 2001/20/EC together with its amendments are considered as the main regulation in Europe which apply to all IMP including radiopharmaceuticals. Many countries have their national regulations set similarly and they are being harmonized by the efforts of the International Conference on Harmonization (ICH). The guidance documents developed through the harmonization process defines the design,conduct, performance,auditing,recording and reporting of clinical trials.

Radiopharmaceuticals may be argued to be different from classical drug products in being radioactive and carrying a small amount of material in a small volume of injection . A radiopharmaceutical is applied to a patient usually only once or a few times in his/her lifetime. In general they spend a short time in the body and no biological effect is expected to occur by the application of a radiopharmaceutical to a human being . On–site preparation and immediate use may be another aspect of radiopharmaceuticals differing from regular drug products .

In spite of these arguments radiopharmaceuticals are considered as medicinal products and any clinical trial performed with radiopharmaceuticals is therefore subject to all the legislation regarding IMP. The radioactive nature of radiopharmaceuticals also makes it necessary to follow the regulations related to ionizing radiation. Euratom Directive 97/43 - protection of individuals against the dangers of ionizing radiation in relation to medical exposure applies to exposure of healthy individuals or patients voluntarily participating in medical or biological,diagnostic or therapeutic,research programmes.

It must be clear that even the use of an authorized radiopharmaceutical for a different indication or given at a different dosage or use of a different route of administration other than that registered are considered as clinical trials . Use of unauthorized radiopharmaceuticals on human beings is certainly subject to permission by the competent authority. This covers home-made radiopharmaceuticals and any change in the method of preparation of a licensed radiopharmaceutical. Studies which involve standard application of approved radiopharmaceuticals to assess the efficacy of a treatment (e.g.bone scan used for monitoring in a chemotherapy trial) are not considered as clinical trials.

Standardization and quality control of an in-house formulation of ^{99m}Tc-(V)-DMSA for imaging and assessment of tumour biology: Work in progress

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Early diagnosis and effective treatment is the key for proper cancer control and nuclear techniques play a key role in differential diagnosis and ongoing follow up in these patients. Tumour imaging properties of various common radiopharmaceuticals have been utilised clinically with varied success. It must be realised that the radiochemical purity and stability of a formulation should be standardised in order to achieve clinically reproducible results. The different success and faliure rates may be due to this reason where one radiopharmaceutical is being used by different groups with sometimes contradictory findings. The objective of the present study is to reproduce an optimum simple method of in house formulation of ^{99m}Tc-(V)-DMSA from the available DMSA (III) kits and use it as a tumour imaging agent in the assessment of tumour biology

Two commercially available kits of DMSA was used. One was from the Board of Radiation & Isotope Technology (BRIT) India and the other was from Amersham (U.K).

Formulation of the BRIT kit was done by adding 7 mg of $NaHCo_3$ in 0.2 ml of water for injection and $^{99m}TcO_4$ simultaneously to the reaction vial. The kit contains 1 mg of DMSA, 0.3 mg of $SnCl_2.2H_2O$. Equal volume of air is withdrawn from the reaction vial. Mixing and incubation was done for 15 min.

Formulation of the Amersham kit (1 mg DMSA, 0.42 mg of $SnCl_2 . 2H_2O$, 0.7 mg ascorbic acid, 2.9 mg sodium chloride and 50 mg inositol) was done by adding 0.2 ml 7.5 % w/v $NaHCo_3$ + $^{99m}TcO_4$. Mixing followed by incubation was done for 15 min.

Radiochemical purity was determined by the method of Westera et al using TLC (T-6145 TLC pre coated plated silica gel with 254 nm fluorescent indicator on polyester supplied by Sigma /silica gel 60F 254 TLC aluminium strip supplied by Merck) with a solvent containing n-butanol acetic acid-water (3:2:3 v/v). Free pertechnetate levels were measured using TLC – SG in saline. Percent labeling of DMSA and free pertechnetate was calculated and the ratio of DMSA (III) & DMSA (V) was evaluated. These were carried out for a period of over 3 hrs.

Initial observations show that the radiochemical purity of DMSA (V) for the Amersham kit is optimum at about 1 hr post formulation whereas that of the kit supplied by BRIT the same radiochemical purity is achieved in 15 minutes (> 90%) The stability of both the kits were checked upto 3 hrs and found to be stable. Amersham kit contains ascorbic acid which acts as a mild antioxidant and is added to the DMSA (III) kit to get 100% pure DMSA (III). The ascorbic acid stops tin from being oxidised which is thought to be responsible for the conversion of DMSA (III) to DMSA (V). To enable the oxidation of tin we tried bubbling of

pure oxygen for 20 min.A high radiochemical purity was achieved in 15 min which remained stable at 3 hours.

Normal human biodistribution studies showed predominantly renal pelvicalyceal excretion in a radiochemically pure preparation with physiological concentration in bladder.The cardiac blood pool concentration is significant at 1 hour which gradually washes away by 3–4 hours time. Physiological tracer concentration was also seen in the liver and ocassionally in spleen.

A pilot study has been carried out so far in 4 patients of histopathologically proved lung carcinoma with the BRIT kit. Increased tracer concentration was seen immediately post injection. The target to non target ratio was maximum in 3 h post injection. Concentration in bone metastasis was optimum at 1 hour post injection. Proportionate washout of tracer from the cardiac blood pool helped in visualising tumour overlying the heart.

These results and the preliminary clinical evaluation show that the BRIT kit formulation achieves a faster conversion to DMSA (V) and achieves good tumour concentration in a radiochemically pure preparation. > 90 % purity is what is desirable for optimum tumour concentration. Amersham kit formulation without oxygen bubbling requires a longer time for conversion to DMSA (V). With the help of oxygen bubbling desired readiochemical purity is achieved in 15 mins.

Thus we have been able to standardise and reproduce an optimum formulation of DMSA (V) which concentrates in the tumour in-vivo.Clinical evaluation will continue side by side with quality control studies and further comprehensive laboratory as well as clinical data will be presented in the meeting.

ACKNOWLEDGEMENT

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Development of centralized radiopharmacies in Spain: A successful experience in Europe

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In Spain, 650.000 nuclear medicine procedures are perfomed every year in more than 120 nuclear medicine departments in public and private hospitals.

Traditionally the doses to be administered to patients were prepared by hospital personnel in small hot labs in the departments. However, during the 90's new legislation was developed that triggered the need for a change in the model. The concept of radiopharmaceuticals brought pharmaceutical requirements to the development, fabrication, commercialization, preparation and administration of radiactive compounds to patients. Additionally, Regulatory Authorities imposed quality assurance rules in nuclear medicine practices.

In what concerns the preparation and administration of doses, those legal requirements established new specific criteria derived from compliance with Good Manufacturing Practices, in several areas like the following:

– Premises and equipment
– Qualification of personnel
– Quality systemes with procedures, records and documentation control
– Traceability.

The implementation of all these changes meant not only significative investments and time but also important modifications in habits and practices developped during many years. Several initiatives were launched to cope with the need of change and the concept of centralized radiopharmacies was among them, particularly in regions with a high density of nuclear medicine departments, were economies of scale bring efficiencies to these pharmacies.

Today a completely new system governs the preparation of unit doses of radiopharmaceuticals in the country.

45% of the units doses are prepared and supplied from 6 centralized radiopharmacies.

20% of the doses are prepared in hospital radiopharmacies but managed and operated by a external contractor.

35% of the doses are prepared in hospital radiopharmacies managed by hospital personnel.

It should be outlined the significative penetration of centralized radiopharmacies what makes Spain the european country where these pharmacies have progressed more in spite of its different population density rates. They are owned and operated by private companies that took the initiative to bring to the spanish radiopharmaceutical sector this preparation and distribution system.

Molypharma has been involved with the development of centralized radiopharmacies in Spain since the beginning. As of today has built, and operates 3 of these radiopharmacies and has an

important stake in another one, being the only company in our contry that owns more than one radiopharmacy. Also and in some limited cases, operates hospital radiopharmacies.

The Molypharma centralized radiopharmacy concept is composed of the following elements:

A radiopharmaceutical unit designed for a sure, safe and efficient operation both for the product and the personnel. It includes a clean room with different areas that fulfills radiactive and pharmaceuticals criteria.

A computer system that supports the operations of the unit, helps the operator in its work and prevent mistakes and human errors. It keeps control of record and traceabilty, and prints all labels and documentation.

An e-business system based upon internet, that allows customers to request electronically every day their needs of unit doses of radiopharmaceuticals for the following day as well as the time of delivery. It also allows customers to have access to the Molypharma date bases to obtain information about their own supplies and statistical summaries.

A quality management system that has obtained not only the ISO certificates but also other recognitions and awards.

Nuclear medicine departments that decided to make the change and obtain their unit doses from the centralized radiopharmacies have obtained benefits summarized as:

– Fulfillment of all regulatory requierements without hospital investmens
– Improvements in flexibility and cost control and reduction.
– Traceabilty
– Improvements in pharmacovigilance and pharmaceutical responsibility.

Customer satisfaction analyses, performed every year have shown a good acceptance from nuclear physicians, nurses and hospital managers. Quality of service has been proven to be one of the most critical factors for success.

Peptide receptor radionuclide targeting of neuroendoctrine tumours with ^{111}In/^{90}Y-DOTATOC

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Peptide receptors are overexpressed in several human cancers, such as somatostatine receptor (SSR) in most of neuroendocrine tumours, allowing the design of radiopharmaceuticals based on peptide receptor radionuclide target for diagnostic image studies and therapeutic treatment by substituting a gamma emitter radionuclide, as ^{111}In, by a pure beta emitter, like ^{90}Y. Because the extremely short plasma half life of Somatostatine itself, interest has been focused on radiolabeling somatostatine receptor analogues.

Tyr^3-Octreotide (TOC), a somastostatine analogue conjugated to the strong chelator 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''tetraacetic acid (DOTA), is commercially available as DOTATOC from Pichem and can be radiolabeled with trivalent radiometals such as ^{111}In and ^{90}Y, which form stable complex with DOTA. Both $^{111}InCl_3$ and $^{90}YCl_3$ are commercially available from MDS Nordion.

^{111}In and ^{90}Y-DOTATOC could be similarly prepared using gentisic buffer (pH 5.05) and mixed with a saline solution of DOTATOC 2μg/μl, in order to have finally a ratio of 10 –15 μg of DOTATOC / mCi of $^{111}InCl_3$ and 1 – 1.5 μg of DOTATOC / mCi of $^{90}YCl_3$. The reaction vial must be heated for 25 - 30 min, in a water bath at 90ºC.

Chemical purity of DOTATOC must be analysed by HPLC and radiochemical purity could be determined by liquid chromatography using Sep-Pak C_{18} Cartridge, activated with methanol and conditioned with acetate buffer (pH 5.5).

The chemical purity of the of the peptide is the determining factor for choosing (DOTATOC μg/mCi of radionuclide) that must be used in order to obtain the radiochemical purity recommended for diagnostic and therapeutic purpose.

The Radiochemical purity obtained for both radiopharmaceuticals labelled using this methods was over 98% for ^{111}In-DOTATOC and over 99% for ^{90}Y-DOTATOC.

To date, over 35 patients have been treated and all of them had positive SSR demonstrated by ^{111}In-DOTATOC scintigraphic image.

This valuable diagnosis and treatment of neuroendocrine tumours has been possible through the strong collaboration with the European Institute of Oncology, Milan – Italy and is now available in Chile.

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POSTER PRESENTATIONS

Radiopharmaceutical production in Albania and its future

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Research and developing in the field of preparing and quality control of radiopharmaceuticals have been concentrated and continue to be at the Institute of Nuclear Physics. The Institute has a radiochemical laboratory, that possesses adequate infrastructure for research and developing including the scope of radioisotopes and radiopharmaceuticals. The radiochemical laboratory and the group of radiopharmaceuticals have izeion with our Nuclear Medical Center, but also with many research institutes in the country and abroad.

Studies on the field of preparing radiopharmaceuticals in the beginning were focused on developing the adequate methods for labelling and quality controls of radiopharmaceuticals more spread used in the 70s. During this time were developed methods for labelling and quality controls of hippurane, oleic acid and rosse-bengale labelled with ^{131}J, dermatological sources with ^{32}P and have done attempts for preparing ^{131}J and ^{32}P with and carrier free from imported targets. At the beginning of the 70s for diagnostic and therapeutic porpoises of thyroid gland was used ^{131}J as “nuclear cocktail”. Actually for diagnoses of thyroid used ^{99m}Tc, meanwhile for therapy ^{131}J as gelatin capsules prepared in the radiochemical laboratory of Institute of Nuclear Physics.

The demand for the technetium kits and the impossibility of importing them on the large quantities that covered needs of nuclear medicine for diagnoses and extents of scope of nuclear medicine were premises for developing the technique of production the cold technetium kits. In the frame of technical izeion with IAEA a laboratory was constructed for these purposes. The laboratory fulfills requirements of GMP and has enough capacity to cover demand of nuclear medicine actually and in the future. Now it is consolidated production of such kits as MDP, DMSA, DTPA, HDPA, Pyrophosphate, Phytate, Heptagluconate [1], etc and continuing before long with more sensitive and complicate kits such as HMPAO, ECD and MIBI, etc.

Last years are undertaken also some studies for preparing ^{90}Y by means of generator ^{90}Sr-^{90}Y with high chemical and radiochemical purity that to be able to use for labelling aminoacides and antibodies [2]. By ^{90}Y, home made, has been prepared ^{90}Y-citrate, which was used successful for therapy of bone metastases. Also by ^{90}Y in the chloride form, are done some studies for labelling antibody Mab B72.3 [3], MDP [4], HEDPA, porphyries [5], etc. with the aim using in the therapy of cancer metastases. Preliminary results encouraged the research group for further studies. In ^{90}Y production it is important content of impurities such as ^{90}Sr. It is develop a fast chromatographic method for identification and determination content of ^{90}Sr [6].

In the field of preparing and quality control of radiopharmaceuticals is izeed in some European research projects for preparing the new radiopharmaceuticals.

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^{99m}Tc-EDDA/HYNIC-TOC in gastroenteropancreatic neuro endocrine tumours

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Somatostatin receptors have been identified on most endocrine tumours, including carcinoid tumours, which generally express a high density of the receptors.

The aim of this work was to analize the ability in detecting GastroEnteroPancreatic neuroendocrine tumours (GEP NET) by somatostatin receptors with ^{99m}Tc-EDDA/HYNIC-TOCscintigraphy.

The conjugated peptide was labeled with ^{99m}Tc using a coligand exchange from tricine to EDDA. The radiochemical purity was higher than 95% measured by HPLC and ITLC.

We studied twenty-four patients (8 men, 16 women; age range, 16–80 y) with either histologically proven or biologically and clinically suspected GEP neuroendocrine tumours. They were derived to our Department to diagnosis and to determine the staging by somatostatin receptor scintigraphy.

Scintigraphy was performed at 3 h (planar and tomographic images) and 24 h (planar images) after injection of 740 MBq of ^{99m}Tc- HYNIC-TOC. We compare the ^{99m}Tc-HYNIC-TOC scintigraphy with the findings of CT, MRI, ultrasonography, and/or selective angiography. In some cases we performed a fusion images with MRI o CT for tumour localization.

Among the tumour location from 24 patients that we included 3 had gastrinoma (Zollinger-Ellison syndrome), 1 MEN I, 1 insulinoma, 3 foregut, 13 midgut and 3 hindgut carcinoids tumours.

^{99m}Tc- HYNIC-TOC showed a positive scan in 17 patients: two of them presented a primary tumour and went to surgery, the others 15 had metastatic disease. Seven present a true negative results. One case was a false-negative showed by the others methods of diagnosis CT, MRI and ^{111}In Octreotide scintigraphy.

This study revealed a higher sensitivity of ^{99m}Tc-HYNIC-TOC as a diagnostic procedure in GEP NET. The scintigraphy allows the localization and staging of tumours, and has benefit in identifying small primary tumours and in detecting metastases.

The other chapter of use of radiopharmaceuticals in a developing country

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Nuclear Medicine is a rapidly growing field of medicine throughout the world. Developing countries are also coming fast either with establishment of new nuclear medicine centers or expansion of its old facilities. However consistent use of radiopharmaceuticals are often hampered in a developing country due to various reasons.

Bangladesh is a developing country of Asia. The country has an old history of nuclear medicine, the first nuclear medicine center being established in as back as 1962. At present the country has got a total of 18 nuclear medicine centers, 15 in government sector and 3 in the private field. There are altogether 28 gamma cameras including some of them with SPECT facilities. Nearly 50,000 nuclear scans are done annually, thyroid being contributing as major share. Besides diagnostic scan radioisotopes like I-131and P-32 are used regularly for therapeutic purpose. A good amount of radiopharmaceuticals are also used in the in vitro laboratories. However the country mainly dependent on import for supply of radioisotopes and radiopharmaceuticals to these nuclear medicine centers. The country's only 3 MW reactor at Savar meet only 20% of the total demand.

Regular and constant supply of radioisotopes and radiopharmaceuticals is a big problem in giving smooth service of nuclear medicine. Often the supply is interrupted or delayed due to various factors. This causes patient suffering. At the same time the cost of the test also goes high. Sometimes the radiopharmaceuticals are not available even on demand. Needless to say some newer radiopharmaceuticals like Sm-153 EDTMP, Re-186 HEDP, Sr-89 chloride, I-131 MIBG, In-111 octreotide are so costly that we can hardly think of to use that. Use of PET tracers is still a dream to many developing countries including Bangladesh.

Constant and regular supply of radiopharmaceuticals is a pre condition of smooth running of a nuclear medicine center. A nuclear medicine center cannot develop until it has the access or capability of use of newer radiopharmaceuticals freely. We have seen developments of many new radiopharmaceuticals including PET tracers in recent years. Use of those radiopharmaceuticals in developed countries has opened a new era for nuclear medicine especially in the field of nuclear oncology. On the other side many developing countries are still fighting for regular supply of some age-old radiopharmaceuticals and radioisotopes for routine procedures in a nuclear medicine center. Time has come to consider this other side of use of radiopharmaceuticals in a developing country for homogeneous growth and development of nuclear medicine throughout the globe.

Historical review of radiopharmacy in Bolivia

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Nuclear Medicine was born in Bolivia 43 years ago, but not until five years later (1968) the radiopharmacy laboratory was implemented in La Paz, Bolivia.

We think that is very important to remark the International Cooperation in the growth of nuclear medicine and of course in the radiopharmacy area in our country. Everything started with the IAEA's cooperation with Grant's, Scientific Experts, Equipement, this allow to start with labelling of the first molecules, for example ^{113m}In -DTPA, PVP, in 1971.

Then in 1976 the use of ^{99}Mo/^{99m}Tc generators for labelling MDP, DTPA, DMSA GH, SC. The improvement of quality control metods and capacitation of more profetionals in the area let the Radiopharmacy grow more and more. Now a day we sinthesize our own kits for labelling with ^{99m}Tc.

But the Nuclear Medicine National Institute (INAMEN), do not stop here, we contributed to the knowdledge and growth of nuclear medicine in the hole country with academic programs and seminaries in the rest of the cities. At the present time in Bolivia are five cities with a Nuclear Medicine Services. (Fig. 1)

Actually we are looking forward to the growth of our Laboratory, we are in the IAEA's programmes to synthesize new radiopharmaceuticals with monoclonal antibodies and radipharmaceuticals for infection detection.

The future perspectives are principally in the field of research and sinthesize of diagnosis and terapeutical radiopharmaceuticals.

FIG.1. Countries with Nuclear Medicine Services.

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Preparation, characterization, and biodistribution study of technetium-99m labelled crotalus venom

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Technetium-99m (^{99m}Tc) is a radionuclide widely used for nuclear medical examination. Called the workhorse of nuclear medicine, ^{99m}Tc is the favoured choice because it has the appropriate physical and chemical characteristics for imaging. The gamma radiation emitted by ^{99m}Tc has the appropriate energy (140 keV) to provide high efficiency detection with the advantage of reduced radiation burden for the patient and environmental (half-life of 6 h). *Crotalus* venom (CV) has been tested in a very few human cases and it has been shown to reduce tumours. Our group has shown that CV has antitumoural effects against some brain tumours in vitro. Pharmacokinetics and tissue distribution studies are very important for clinical use. The aim of the present study was to obtain an analogue of CV labelled with ^{99m}Tc which preserves its biological activity for use in biodistribution and binding studies [1].

A direct method for ^{99m}Tc–labelling venom has been evaluated according to Pauwels, et al. [2]. The method employs stannous chloride and sodium borohydride as reducing agents. By altering the reaction conditions high yield of labelled venom was achieved. Labeling yield was estimated using chromatographic systems. Biological activity was assessed by haemolytic activity study. Comparing haemolytic activities of labelled and unlabeled venom we observed that neither ^{99m}Tc-CV nor CV caused direct lysis on washed erythrocytes. However, both of them caused indirect hemolysis provided that the incubation medium contained an exogenous source of lecithin. So, the biological activity of CV was preserved after labeling.

Biodistribution of ^{99m}Tc-CV was evaluated in mice. Male swiss mice were injected i.p. with ^{99m}Tc-CV, vital organs were isolated and their respective radioactivities were measured. High radioactivity was found in the kidneys suggesting renal excretion. On the other hand, this radioactivity was displaced in animals pre-treated with excess of non labelled CV (not shown). These data show that the uptake of ^{99m}Tc-CV is specific.

In this paper we reported the radiolabelling of CV with ^{99m}Tc and showed that ^{99m}Tc-CV kept its biological activity. Our results showed that ^{99m}Tc-CV can be a useful tool for biodistribution and binding studies.

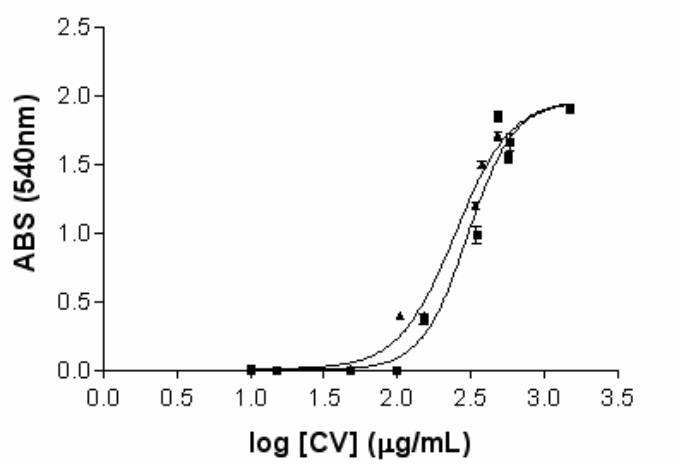

FIG. 1. ^{99m}Tc-CV kept its hemolytic activity. (■) Curve dose-response of the indirect hemolytic (phospholipasic activity) of crude venom (IC_{50} = 301.5 ± 73 µg/mL) and (▲) ^{99m}Tc-CV (IC_{50} = 247.7 ± 49 µg/mL).

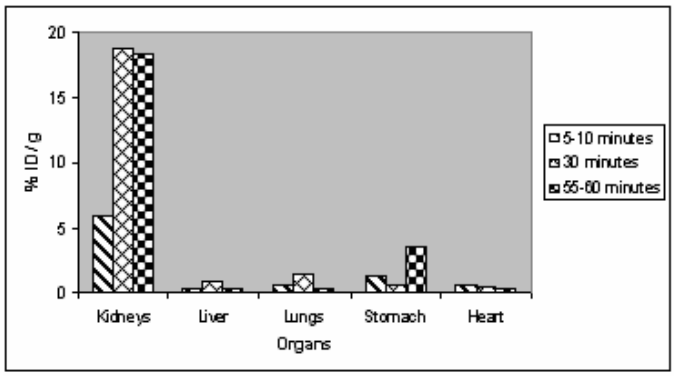

FIG. 2. Biodistribution of ^{99m}Tc-CV in male Swiss mice (22–30g). Animals were injected i.v. with ^{99m}Tc-CV (0,1MBq). CV showed high kidney uptake. The biodistribution pattern of ^{99m}Tc-CV was different compared with colloid (TcO_2) (not shown).

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Labelling of thymidine with ^{99m}Tc-carbonyl and in vivo biodistribution studies

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Labeled thymidine is used for tumour imaging, since it is incorporated into DNA and therefore provides a measure of cell proliferation. It is reported that high human thymidine kinase levels occur in breast carcinomas, in lung cancer and other proliferating and malignant cells. For the current study thymidine was functionalized at the C5'position of the sugar moiety with the tridentate iminodiacetic acid chelator for complexation and radiolabeling with ^{99m}Tc(I)-tricarbonyl core. The aim of this study was the labeling of thymidine analogue with ^{99m}Tc-tricarbonyl technique, and the exploration of its potential as radiopharmaceutical in vivo.

The preparation of the ^{99m}Tc-precursor consisted in flushing the mixture of 4.4 mg of sodium carbonate, 5.5 mg of sodium borohydride and 20 mg of sodium-potassium tartrate tetrahydrate with CO gas during 30 min. Pertechnetate was added and the vial was heated for 35 min at 75 ºC. The reaction was stopped in ice bath, and pH was adjusted to 7. Then 50 μL of the precursor was added to 10-4 M of the ligand and heated again for 50 min at 75 ºC. Radichemical purity of the precursor and the product was checked by HPLC, TLC and paper chromatography. Biodistribution studies were performed in normal Swiss mice at 1, 4 and 24 h post injection of the drug and also in breast-cancer-bearing Sprague-Dawley rats (induced by dimethylbenzaanthracene), 1h after administration of the drug.

Results: Yield of the ^{99m}Tc(CO)3-thymidine complex was 98.3 ± 0.8%. Radiochemical purity of the product was 97.3 ± 0.4%. Biodistribution studies showed the highest uptake by intestine, followed by liver and kidneys. A low amount of radioactivity was observed in the blood 1 hour post-injection (0.1 %ID/mL). Biodistribution studies in tumour-bearing rats showed a higher uptake in the kidney than in the liver, probably because these animals were anesthetized. In these animals it was observed that 0.32 ± 0.05 % ID/g remained in the blood 1 hour post-injection. Tumour/blood and tumour/muscle ratios were 0.56 and 1.38, respectively. Uptake by the tumour was 0.18 ± 0.2 %ID/g.

Preparation of the ^{99m}Tc-precursor and labeling of thymidine were achieved with very high yield. Uptake by the breast-tumour-bearing rats was low. Other tumour models and labeling techniques should be used to permit better results.

Management and supervision of radiopharmaceuticals in China

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Nuclear technology has been widely applied in China, in which radiopharmaceuticals is a very important field. Chinese government emphasizes radiation, medical and biological safety of radiopharmaceuticals on human while promoting its application for public health. China has established full government infrastructure and corresponding legal system for safety of radiopharmaceuticals. State Food and Drug Administration (SFDA), China Atomic Energy Authority(CAEA), Ministry of Health (MOH), State Environment Protection Administration (SEPA) and other related departments have made great endeavor to ensure the safety of radiopharmaceuticals in all stages.

Main roles and responsibilities

SFDA is main safety regulatory body in this field in China, its responsibilities are, as follows:

- To draft law and administrative regulations on drug administration and supervise their enforcement.
- To draft law and regulations on administration of medical devices and supervise their enforcement; take charge of registration and regulation of medical devices; draft relevant national standards, draw up and revise professional standards of medical devices, manufacturing practice and supervise their implementation.
- To be in charge of drug registration, draw up, revise and promulgate national standard of drugs; be responsible for drug reevaluation, review drugs to be withdrawn.
- To draft and revise good practices for drug research, manufacturing, distribution and use, and supervise their implementation.
- To control the quality of drugs and medical devices in manufacturers, distributors and medical institutions; release national quality bulletin on drugs and medical devices on a regular basis; investigate and punish illegal activities of producing and selling counterfeit and inferior drugs and medical devices in accordance with law.
- To regulate radiopharmaceuticals and devices in accordance with law.
- To draw up and improve qualification system for licensed pharmacist, supervise and direct the registration of licensed pharmacist.

CAEA is the competent authority of nuclear industry in China, The responsibilities of CAEA in this field are, as follows:

- To deliberate and draw up policies and regulations on peaceful use of nuclear energy.
- To deliberate and draw up the development program, plan and industrial standards for peaceful use of nuclear energy.

– To organize argumentation and give approval to China's major nuclear R&D projects; supervise and coordinate the implementation of the major nuclear R&D projects.

– To deal with the exchange and co-operation in governments and international organizations, and take part in IAEA and its activities in the name of the Chinese government.

– To participate in regulation of radiopharmaceuticals.

MOH is responsible for formulating hygienic rules and standards related to personnel of related facilities and general public, reviewing and approving the evaluation of the health effects on human body due to nuclear contamination, prevention and cure of radiation injury, participating in emergency response activities.

SEPA is responsible for radiation safety surveillance of radiopharmaceuticals application; monitoring of radiological environment of its manufacture, distribution and use; investigation and treatment of radiation accidents in this field.

Legislation and regulation

According to the experience combined with the newest domestic and international requirements, China continually perfects its laws and regulations in this field. The system of laws, regulations and guides consists of state laws, administrative regulations of the State Council, department rules, guiding documents and reference documents.

Related national laws:

– Drug Administration Law of the People's Republic of China(1984)
– Act of Protection and Remedy of Radioactive Contamination of the People's Republic of China (2003)
– Atomic Energy Act (being legislated), etc.

Related regulation:

– Regulation for Implementation of Drug Administration Law (2002)
– Regulation for the Supervision and Administration of Medical Devices (2000)
– Administration regulation of radiopharmaceuticals (1989)
– Regulation on Radiation Protection of Radioactive Isotope and Radiation Installation(1989, being revised), etc.

Management and supervision practice

The government supports R&D of radiopharmaceuticals, especially for new products, processing and techniques, conducts review and approval of its research and clinical trial, manages its registration, adopts a licensing system for its manufacturing, distributing and using, exercises review and approval for GMP of its manufacturer, conducts review and approval of its import and export, and supervises their implementation.

We propagandize the basic knowledge and information about radiopharmaceuticals to the public, require licensees to organize special training for related workers and some important

professional shall get qualification from government, and do some inspection regularly. we sponsor symposium to promote exchange in this field.

The Chinese government greatly emphasizes the IAEA's positive role in promoting international cooperation in the peaceful uses of nuclear technology and safety management of radiopharmaceuticals. We will stick to support the IAEA's efforts in this area, sincerely hope to keep expanding cooperation and exchanges with other countries in this field, and continuously make joint efforts to widen its application effectively and to benefit public health.

Internal dose assessment of ^{99m}Tc-HTOC

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Octreotide is a synthetic octapeptide analog of somatostatin. Its major effects inhibit the release of pituitary growth hormone and the endocrine secretions of the pancreas, stomach, and intestine. So it is recommended to control symptoms associated with neuroendocrine tumours.

In therapy, it is necessary to estimate patient-specific absorbed dose, especially to dose-limiting risk organs and to the tumour tissue. Kinetic analyses need to be carefully planned, meanwhile absorbed fraction are most similar to the subject in question should be chosen. However, the result of dose assessment is not sufficiently accurate or detailed to guide clinical decision-making, and not well correlated with observed effects on patient's organs and tumours.

In this study, ^{99m}Tc-HTOC is an excellent indicator that displays the distribution of ^{188}Re-HTOC in patient's body. The kinetic information of $^{99}Tc^{m}$ -HTOC in patient's body can be obtained by SPECT in 1,4 h after injection. The activity in patient's tumours and organs can be calculated by lined the time-activity curve. In the study, the data of 86 cases can be collected. Although the basic formula of dose assessment is based on that of the medical internal radiation dose committee (MIRD), the absorbed fractions have been adjusted to be more patient-specific by patient's CT image and other data. It make internal dose of patients more accurate by the method.

Evaluation of low and high molecular weight ^{99m}Tc-radiopharmaceuticals as a radiotracer for the diagnosis of inflammatory process

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The purpose of this work was the comparison of four ^{99m}Tc-radiopharmaceuticals for visualization of aseptic inflammation and infection process [1,2]. The compounds were divided in two groups according to their molecular weights and nature. The first group included two immunoglobulins, the ^{99m}Tc-human-IgG and ^{99m}Tc-ior t3 murine-MoAb, biomolecules of high molecular weight (MW- 150 kDa) and complex structure. The second group included two compounds of relative simple structure and low molecular weights(MW< 600 Da), the ^{99m}Tc-Ciprofloxacine and the ^{99m}Tc (V)-DMSA [3].

Radiolabeling of immunoglobulins was made by a direct method. The reduction of disulfide bridges was developed with sodium metabisulphite (SMB), which was utilized for first time in the reduction of a MoAb, and the classic 2-mercaptoethanol, whose the main disadvantage is the purification step after the reduction. These reducing agents produced a similar amount of free thiols in both molecules of IgG, the values varied around 21.0-27.0 %. The results obtained with the SMB demonstrate its possibility as a reducing agent for the direct radiolabeling of immunoglobulins with ^{99m}Tc.

The radiopharmaceuticals were submitted to different quality controls and biodistribution studies in two models, the first represents a septic inflammation produced by E. Coli and the second to an aseptic inflammation induced by sterile carrageenan.

The four radiopharmaceuticals were obtained with high radiolabeling efficiency (>90.0%) and strong binding of ^{99m}Tc-Ligand. The reduction process produced fragments which were detected by gel electrophoresis and were confirmed by biodistribution studies. Also aggregates were obtained which were not detected by "in vitro" tests, but were corroborated by "in vivo" studies.

The behaviour of low molecular weight radiopharmaceuticals, showed a high binding to plasma proteins and low binding to the E. Coli. However, the high molecular weight radiopharmaceuticals showed a higher binding to the E. Coli.

The immunoglobulins showed high incorporation in inflammation focus by "in vivo" studies. The higher results was obtained with 2-mercaptoethanol as a reducing agent. The main disadvantage of the immunoglobulins is that they do not discriminate between the aseptic and septic inflammatory process (Figs. 1, 2). The low molecular weight compounds showed a lower uptake in the inflammatory process in comparison to the high molecular weights. The

^{99m}Tc-ciprofloxacine was unable to distinguish between aseptic and septic inflammatory process, and the 99mTc (V) – DMSA produced a difference between these two inflammatory process, being of these four different radiopharmaceuticals the only one which may discriminate aseptic from septic process.

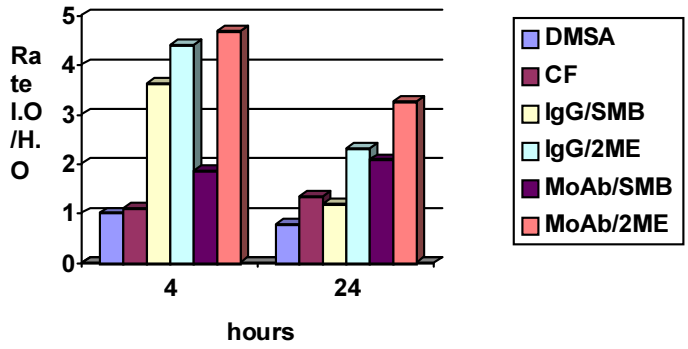

FIG.1. Rate inflamed organs/ healthy organs in aseptic inflammation.

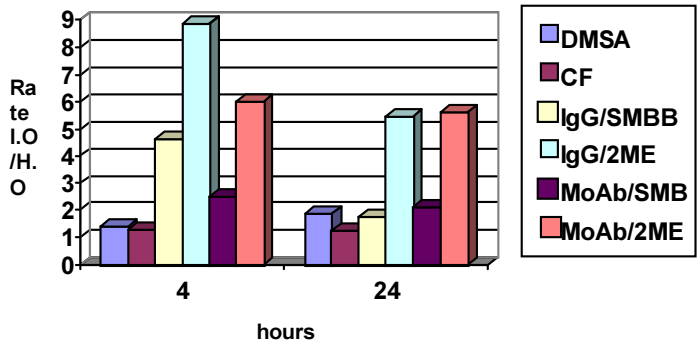

FIG.2. Rate infected organs/ healthy organs in septic inflammation.

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Colloidal bismuth subcitrate, a new agent for the scintigraphy of inflammatory bowel diseases

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A correct diagnosis and adequate assessment of disease activity is crucial in the management and surveillance of inflammatory bowel diseases (IBDs). However, in certain occasions the obtained results by traditional techniques are not correlated specifically with the mucosal wall inflammatory state, mainly in case of the small bowel, due to its no accessibility. On the order hand, some of them such as the endoscopy and the histopatological studies have the inconvenience of being bloody and traumatic for the patient [1].

Radioactive isotopes techniques have been used with advantageous in relationship with conventional ones. They are able to detect inflammations along the whole gastrointestinal tract; radiation level is low and tolerated by extreme care patient and children. One of these techniques relates the inflamed state of the mucous with the ^{111}In-leukocytes uptake level of the injured area [2].

Another radioisotopic technique developed by Vázquez et al. [3], consists on detecting the gastric lesion by means of the use of a local action anti ulcerous agent labelled with Tc-99m. This one is simple, cheap and its results are similar to those of conventional techniques.

Taking into account that the inflammatory intestinal illnesses develop inflamed and ulcerated areas in the mucous and that Colloidal Bismuth Subcitrate (CBS) $K_{55}(NH_4)_{36}(C_6H_7O_7)_{22}[Bi_{30}(OH)_{55}(C_6H_5O_7)_{20}]$ possesses the capacity to form with the mucus a complex that adheres on ulcerated mucous through a reaction with the detritus derivate from the tissue necrosis [3]. The present work has been proposed as a method to assess disease activity in patients with inflammatory diseases along the entire alimentary tract.

This investigation constituted a multicentric clinical trial phase I-II. Forty-four patients were prospectively evaluated with the objective of determining the validity of the scintigraphy with ^{99m}Tc-Sn-CBS for the diagnosis of illnesses with ulcerations of the intestinal tube. Of the evaluated patients, 20 presented Cohn's Disease (in 1 of them the scintigraphy was not useful), 1 Idiopatic Ulcerative Colitis, 3 gastric ulcer and 20 patients were seemingly healthy, conformed the control group.

Five hundred mgs of CBS were labelled with 370 MBq (10 mCi) of ^{99m}Tc and administered via oral as unique dose. The formulation of the freeze-dried kit was previously adjusted in pre-clinical studies, where ph and the NH_4^+ ions played an important role in the complex stability [4].

The diagnostic test had three days duration for each patient: first day for the patient's preparation with soft diet and mannitol 20%, in order to clean intestine and the next two days for the drug administration and image registrations. The images were carried out at 2, 4, 6 and 24 h post administered the product. Fig. 1 shows a) Patient 10, control group, where no defined uptake is observed and b) Patient 42 with Cohn's Disease more than 5 year evolution, several gastric and small bowel lesions including terminal ileum are observed.

All procedures were performed by experienced examiners, who were blinded to the clinical data and other results. It was determined the sensibility and specificity of this technique taking colonoscopy, gastroduodenoscopy, intestinal transit and/or biopsy as reference test, achieving a 91,3% sensibility and 90% specificity. The EPIDAT test (version 3.0) was used for statistical analysis. During the whole study an adverse event appeared, which had not probable causation with drug administration according to Karch and Lasagna scale .

It can be concluded that the diagnostic criteria established in this study can be useful for the evaluation of inflammatory bowel diseases by scintigraphy with ^{99m}Tc-Sn-CBS.

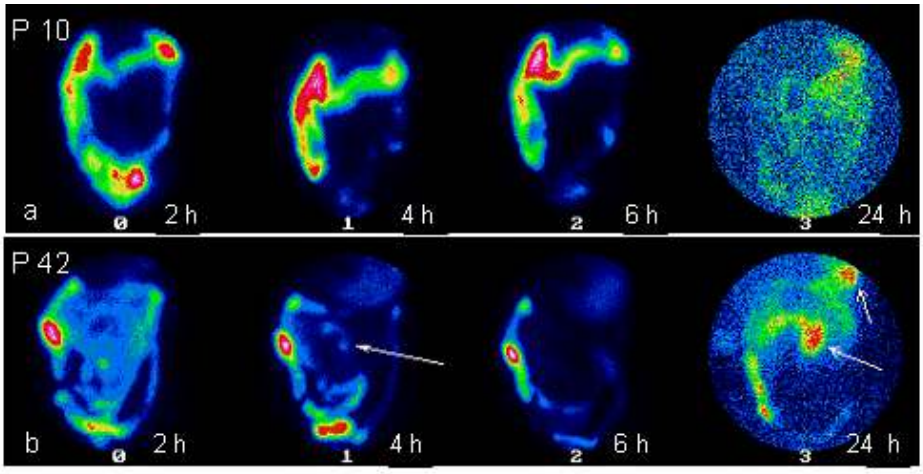

FIG: 1. Images at 2, 4, 6, and 24 h post administration of ^{99m}Tc-CBS a) Patient No. 10 of control group, b) Patient No. 42 of over5 year evolution Crohn's Disease, where lesions in the stomach and smal bowel are observed.

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Use of stannous fluoride (II) like reducer agent for ^{99m}Tc-labelling ciprofloxacin. Animal pharmacokinetic and biodistribution in animals

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The present work is based on ^{99m}Tc-labelling of ciprofloxacin using stannous fluoride (SnF_2) like a reducer agent and to evaluate the biological activity, pharmacokinetic and biodistribution of the radiopharmaceutical obtained. Stannous (II) chloride ($SnCl_2$) **(1)**, formamidine sulfinic acid (FSA) as an alternative to stannous chloride **(2)** and redox polymers **(3)** were used like reducers agents of pertechnetate but SnF_2 is an advantageous agent due it high stability.

Five to sixty micrograms (μg) of SnF_2 and 2 milligrams (mg) of ciprofloxacin lactate were mixed in a vacuum vial in 1 milliliter (ml) of NaCl 0.9%. Freshly eluted sodium pertechnetate solution, 370 megabecquerel (MBq), were added and incubated for 10 minutes (min) into boiling water. The pH of lactate ciprofloxacin commercial solution doesn't was change. After purification through sephadex G50 column, UV-visible spectrum was made to the fraction of larger activity. Quality control and in-vitro stability study of labelled up to 6 h (h) using tin layer chromatography (TLC-SG) and Watman 3 was carrier out.

Six male wistar rats were divided into two groups to made abscess model: Three were inoculated with Staphylococcus aureus (ATCC 6538) and the others with Escherichia coli (ATCC 10536). After 18–20 h, 666-740 MBq/ kilograms (MBq/kg) of ^{99m}Tc-ciprofloxacin were administered. Planar scintigraphic images were acquired at 1, 2, 4 and 18 h using a gamma camera. Abscess to counter-lateral thigh (A/C) ratio of activity was calculated.

Pharmacokinetic study in blood, serum and plasma was performed in 30 wistar rats males, separated in 10 groups. They took samples of blood at different times intervals up to 24 h after injection of 666–740 MBq/Kg of ^{99m}Tc-ciprofloxacin. A model of two compartments was assumed. The calculated pharmacokinetic parameters were volume apparent of distribution (Vd) and half time of clearing of the distribution and elimination phase's of the blood depuration curve.

Organ biodistribution study was carried out in 24 male healthy wistar rats divided into 8 groups. The animals were killed at different times intervals up to 24 h after injection of 666 - 740 MBq/Kg of ^{99m}Tc-ciprofloxacin. Was measured the counts per minute (cpm) in liver, spleen, kidneys, lungs, heart, muscles, long intestine, small intestine, bone (femur), knee and urine. The results were expressed as the percent of the injected dose per gram of tissue (%DI/g). All the values were expressed in mean value ± sd.

The higher radiochemical purity of radiopharmaceutical was attained in a range from 17 to 23 μg of reducing agent (97.85 ± 0.34%), Fig. 1. The stability of ^{99m}Tc-labelled-ciprofloxacin

was >90% up to 6 h (92.00± 0.73%). The use of a commercial lactate ciprofloxacin solution for injection represents a considerable advantage, since there is not need to modify its pH.

The UV-visible spectrum obtained of the maximal ^{99m}Tc-ciprofloxacin-concentration fraction was quallitatly indent to the control.

Significant values of A/C thigh ratios determined for Escherichia coli and Staphylococcus aureus modeles were found at 1, 2, 4 and 20 h. The A/C thigh ratio was rising up to 4.21±1.13 and 5.00±0.29 at 20 h for Escherichia coli and Staphylococcus aureus, respectively. The presence of life bacterial at the lesion was confirmed at all animals. The kinetic in blood after injection of radiopharmaceutical showed a fast plasmatic clearing, following a kinetic biexponential, typical of a two compartments model, you can appreciate that significant differences didn't exist in the $T_{1/2}$ of incorporation phase in the three types of samples (blood total, serum and plasma) ($p>0.05$). However the $T_{1/2}$ of elimination phase in blood total was twice as much that in serum and plasm, where $p<0.05$ compared with each sample type. The distribution volume was relatively large for (38 ml). Marked accumulation in kidneys and bowels the all time it was observed. For renal way 41±1% of the dose was eliminated.

^{99m}Tc-labelling ciprofloxacin method using SnF_2 like reducer of pertechnetate it provide us a radiopharmaceutical biologically effective, stable, economic and perfectly reproducible.

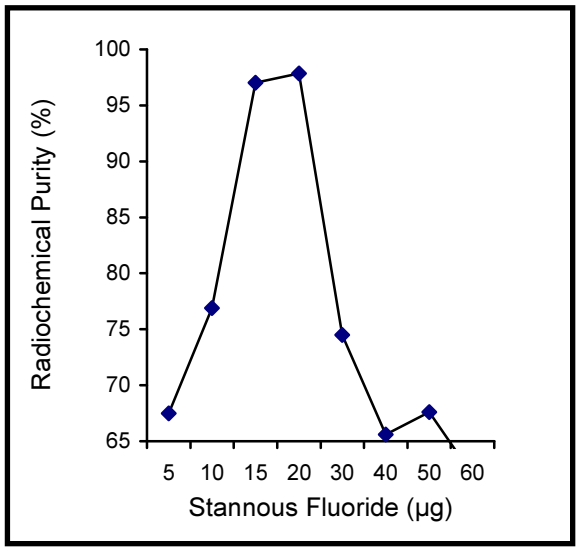

FIG. 1. Yield of ^{99m}Tc-ciprofloxacin as a function of the amount of stannous fluoride.

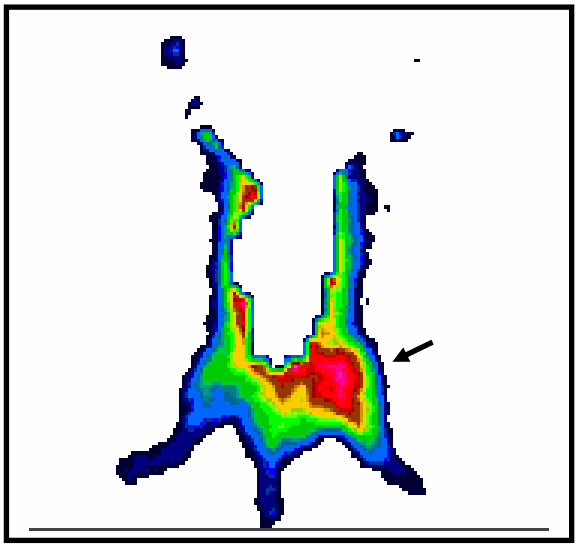

FIG. 2. Scintigrahic image acquired at 1 h post-administration ^{99m}Tc-ciprofloxacin to a rat bearing an abscess in the thigh by Staphilococcus aureus. Arrow shows the uptake of ^{99m}Tc-ciprofloxacin in the bacterial infection site.

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Differential chemical behaviour of humanized monoclonal antibodies radiolabelled with ^{99m}Tc

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In the radiopharmaceutical field the substitution of murine monoclonal antibodies (m-mAbs) by humanized monoclonal antibodies (h-mAbs) is one of the main works in our days. The use of h-mAbs involves many advantages, because the HAMA response is one of the principal drawbacks in the use of m-mAbs. However, the transference of the procedures from the m-MoAbs to the h-MoAbs is a cumbersome task, because these have a differential behaviour once reduced and radiolabeled with ^{99m}Tc.

In Cuba the biotechnological industry has developed a great diversity of monoclonal antibodies, including new humanized antibodies. The use of these in therapy and diagnosis is very attractive.

The procedures for radiolabeling the m-mAbs have been established and these are used in Cuba [1], but the radiolabeling of h-mAbs is a recent topic for our radiopharmacists.

This work develops a simultaneous comparison of immunoglobulins from different origin. For this purpose the human-IgG, two h-MoAbs and a m-MoAb were employed. Our goal consists to demonstrate that there are differences in the behaviour of these immunoglobulins after their reduction with sodium metabisulfite (SMB) [2] and radiolabeling with ^{99m}Tc. The four immunoglobulins were the human-IgG, the R3 and T1 humanized monoclonal antibodies (R3 and T1 h-mAbs), and the ior t3 murine monoclonal antibody (ior t3 m-mAb).

The formation of fragments and aggregates, and the immunoreactivity of the monoclonal antibodies were studied once concluded the reduction process. Also, comparative studies of the radiochemical stability and challenges were carried out after radiolabeled. Furthermore four auxiliary ligands were compared in the radiolabelling of the h-IgG.

The comparison of four ligands, showed a differential behaviour in the radiolabeling process. The tartrate and gluconate were the most suitable for the human-IgG.

In the comparison, of the chemical behaviour of the h-IgG vs the humanized and murine monoclonal antibodies radiolabeled with ^{99m}Tc. The results showed differences in the collected data of these h-mAbs after the radiolabeling, the highest radiochemical purities were obtained with the human-IgG and m-mAb in comparison with the h-mAbs, also we observed a small difference between the h-mAbs which was confirmed statistically. However, for all immunoglobulins the radiochemical purity was higher to 90.0 % and the amount of ^{99m}Tc-radiocolloid was lower than 5.0%.

The challenges showed the lowest dissociation for the ^{99m}Tc-h-IgG in comparison to the rest. The h-mAbs presented a differential behaviour, because the R3 h-mAb had lower dissociation in relation to the T1 h-mAb. These h-mAbs showed furthermore a higher dissociation compared to the m-mAb, this difference was confirmed statistically.

The PAGE study showed a lower generation of fragments for the human IgG and the m-mAb. The humanized mAbs presented a higher generation and the T1 h-mAb showed more fragments in relation to the R3 h-mAb.

There were not detected a molecular aggregates of the immunoglobulins by HPLC studies. However, the profile of the T1 h-mAb confirmed the results obtained in the fragmentation study, where appeared fragments of this MoAb. Ellman assay showed a significative difference among h-MoAbs and the rest of the inmunoglobulins. The immunoreactivity study showed the preservation of biological activity for all MoAbs.

The results indicated differences in the chemical behaviour of the immunoglobulins after the reduction process. The percentage of fragmentation and generation of free sulphydryls groups for the evaluated immunoglobulins, demonstrated that the humanized MoAbs were more sensitive to the reduction process; this fact influenced without doubt their behaviour in the radiolabeling with ^{99m}Tc.

After the radiolabeling, the humanized MoAbs showed a differential behaviour, which was consistent with the results of the pre-labeling evaluation, and demonstrated that the structural modification influenced in a determinant manner in their chemical and radiochemical behaviour.

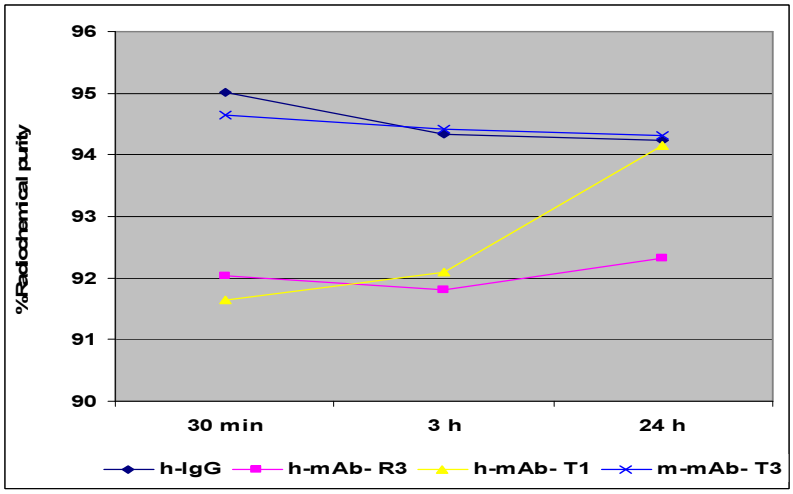

FIG. 1. Immunoglobulin comparison.

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The effect of certain labelling parameters on the ^{99m}Tc-HMPAO complex formation - A chromatographic study

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Technetium-99m complex of hexamethyl propylene amineoxime (HMPAO) (which is called also exametazime) in addition to its using in leukocytes labeling techniques is widely used as an efficient brain perfusion agent due to the ability of the primary ^{99m}Tc-HMPAO complex to penetrate the intact blood-brain barrier (BBB) which is believed to be attributed to its lipophilic property [1].

The present work is based on the assessment of the effect of different factors on the radiolabelling efficiency of (HMPAO) in a trial to evaluate the radiochemical purity concept. The radiochemical purity of the ^{99m}Tc-HMPAO preparation could be recognized by using a combination of three thin layer chromatographic (TLC) systems [2].

These factors include:

1. Age of sodium pertechnetate
2. Source of sodium pertechnetate
3. Order of generator elution
4. Activity added to the kit
5. Specific concentration (activity per volume)
6. Intra-lot variability.

and these chromatographic systems are:

1. ITLC-SG / saline system (Nacl)
2. ITLC-SG / Methyl-ethyl keton
3. Whatman no.1 / 50% Acetonitrile: 50% H2OE.

Our conclusion could be summarized in Table I.

TABLE I. THE MOLECULAR PARAMETERS AFFECTING THE RADIOCHEMICAL PURITY OF ^{99m}Tc-HMPAO COMPLEX

Parameters		Radiochemical purity of			
		Primary-HMPAO	Secondary-HMPAO	$^{99m}TcO^-_4$	$^{99m}TcO^-_2$
Age of sodiume pertechnetate	1-5 h.	Not affected	Not affected	Not affected	Not affected
	> 5 h	Decreased	Not affected	Increased	Not affected
Source of sodium pertechnetate	Elutec & Amertec	Not affected	Not affected	Not affected	Not affected
Order of generator elution	1^{st}-11^{th} day	Not affected	Not affected	Not affected	Not affected
Activity added to the kit	20-50 mCi	Not affected	Not affected	Not affected	Not affected
	>50 mCi	Decreased	Increased	Increased	Increased
Specific concentration	5-10 mCi/ml	Not affected	Not affected	Not affected	Not affected
	>10 mCi/ml	Slightly decreased	Highly increased	Slightly increased	Slightly increased
Intra-lot variation	Lot no. 1-3	Not affected	Not affected	Not affected	Not affected

All of the previous tests was done with the following standard values:

* Age of sodium pertechnetate ≡ 1 h
* Order of generator elution ≡ 5^{th} day
* Source of sodium pertechnetate ≡ elutec generator.
* Activity added to the kit ≡ 40 mCi
* Specific concentration ≡ 20mCi/ml.
* Lot of HMPAO kit ≡ Lot no 2.

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A convenient synthetic procedure yielding 2-picolinamino-N,N-diacetic acid monoamide derivatives, for labelling with the fac-M(CO)3 +core

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Recently, the introduction of the low valent *fac*-$[M(CO)_3(H_2O)]^+$ synthons (M=Tc or Re) offered a new impetus in the development of diagnostic ^{99m}Tc(I) and therapeutic $^{186/188}$Re(I) radiopharmaceuticals. The aqua ligands of the *fac*-$[M(CO)_3(H_2O)]^+$ cation are labile and readily substituted by a variety of functional groups including amines, thioethers, imines, thiols, and phosphines [2]. Furthermore, the small size and the chemical inertness of the *fac*-$[M(CO)_3]^+$ core provide a convenient platform for the development of efficient radiopharmaceuticals.

Previous studies on the coordination chemistry of the *fac*-$[M(CO)_3]^+$ core suggested that an ideal bifunctional chelating system should be tridentate because it forms complexes with more favourable pharmacokinetics compared to a bidentate one. Picollinamino N,N diacetic acid (PADA), **1,** acts as tridentate NNO ligand and coordinates efficiently with the organometallic core at very low concentrations leaving a carboxylic group out of the coordination sphere of the metal. This carboxylic group can serve as an attachment site for functionalization with biologically active molecule rendering specificity to the overall complex. [3].

In this investigation we report a convenient, one-pot synthesis of a wide variety of monoamide derivatives of PADA, a simple method for tethering of a bioactive molecule that contains an amino group to the PADA ligand. The synthetic scheme includes the preparation of the anhydride of PADA, 2, and its subsequent reactions with the appropriate amines for the formation of the corresponding ligands, 3a–3c**,** (Fig. 1). Pyrrolidine and aniline were used as model compounds leading to the formation of ligands of 3a and 3b. Subsequently, 2-methoxyphenylpiperazine, which is a fragment of the true 5-HT_{1A} antagonist WAY-100635 and displays affinity for the 5-HT_{1A} receptors, reacted with the anhydride of PADA leading to the formation of 3c.

These ligands were reacted successfully with the *fac*-$M(CO)_3$ cores. The rhenium complexes 4a-c (M=Re) were prepared first by ligand exchange reactions using $[Et_4N][Re(CO)_3Br_3]$ as precursor. All complexes were characterized by elemental analysis and spectroscopic methods. Complex 4**a** was further characterized by X ray crystallography. The analogues Tc-99m complexes 5a-c were prepared by ligand exchange reactions using $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as precursor. The identity of the ^{99m}Tc complexes was established by comparative HPLC analysis using the well characterized rhenium 4a-c complexes as reference. All technetium complexes were stable in vitro for 6 h.

For biodistribution studies, the radiolabelled complex 5c was administered in Swiss Albino mice. Before injection, 5c was purified by HPLC to remove the excess of unlabelled cold ligand. The complex exhibits rapid blood clearance with the activity eliminating through the hepatobiliary system. Only a very small fraction of the injected dose (0.10 % ID at 2 min p.i.) crosses the brain blood barrier.

In conclusion, this work describes the easy one step synthesis of a bifunctional chelating agent, 3, which carries the efficient NNO chelating system and, as expected, leads to highly stable technetium and rhenium complexes. The application of this agent for the synthesis of a Tc-99m complex, 5c, carrying a target specific group for imaging of 5-HT_{1A} receptors is also reported. This method may be further applied for the attachment of drugs or small bioactive peptides into the PADA moiety leading to bifunctional chelating agents for the development of target-specific diagnostic or therapeutic radiopharmaceuticals.

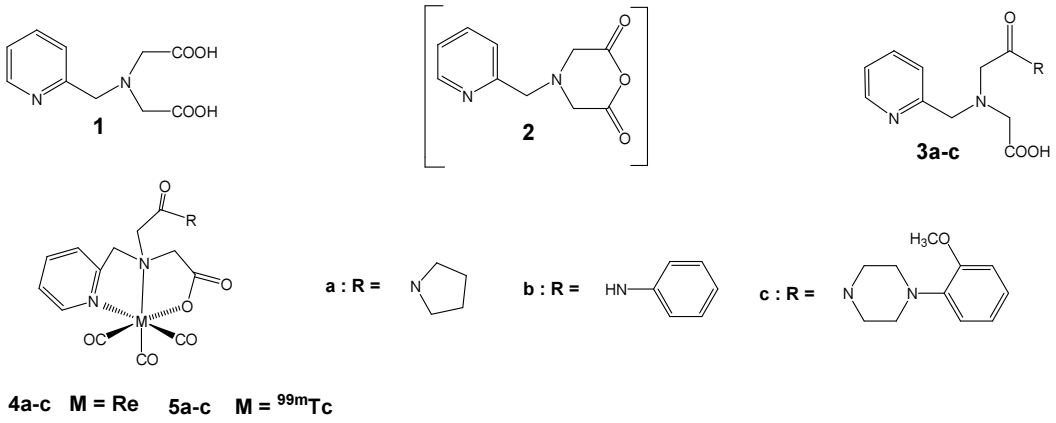

FIG. 1. Structures of the ligand and complexes synthesized.

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S-(2-(2'-pyridy)ethyl)mercaptoacetic acid and S-(2-(2'-pyridy)ethyl) mercaptopropionic as ligands for the "fac-[M(CO)3]+" core (M = Re, ^{99m}Tc)

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The introduction of the low valent $[M(CO)_3(H_2O)_3]^+$ (M = Tc or Re) synthons gave a new impetus in the development of diagnostic ^{99m}Tc(I) and therapeutic $^{186/188}$Re(I) radiopharmaceuticals. The aqua ligands of the $[M(CO)_3(H_2O)_3]^+$ cation are labile and readily substituted by a variety of functional groups. Furthermore, the small size of the *fac*-$[M(CO)_3]^+$ core provides a convenient platform for the development of efficient radiopharmaceuticals. Several chelating agents which allow the stabilization of the metallic center and attachment to relevant biomolecules have been explored [1]. However, further chelating agents are still needed in order to prepare complexes with different physicochemical properties that will improve their *in vivo* stability and pharmakokinetcs.

In the present study we describe the products of the reaction of $[NEt_4]_2[Re(CO)_3Br_3]$ and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursors with the L_1 and L_2 ligands (Fig. 1). Both ligands have been prepared by pyridinethylation [2] of the corresponding thiol (mercaptoacetic acid for L_1 and 3-mercaptopropionic acid for L_2) and characterized by spectroscopic methods.

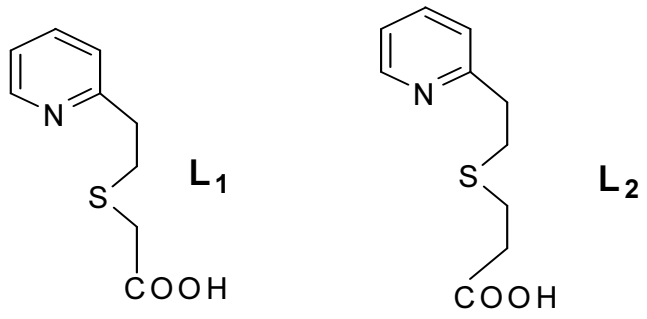

FIG. 1. The structures of the ligands L_1 and L_2.

The reaction of $[NEt_4]_2[Re(CO)_3Br_3]$ with the NSO tridentate L_1 in methanol, in the presence of NaOH, leads to the formation of the expected $[Re(CO)_3(NSO)]$ complex, **1** (Fig. 2). X ray

analysis shows that the nitrogen of the aromatic amine, the sulphur of the thioether and the oxygen are facially coordinated to the metal forming one five membered and one six-membered ring. At technetium-99m level, the corresponding complex *fac*-[$^{99m}Tc(CO)_3(NSO)$], **1´**, was obtained almost quantitatively (>95%) by heating the *fac*-[$^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor at 70 ^oC in water and in the presence of a 10^{-3} M concentration of L_1. In the case of rhenium, when the reaction is carried out in the absence of NaOH, the formation of the same complex, as the main product, was detected by HPLC.

The reaction of $[NEt_4]_2[Re(CO)_3Br_3]$ with the ligand L_2 in methanol, in the presence of NaOH, leads to the formation of the $[Re(CO)_3(NSO)]$ complex, **2**, practically of the same structure as complex **1**. However, in the absence of NaOH, the reaction leads to the formation of the $[Re(CO)_3(NS)Br]$ complex, **3**, that is the major product of the reaction as shown by HPLC (85%). X ray analysis of complex **3** shows that the L_2 ligand acts as bidentate. The nitrogen of the aromatic amine, the sulphur of the thioether and a bromine atom are facially coordinated to the metal forming one six-membered ring. The carboxylic acid remains uncoordinated. Complex **2** is also formed in a small amount (15%).

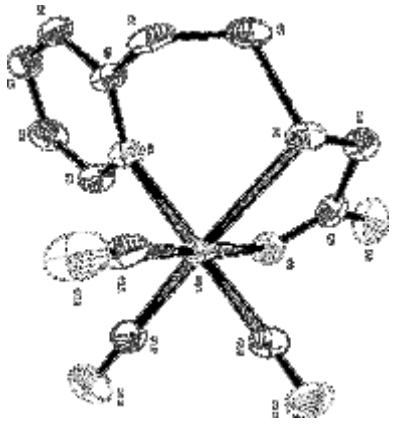

*FIG. 2. Molecular structure of **1** with the atomic labeling.*

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Complexes of 4-(aminophenyl) benzothiazole derivatives with technetium and rhenium, and assessment of their properties as diagnostic and/or therapeutic radiopharmaceuticals

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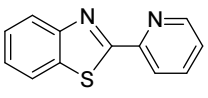
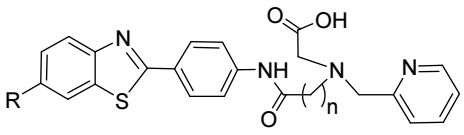
4-(Aminophenyl)benzothiazole derivatives have promising qualities for radiopharmaceutical chemistry, since they display both anticancer activity [1] and affinity for amyloid plaques [2] of Alzheimer's disease (AD). This chemical class is therefore an important candidate for the development of technetium (Tc) and rhenium (Re) radiopharmaceuticals for tumour imaging and/or radiotherapy, as well as, *in vivo* diagnosis of AD.

In view of the interesting properties of the 4-(aminophenyl)benzothiazole derivatives and our long involvement in the chemistry of Tc and Re, we proceeded in the design and synthesis of new 4-(aminophenyl)benzothiazole ligands **1-5** (Fig. 1) properly modified for chelation with the $M(I)^{+}$ and/or $MO(V)^{3+}$ (M = Tc, Re) metal cores and the formation of stable complexes.



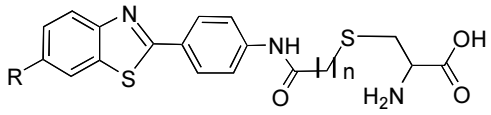
1



	R
2	H
3	CH_3

n=1, 4, 5

2, 3



	R
4	H
5	CH_3

n=1, 4, 5

4, 5

FIG. 1. Structures of the ligands.

Ligand **1** is designed for direct attachement to the MO^{3+} core through the heterocyclic nitrogens. In ligands **2**–**3** which are designed according to the conjugate approach, the 4-(aminophenyl)benzothiazole is joined through a 2-6 carbon atom chain to the [(Pyridin-2-ylmethyl)-amino]-acetic acid (PAMA), a chelate known to give stable complexes with the $M(I)^+$core [3]. The purpose of the variable chain length is to test the effect of steric freedom in the interaction of the 4-(aminophenyl)benzothiazole moiety with its target. In addition, ligands **4**–**5** that carry the cysteine moiety in the place of PAMA are being synthesized.

All ligands are being tested *in vitro* for their anticancer activity by cell uptake and inhibitory effect studies against the MCF-7 breast cancer cell line, as well as for their binding affinity to amyloid plaques in human AD brain sections.

Complexes of ligands **1**–**5** are being prepared by ligand exchange reactions using either the Tc-gluconate and $ReOCl_3(PPh_3)_2$ as precursors for the MO^{3+} core or the organometallic Tc and Re tricarbonyl precursor $[M(OH_2)_3(CO)_3]^+$ for the $M(I)^+$ core (M = Tc, Re).

Indicatively, the structure of complex **6**, product of the reaction of ligand **2** with the $Re(I)^+$ core, is shown in Figure 2. The complex is stable, neutral and was fully characterized with NMR spectroscopy and elemental analysis. Its radioactive ^{99m}Tc analogue was also

synthesized proving that the complexation reaction is successfully transferred at Tc tracer level. Complex **6** has displayed satisfactory uptake in the MCF-7 cell line.

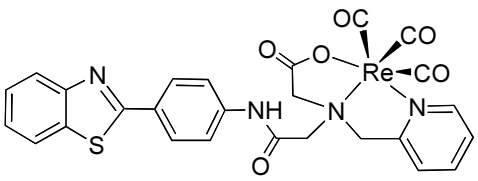


FIG.2. Structure of complex 6.

The results of the synthesis of Tc and Re complexes with all ligands and their biological properties will be presented.

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Utility of ^{99m}Tc labelled tetrofosmin in evaluation and surgical management of parathyroid adenoma

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In the last 10 to 15 years surgery of primary hyperparathyroidism moved from the wide bilateral neck exploration to various types of limited neck exploration ranging from unilateral neck surgery to minimally invasive approaches as the minimally invasive radioguided parathyroidectomy. In contrast with the bilateral neck exploration, an accurate preoperative localizing imaging, which is mainly based on Tc99m -sestamibi or Tc99m-Tetrofosmin scintigraphy, is mandatory when planning a concise parathyroidectomy. The present study was aimed to determine the role of Tc99m-Tetrofosmin scintigraphy in evaluation and surgical (minimally invasive radioguided parathyroidectomy) management of parathyroid adenoma.

The study population included a total of 5 consecutive patients with primary hyperparathyroidism who had undergone minimally invasive parathyroidectomy. Preoperative imaging consisted of Tc99m-Tetrofosmin scintigraphies, which were obtained, in all patients before surgery. The intra-operative technique was based on the injection of a 5 mCi (185 MBq) of Tc99m-tetrofosmin about 30 minutes before the beginning of surgery and on the use of a collimated gamma probe.

Minimally invasive radioguided parathyroidectomy was successfully performed in all patients with primary hyperparathyroidism with adenoma. The new surgical technique required a significantly less mean operating time and a mean hospital stay as compared to traditional wide bilateral neck exploration. No major surgical complications were recorded during and after surgery.

Minimally invasive radioguided parathyroidectomy can be accurately planned in patients with a solitary parathyroid adenoma on the basis of Tc99m-Tetrofosmin scintigraphy. Minimal invasive radioguided parathyroidectomy has been proven to be safe and effective in our experience. The main advantages of minimally invasive radioguided parathyroidectomy over the traditional wide bilateral neck exploration can be resumed as follows: a shortening in the operating and recovery time, possibility of local anesthesia, possibility of ambulatory surgery or same-day discharge, less postsurgical hypocalcemia, less postsurgical pain, favourable cosmetic results, benefits from a cost-analysis point of view.

Synthesis and radiolabelling of a fatty acid xanthate with [^{99m}TcN]+2 core for its possible use in myocardial imaging

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Free fatty acids circulate in the plasma bound to albumin and cross the myocardial cell membrane by passive diffusion. Once inside the cell, fatty acids can either back-diffuse or become activated by acyl-CoA synthetase. Once the latter step occurs, fatty acids become polar and are trapped inside the cell where they can either undergo beta-oxidation or be incorporated into the intracellular lipid pool. Currently ^{123}I-iodophenylpentadecanoic acid is being used employing this mechanism for myocardial perfusion imaging. Because of the limited accessibility of ^{123}I, search for a technetium analogue is on for a long time. In line with this idea we synthesized xanthate derivative of 15-hydroxypentadecanoic acid (HPDA). Overnight reaction of known amount of HPDA with excess of carbondisulphide in 1M sodium hydroxide solution resulted in the formation of xanthate of HPDA. Excess carbondisulphide was removed under vacuum and the crude product was used as such without further purification for the radiolabeling studies.

The xanthate of the fatty acid was then labeled with $[^{99m}TcN]^{+2}$ intermediate. A brief protocol for the preparation of $[^{99m}TcN]^{+2}$ intermediate involved mixing 1 mL of freshly eluted sodium pertechnetate to a commercially available kit vial, vortexing the mixture for a minute and then incubating it for further 20 min. The $[^{99m}TcN]^{2+}$ intermediate was prepared in >95% yields and was characterized by TLC using ethanol:chloroform:toluene:0.5M ammonium acetate (6:3:3:0.5 v/v) as well as saline as developing solvents. Optimized protocol for the labeling of HPDA-xanthate involved mixing 5 mg of the xanthate in 0.5 mL of saline with 0.5 mL of freshly prepared $[^{99m}TcN]^{+2}$ intermediate and incubating the reaction mixture for 15 min. The pH of the reaction mixture was maintained above 9 to avoid decomposition of the xanthate. After 15 min the pH was brought 7. By Paper electrophoresis more than than 95% complexation was observed. Biological studies are yet to be carried out.

Synthesis, characterization and in vivo skeletal localization of a new ^{99m}Tc based bifunctional multidentate phosphonate chelate: 5-amino-1,3-bis(ethylamine-(N,N dimethyl diphosphonic acid) acetamido) benzene

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A new bifunctional multidentate phosphonate ligand was designed, synthesized and characterized by IR, Mass and NMR spectroscopy. The complex was prepared with above 97 % radiochemical purity producing stable ^{99m}Tc-chelates with 1:1 complexation with greater numbers of phosphonate groups per molecule than diphosphonates and therefore better-defined preparations.

Blood clearance showed a quick wash out from the circulation and biological half life was found to be $t_{½}$ (F)=29 min; $t_{½}$ (S)=3 h 48 min. Excellent quality bone images of rabbit were recorded showing rapid clearance of background activity, visualization of skeleton at 1h and clearance from kidneys. No significant activity in any other soft tissues was noted. Biodistribution carried out in Balb/c mice revealed 5.39 ± 0.96 %ID/g and 7.03 ± 0.14 %ID/g radiotracer uptake in bone at 1h and 4 h respectively which resulted in very high bone-to-muscle ratio. Accumulation in kidneys was found to be 2.67 ± 0.05 % ID/g and 1.96 ± 0.23 % ID/g at 1h and 4 h post injection respectively.

The synthesized multidentate phosphonate ligand exhibited good metal ion control properties with very high *in vitro* stability and possess other useful properties such as high water solubility and chemical stability.

This functionalized α-aminomethylene phosphonic acid derivative is suitable for bone imaging as high and selective *in vivo* bone uptake was seen and can be conjugated to wide variety of vector molecules for therapeutic application.

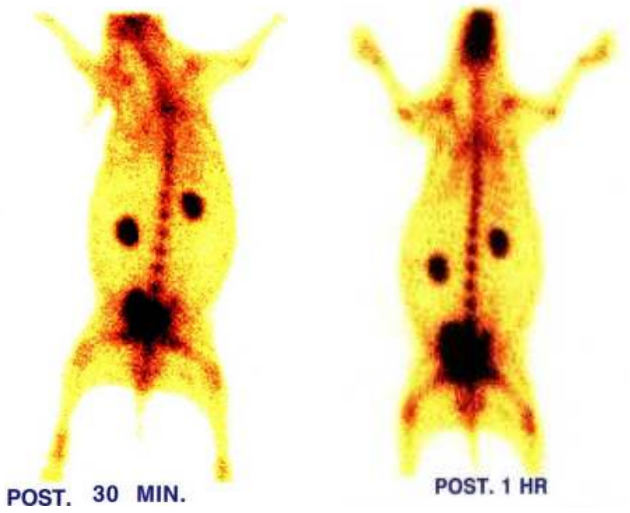

Fig. 1. Whole body scintigraphy in normal rabbit with ^{99m}Tc-5-amino-1,3-bis(ethylamine-(N,N dimethyl diphosphonic acid) acetamido) benzene.

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^{99m}Tc-EDDA-Tricine-HYNIC-TOC versus ^{99m}Tc-EDDA-Tricine-HYNIC-TATE: Synthesis and preclinical result of two new radiopharmaceuticals for somatostatin receptor scintigraphy

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Radioisotope Department, Nuclear Research Center,
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Tehran, Iran

Somatostatin receptor (SSTR) scintigraphy with indium-111 DTPA-Octreotide has become a routine diagnostic procedure in oncology. However, it suffers from some drawbacks concerning the limited availability, suboptimal imaging properties and elevated radiation burden of ^{111}In [1].

In this study the preclinical evaluation of two somatostatin analogues Tyr^3-Octreotide and Tyr^3 - Octreotate labeled with technetium 99m using bifunctional chelators (BFCs) based on the hydrazinonicotinamide (HYNIC) system in comparison with each other is described.

Conjugates of both peptides with HYNIC were prepared and radiolabelling performed at high specific activity using EDDA/Tricine as co-ligands for HYNIC conjugates [2].

In receptor binding study Tyr^3- Octreotate showed higher affinity to sstr2 (IC50 = 1.1±0.2) than that Tyr^3- Octreotide (IC_{50} = 1.3±0.3), while for sstr3 (IC_{50} = > 1000) and sstr5 (IC50 = 80±10) Tyr^3- Octreotate had lower affinity than Tyr^3- Octreotide (IC_{50} = 128±22) and (IC_{50} = 50±12).

^{99m}Tc-EDDA-Tricine-HYNIC-TATE showed a specific and high rate of internalization into AR4-2J rat pancreatic tumour cells which, after 4 h, was about one and half time higher than that of ^{99m}Tc-EDDA-Tricine-HYNIC-TOC.

Biodistribution studies in AR4-2J tumour-bearing rats showed rapid clearance from all sstr-negative tissues except the kidneys for two analogues. At 4 h the uptake of ^{99m}Tc-EDDA-Tricine-HYNIC-TATE in kidneys and liver was lower than for ^{99m}Tc-EDDA-Tricine-HYNIC-TOC, but in the tumour and sstr-positive tissues, such as adrenals, stomach and pancreas, was more than two time higher than ^{99m}Tc-EDDA-Tricine-HYNIC-TOC.

Clinical Comparison of these two peptide based Radiopharmaceuticals is underway.

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An anti-MUC1 monoclonal antibody for radioimmunoscintigraphy of breast cancer: Indirect labelling of antibody with ^{99m}Tc via the HYNIC; in vitro and in vivo studies of the new radiopharmaceutical

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Breast cancer is the second leading cause of cancer death in women. More than 180,000 women are diagnosed with breast cancer each year in the United States. Human epithelial mucin, MUC1, is commonly over expressed in adenocarcinoma that includes more than 80% of breast cancers and represents a useful target for radioimmunoscintigraphy (RIS). The PR81 is a new murine anti-MUCI monoclonal antibody that reacts with the membrane extracts of several human breast cancerous tissues and cell surface of many MUC1 positive cell lines. In this study we have developed an efficient method for indirect labeling of this MAb with ^{99m}Tc *via* the HYNIC as a chelator. The quality control of new radiopharmaceutical and immunoscintigraphy studies in BALB/c mice bearing breast tumours were also performed.

A 20 molar excess of freshly dissolved succinimidyl 6-hydrasino nicotinate hydrochloride (30 mM in DMSO) was added to a solution of Ab (in 0.1 M borate buffer, pH 8.5). The solution was stirred gently for 5 hr at room temperature protected from light. This was followed by dialysis against 0.1 M citrate buffer, pH 5.2 at 4˚C over night. The resulting conjugate was labeled with ^{99m}Tc using tricine (36 mg/ml, pH 7.1) as a co-ligand. The labeling efficiency was determined by ITLC. The amount of radiocolloids was measured by cellulose nitrate electrophoresis. Stability of labeled product was checked at room temperature by ITLC and in human serum by gel filtration chromatography (FPLC) over 24 h. The integrity of labeled MAb was checked by means of SDS-PAGE. Cell-binding assay was used to test binding ability of ^{99m}Tc-HYNIC-PR81 to MCF 7 cells, a kind of human breast adenocarcinoma cell line. Biodistribution was studied in normal BALB/C mice at 4 and 24 h post-injection. The tumour imaging was performed in BALB/c mice with breast xenograft tumours at 24 h after the radiopharmaceutical injection.

The labeling efficiency was 89.2%±4.7 and radiocolloids were 3.4%±0.9. *In vitro* stability was 85.3%±3.6 and 78.6%±5.7 at room temperature and in human serum respectively over 24 hr. There was no significant Ab fragmentation due to labeling procedure. Both labeled and unlabeled PR81 were able to compete for binding to MCF 7 cells. Biodistribution studies in normal BALB/c mice showed that there was no significant accumulation in any organ. The immunoscintigraphy studies demonstrated definite localization of the preparation at the site of tumours with high sensitivity.

The findings showed that the new radiopharmaceutical is a promising candidate for RIS of human breast cancer.

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Preparation and bulk production of ^{99m}Tc-sestamibi with new formulation

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Technetium 99m-hexakis-2-methoxy-isobutyl-isonitril (Sestamibi or MIBI) was developed as a myocardial perfusion imaging agent. It was subsequently used as a tumour imaging agent for lung, mammary, thyroid, parathyroid and brain tumours and lymphoma because it accumulates in tumour cells. ^{99m}Tc-sestamibi is a number of a chemical family referred to as isonitril. This radiopharmaceutical is a monovalent cation in which ^{99m}Tc is surrounded by six isonitril ligands. Regarding to climate in our country, sometimes we had some problems with current sestamibi kit formulation in nuclear medicine centers as low radiolabeling efficiency, so we try to introduce new formulation for this kit respect to our climate.

In this new formulation, we changed some materials and decrease the amount of MIBI to half. After preparation of this formulation kit, as lyophilized, following quality control has been done: radiochemical purity, stability at room temperature, biodistribution in mice, determination of shelf life and sterility, pyrogenicity tests. Also, this kit has been sent to hospitals for clinical applications.

Radiolabeling efficiency was more than 95 percent with up to 200 mCi of $^{99m}TcO_4^-$. This complex was stable at 6 hrs at room temperature. Biodistribution data in mice showed that 1.36 ± 0.11 percent of injected dose accumulated in heart after 30 min. The shelf life of lyophilized cold kit was more than one year. Clinical application had reasonable scan and during this period, there isn't any unfavorable reports.

Our results showed that this kit is a suitable radiopharmaceutical for heart perfusion imaging. The study demonstrated that our formulation with half amount of MIBI regarding to current MIBI kit, has capacity for complexation of 200 mCi of pertechnetate, even one year after production of kit.

Labelling efficiency and stability of some ^{99m}Tc radiopharmaceuticals in the presence of a competitive labelling agent

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At micromole concentration of chelating agent and stannous chloride, the labelling efficiency and stability of ^{99m}Tc – DTPA, ^{99m}Tc - MDP and ^{99m}Tc - GH in the presence of a constant amount of Sn-colloid has been studied, using GCS method.

It has been suggested that Sn-colloid offers an abundant amount of Sn^{2+} in the preparation , resulting a variation in the labelling efficiency and in vivo behavior of the radiopharmaceutical .The labelling efficiency of the different ^{99m}Tc - chelates as a function of chelating agent and Sn^{2+} concentration has been determined .

The molar ratios, 1.25,3.01 and 12.08 were found to be the lowest chelating agent / Sn^{2+} ratio limits and 0.72, 0.30 and 0.07 the highest Sn^{2+}/ chelating agent limits, that can afford high labelling efficiency of ^{99m}Tc -DTPA , ^{99m}Tc - MDP and ^{99m}Tc - GH respectively .The non significant difference in the labelling yield and stability of ^{99m}Tc -DTPA and ^{99m}Tc - MDP at the experimental pH-range emphasizes the strong chelating behavior of DTPA and MDP with reducing technetium. While the less stable ^{99m}Tc - GH preparation at pH higher than the optimal was attributed to the relatively weak ^{99m}Tc-GH chelate .

The organ distribution results as a function of pH in mice, showed that the relatively low kidney/liver ratio of ^{99m}Tc- DTPA preparation was not completely dependent on the experimental pH range, but the Sn^{2+} excess bioundto the chelate undergoes in vivo hydrolysis resulting a higher activity accumulation in the liver the low femur/liver and kidney/liver activity ratios of ^{99m}Tc – MDP and ^{99m}Tc-GH preparations, respectively, at the pH higher than the optimal reveal in vivo instability of ^{99m}Tc – MDP and ^{99m}Tc-GH. Higher blood and gut activity were observed in both chelate preparations.

However, the three technetium chelates at optimal pH without Sn^{2+} excess showed the highest target organ/liver ratios.

Design and synthesis of a novel semi-rigid multidentate ligands with amido, or amino donor groups: Versatile compounds for the preparation of rhenium and technetium radiopharmaceuticals

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Large number of chelates of technetium and rhenium have been prepared in the search for novel, selective, and effective agents for diagnostic imaging and therapy[1,2]. Variety of monodentate ligands can be combined with tetradentate Schiff-base ligands to give mixed-ligand rhenium complexes. The N_2O_2-calix[4]arene Rhenium Complexes was synthesized from 1,3-bis(methylamino)tetrapropoxycalix[4]arene[3].

This work include a synthetic pathways to a range of novel multidentate glycyl ligands, with amido, or amino donor groups, designed to coordinate to rhenium and technetium-99m have been developed. Using formyl substituted anilines as the basic compounds containing the active proton center to follow Manich reaction. The method was successful and was very promising to design ligands of various molecular structures (I-IV). These ligands coordinate to rhenium metal ions in aqueous solutions through ligand exchange with the complex $ReOCl_3(PPh_3)_2$ to give new rhenium complex $ReOCl_3L$. These ligands were identified and studied, so as their complexes with rhenium by absorption spectrophotometry, as well as the other classical techniques such as microanalysis and molecular weight determination by depression of freezing Point.

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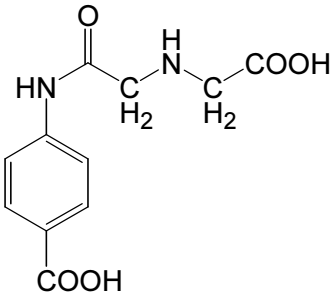
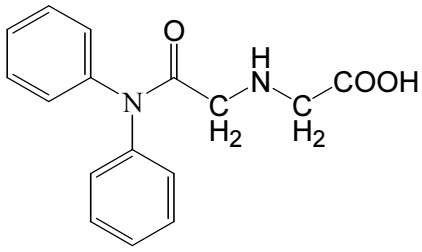


(I)



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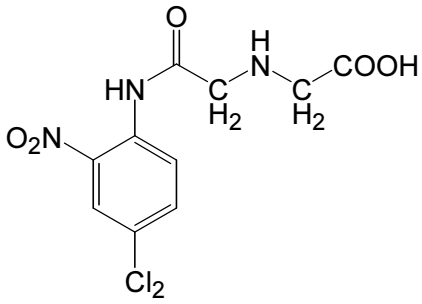

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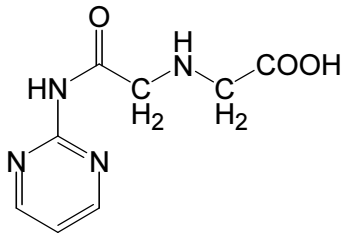

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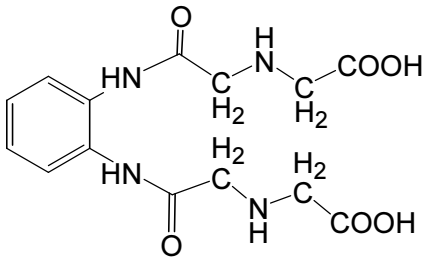

(V)

(I) *N*-Glycylacetyl *p*-aminobenzoic acid
(II) *N*-Glycylacetyl diphenylamine.
(III) *N*-Glycylacetyl 4-chloro-2-nitroaniline.
(IV) *N*-Glycylacetyl 2-pyrimidine.
(V) *Bis*(*N*-Glycylacetyl) phenylene diamine.

Imaging of osseous lesions by ^{99m}Tc(V)-DMSA

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Diphosphonate radiolabelled with technetium-99m are the most diffuse bone-seeking agents with an affinity for areas of active mineralisation. Another 99m-Tc radiopharmaceutical, Tc-99m-DMSA is becoming popular since 1984 for imaging of different primary tumours and bone metastases.

In the preparation of 99mTc labelled compounds, the reduction of 99mTc-pertechnetate is routinely performed by the addition of stannous chloride, because of the simplicity of its handling in the preparation of kits for labelling. [1].

In order to avoid the presence of Tc(III) in the complex, we have optimized the preparation of TC(V)-DMSA [2]. The biodistribution in patients affected with medullary thyroid cancer, head and neck tumour, osseous metastases from prostate and breast cancer generally a much lesser concentration in liver and a lesser uptake for kidney

The images obtained 3-5 h after injection are generally much "cleaner" than by commercial complexes in the visualization of tumour tissues, and especially for osseous metastases the accuracy is comparable with 99mTc-MDP scintigraphy, but with a much reduced uptake from healthy skeleton.

The pentavalent technetium complex of dimercaptosuccinic acid [Tc(V)-DMS] showed a noticeable osteotropic character in bone pathologies (bone metastases, Paget's diseases) and lacked accumulation in normal mature bone.

Technetium-99m (V) DMSA showed high uptake by all chrondrosarcomas, but low or no uptake always indicated benign chondrogenic tumours. Technetium-99m (V) DMSA scintigraphy may be superior to Tc-99m HMDP scintigraphy for distinguishing benign and malignant chondrogenic tumours, and could also be useful for diagnosing the malignant transformation of chondrogenic tumours [2].

The effect of glucose-mediated acidification on the skeletal distribution of the Tc agents in the mice provided valuable hints regarding the differential mediation of bone cells in skeletal tissue affinity for the agents. Very specific pH-sensitive Tc(V)-DMS accumulation only in the osteoclastic system was detected, and use of Tc(V)-DMS in the differential detection of osteoblastic and osteoclastic metastases is discussed. [3]

Skeletal metastases arising from a wide variety of malignancies were evaluated using (99)Tc(m) MDP bone scanning and (99)Tc(m)(V)DMSA scintigraphy. Whole body planar scans were obtained at 3 h after injection of (99)Tc(m) MDP and of (99)Tc(m)(V)DMSA.

A qualitative as well as quantitative comparison was made between the (99)Tc(m) MDP

bone scan and the (99)Tc(m)(V)DMSA scan in detection of osseous metastases.

Avid (99)Tc(m)(V)DMSA concentration in skeletal metastases from a wide variety of malignancies was seen, so confirming the potential therapeutic indications for 188/186 Re(V)DMSA.

In addition, a (99)Tc(m)(V)DMSA scan detected a number of metastatic lesions in and around joints and regions with previous surgical intervention that were inconclusive in the bone scan.

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Design of GMP compliance radiopharmaceutical production facility in MINT

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In 1985, MINT built the only radiopharmaceutical production facility in Malaysia. The facility was designed based on IAEA (International Atomic Energy Agency) standard guidelines, which provides radiation safety to the operator and the surrounding environment from radioactive contamination. In 1999, BPFK (Malaysia National Pharmaceutical Control Bureau) enforced the guidelines from Pharmaceutical Inspection Convention Scheme (PICS) to meet the requirements of the Good Manufacturing Practice (GMP) for Pharmaceutical Products. Among others, the guidelines require the pharmaceutical production facility to be designed based on cleanroom environment. In order to meet this requirement, the design of a radiopharmaceutical production facility shall combine the concept of radiation safety and cleanroom to ensure that both requirements from GMP and IAEA are met. This design requirement is necessary to ensure a radiopharmaceutical production facility which is safe, has high production quality and in compliance with the Malaysian and International standards.

In order to obtain the license to continue producing radiopharmaceutical products, MINT shall closely follow the guidelines from PICS: GMP and nuclear facility requirements. To meet those requirements, the layout of MINT facility was redesigned to ensure the contaminants or particulates from outside area will not enter the production area.

The flow path of personnel to the production room is equiped with air lock and change room in order to avoid contamination from outside environment. The airlock and changing room provide the physical separation of different stages of cleanroom class and between clean area and non-clean area. The cleanliness level or class for each room is determined by the criticality of exposure of the process and products to the environment. The clean class which is grade A is used for critical process and the other grade is for non-critical process or as a background.

The concepts of nuclear facilities that have been incorporated in the cleanroom are:

The function of hotcell as a processing equipment is maintained in order to avoid contamination. Since the hotcell is the surrounding area where the radiation material is exposed, the hotcell pressure is maintained in negative pressure. Although the pressure is negative, the cleanliness shall be of A grade.

In emergency situation, the room pressure will switch from positive to negative pressure when the operator or personnel pushes the emergency button. The emergency button is located inside and outside the production room. This feature can prevent the contamination from spreading out in the building.

The pressure of the area which adjacent to the production room is slightly negative. This will act as a buffer to prevent the radioactive substances contaminating the whole building.

The function of air treatment plant is maintained in order to filter the radioactive substances, which is released to the atmosphere.

Although the function of hotcell is maintained, a few modifications had been made. The modifications involved the source entering system and liquid waste handling system to ensure it will meet the required cleanliness standard.

The above characteristics of a clean room design will be able to ensure the cleanliness of the pharmaceutical products as well as the nuclear safety of the facility.

Tricarbonylrhenium (I) complexes with neutral bidentate N-methyl-2-pyridinecarboamide as a precursor of therapeutic radiopharmaceuticals

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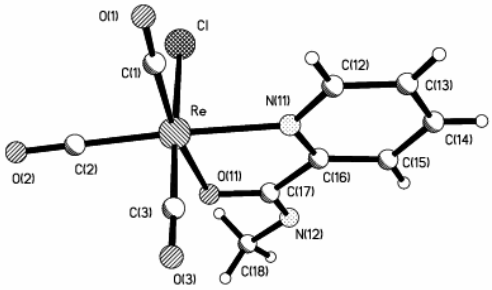
Coordination chemistry of technetium and rhenium attracts considerable interest due to the nuclear medicine applications of their radionuclides. ^{99m}Tc (γ-emitter) is the "working horse" in diagnostic nuclear medicine, used in more than 80 percent of routine studies in this field. The β^--emitting rhenium isotopes, ^{186}Re and ^{188}Re, are used in radioimmuno therapy. The coordination behaviour of tricarbonylmetal(I) with chelating ligands, important for designing new metal-based radiopharmaceuticals, has been intensively studied by numerous authors. Particularly stable bonds have been found between the $[M(CO)_3]^+$ core (M = Re, Tc) and aromatic nitrogen donor atoms of multidentate ligands [1].

In this paper we report the complex formation (in mg quantities) of the tricarbonylrhenium(I) moiety with N-methyl-2-pyridinecarboamide (**1**), an analogue of the thio-compound recently studied [2]. Ligand **1** was obtained according to the general procedure described in [3]. The Re(I) precursor (**2**) was obtained according to [4].

The investigated complex $[Re^I(CO)_3LCl]$ (**3**), where L denotes **1**, was obtained from **2**, following the synthetic procedure worked out at the Paul Scherrer Institute.

Molecular structure of the investigated complex **3** is presented in Figure 1. As it was expected, the ligand coordinates the cation bidentately, *via* the pyridine nitrogen and the oxygen atoms, and forms a five-membered ring with the metal centre.

Stability of **3** towards oxidation was studied in the aerated 0.9% NaCl aqueous solution by registering its UV-Vis spectra as a function of time. Within one week no distinct change in the spectrum was detected.



Infrared spectra of the ligand **1** and the complex **3** are shown in Figure 2. All the main bands of the "fingerprint region" of the pyridinecarboamides (1300 ÷ 700 cm^{-1}) can be found in both spectra. The spectrum of **3** shows the typical pattern for a facial tricarbonyl moiety with bands at 2085, 1998 and 1879 cm^{-1} (CO ligands vibrations), and a very sharp signal of valency stretching of C=O in the amide group at 1639 cm^{-1}.

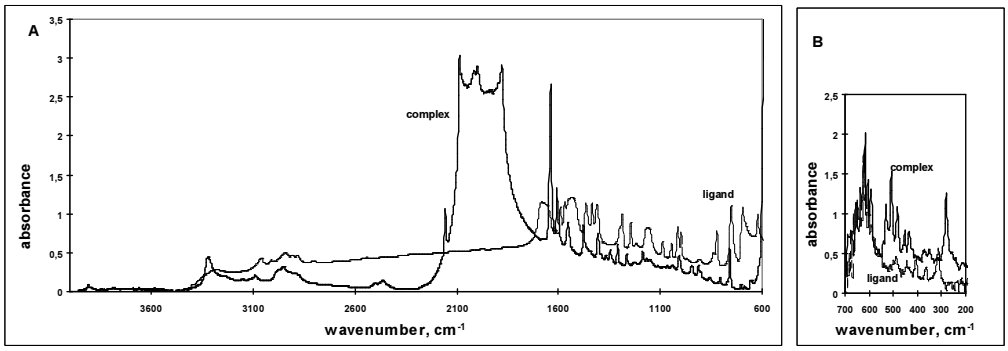


*FIG. 2 FT-MIR (A, KBr pellets) and FT-FIR (B, CsJ pellets) spectra of ligand (**1**) and complex (**3**).*

The far infrared spectrum of **3** (Fig. 2-B) reveals six bands which have been interpreted to arise from vibrations of Re-C bonds at 530, 510 and 482 cm^{-1}, Re-N bond at 450 cm^{-1}, Re-O bond at 433 cm^{-1} and Re-Cl bond at 281 cm^{-1}.

The value of partition constant of **3** in the *iso*octanol – 0.9% NaCl aqueous solution system, $\log P_{\mathbf{3}} = 0.85 \pm 0.09$, is greater than the corresponding value for its thio-analogue, $\log P = 0.57 \pm 0.15$ [2].

Further studies on functionalization of **3** with glutathione and/or N-acetyl-cysteine are in progress.

Crystallographic data for the structural analysis have been deposited at the Cambridge Crystallographic Data Centre and obtained CCDC No. 270202.

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^{99m}Tc-EDDA/HYNIC-TOC and ^{99m}Tc-EDDA/HYNIC-TATE – from CRP to extended clinical application

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Somatostatin is 14-amino acid peptide naturally occurring which controls the secretion of many hormones. A high density of somatostatin receptors have been found on tumours mainly of neuroendocrine origin. Somatostatin has a very short biological half-life of several minutes, hampering sufficient receptor binding to allow adequate imaging. Derivatives of somatostatin represent the most important peptides for receptor targeting in oncological applications. In this study two promising radiopharmaceuticals ^{99m}Tc-HYNIC-TOC and ^{99m}Tc-HYNIC-TATE with the potential to replace ^{111}In-DTPA-D-Phe^{1}-Octreotide in somatostatin receptor scintigraphy are presented

HYNIC – Tyr^{3}-octreotide (HYNIC – TOC) and HYNIC – Tyr^{3} – Octreotate (HYNIC - TATE) were synthesized in our laboratory on solid phase using the Fmoc strategy. Then for the HYNIC chelators coupling, the peptides were cleaved from the resin, cyclization and coupling was done after that in solution. The overall yields after deprotection with preparative HPLC were between 14% and 20 %. Wet ^{99m}Tc-labelling of HYNIC-TOC and HYNIC-TATE were performed to optimize the amount and concentration of reagents, temperature and reaction time which was then transferred to HYNIC-TOC and HYNIC-TATE dry kit formulation. The kit contains two vials: the first vial: 20 μg HYNIC-TOC or HYNIC-TATE, 40 μg $SnCl_2$, 50 mg tricine, 10 mg mannitol, the second vial: 10 mg EDDA(ethylenediamine-N,N'-diacetic acid). Radiolabelling was carried out by the addition 1 ml of generator eluate (20-40mCi radioactivity) to kit and 0,5 ml EDDA followed by 30 min incubation at 80^{o}C. Radiochemical purity of ^{99m}Tc-HYNIC-TOC and ^{99m}Tc-HYNIC-TATE, controlled by TLC and HPLC methods showed over 90% radiochemical yield and percentage of unbound free ^{99m}Tc-pertechnetate as well as colloidal forms of ^{99m}Tc was in the range of 2 - 3%. The stability of the freeze-dried kits during storage at 2-8^{o}C was performed once a month by radiolabelling and determination of the radiochemical purity by HPLC. The stability of these kits was determined for 1 year. Stability of the obtained kits allowed extensive clinical studies of ^{99m}Tc-HYNIC-Tyr^{3}-octreotide and ^{99m}Tc-HYNIC-Tyr^{3}-Octreotate.

Biodistribution studies in normal mice showed rapid urine excretion of unbound radioactivity. Accumulation in kidneys was at the level of 5% at 3.5h p.i. Activity accumulated in pancreas, organ reach in somatostatin receptors, was 2.8 times higher than in lungs in normal animals and 2.2 times lower than in lungs in blocked animals, respectively. Similar accumulation in adrenals was observed. The internalization experiments were tested using AR42J rat pancreatic tumour cell membranes. The ^{99m}Tc-labelled peptides showed the following order in the rate of internalization: ^{99m}Tc-HYNIC-TOC - 10.9%, ^{99m}Tc-HYNIC-TATE – 18.7%

Hynic-Tyr^{3}-octreotide and Hynic-Tyr^{3}-octreotate conjugates obtained in our laboratory were successfully labelled with technetium-99m with the yields over 90% and tested *in vitro* for human serum stability and internalization. To make labelling procedure easier, dry kits were produced with Tricine and EDDA as co-ligands. The *in vitro* and *in vivo* features of the ^{99m}Tc-Hynic-Tyr^{3}-octreotide and ^{99m}Tc-Hynic-Tyr^{3}-octreotate confirmed their diagnostic potential.

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Use of ^{89}Sr for bone pain palliation, experience in our Department

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Bone metastases are the most common cause of pain in patients with cancer, and postmortem studies have been shown that up to 85% of patients with breast or prostate cancer have bone metastases at the time of death. The palliation of bone pain is one of the goals of treatments in oncologic patients, due to the fact that patients with bone metastases may survive many years with severe pain and serious impaired mobility. Bone pain palliation can be attempt by using different modalities – analgesics (nonsteroid, opioides), biphosphonates, external beam radiotherapy or radionuclide therapy.

The aim of our study was to review the database and to analyze the results of the treatments performed in our department, in the field of radionuclide metastatic bone pain palliation with ^{89}Sr

Eleven patients (47–73 years old) were treated with ^{89}Sr in the past two years in our department. All patients had confirmed prostate adenocarcinoma and refractory bone pain due to skeletal metastasis involving more than one site, associated with osteoblastic response on bone scan. All patients had a good hematological and renal status (Hb> 9 mg/dl, leukocytes > 4000/ μl, platelets > 150.000/ μl, GFR > 30 ml/min), an increased alkaline phosphatase and recent bisphosphonate therapy interrupted within 48h before treatment. The standard administered dose was 150 MBq [4 mCi] of ^{89}Sr (Metastron – Amersham). In 8 cases single dose was given, while in 3 patients a second dose was administered at 4, 6 and 9 months after the first injection. Clinical and biological evaluation was repeated at 3 weeks and 3-6 months after treatment.

The most important criteria of pain relief was objective pain score. Significant improvement of life quality was seen in 4 patients with major reduction of analgesic needs. In 3 patients in addition to external beam radiotherapy we obtained a stable effect of more than 6 months. 3 patients had moderate response, one of them with an early need of a second dose at four months. No response was seen in one patient. Pain flare occurred in two cases. Temporary myelosuppression was seen in 5 cases (45,46%) with recovery in 2-11 weeks

Respecting the criteria of evaluating patients who might be candidates for treatment using ^{89}Sr, the significant clinical improvement of life quality can be obtained with an easy procedure, minor side effects and a good compliance of patient. In a country with limited resources for an extensive medical research it is important to introduce new strategies of treatment that have proved their efficacy, to try to promote the cost effective use of high quality therapeutic procedures and the recommended guidelines.

Radiopharmaceuticals production in Saudi Arabia: The status and prospects

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One of the factors influencing the tissue localization of radiolabeled molecules is their lipophilicity. The replacement of a benzene ring with pyridine has been reported to decrease significantly the lipophilicity of the resultant molecular entity. Fluorobenzoates have been used extensively as prosthetic groups for labeling bioactive molecules. Very few attempts have been made to develop pyridine derivatives for the same application. We have therefore, embarked on the development of fluorinated pyridine derivatives as potential prosthetic groups for fluorination of protein and peptides. We report here an efficient synthesis of 6-fluoronicotinic and 2-fluroisonicotinic acid and their N-succinimidyl esters. In addition, N-succinimidyl activated ester of the 2-[^{18}F] fluoronicotinic acid was used to label several peptide analogues.

The radiochemical synthesis of ethyl 6-[^{18}F]fluoronicotinate and ethyl 2-[^{18}F]fluoroisonicotinate were accomplished by catalyzed nucleophlic no-carrier-added fluorination. Treatment of the 6-N,N,N-trimethylammonium ethylnicotinate and 2-N,N,N-trimethylammonium ethylisonicotinate triflate precursors with radiofluoride and Kryptofix 222 in anhydrous acetonitrile at 100^{o}C gave ethyl 6-[^{18}F]fluoronicotinate and 2-[^{18}F]fluoroisonicotinate intermediates in greater than 90% radiochemical yield within two minutes reaction time. The fluorination reactions were consistently higher than 90% when studied over a time range of 2–15 minutes and deferent amount of triflates. These intermediates were converted to the corresponding acids followed by the reaction with O-(N-succinimidyl) N,N,N,'N'-tetramethyluronium tetrafluoroborate (TSTU) in acetonitrile for 10 minutes at 100^{o}C. The resulted activated esters of the N-succinimidyl 6-^{18}F-nicotinate and 2-^{18}F-isonicotinate were purified using silica Sep-Pak cartridge. These purified activated esters have been successfully used to label chemotactic peptide and other bioactive molecules through their amine moieties and their biological evaluation are in progress. The overall radiochemical yields ranged between 60–70% (decay corrected) with preparation time of about 50 min.

This method in comparison with the replacement of halogen or nitro groups by fluoride procedure appear to be advantageous in the synthesis of high radiochemical yield fluorine-18 labeled compound in shorter time. Hence, this technique may be applied to obtain high specific activity products, a prerequisite for studying low capacity and saturable sites.

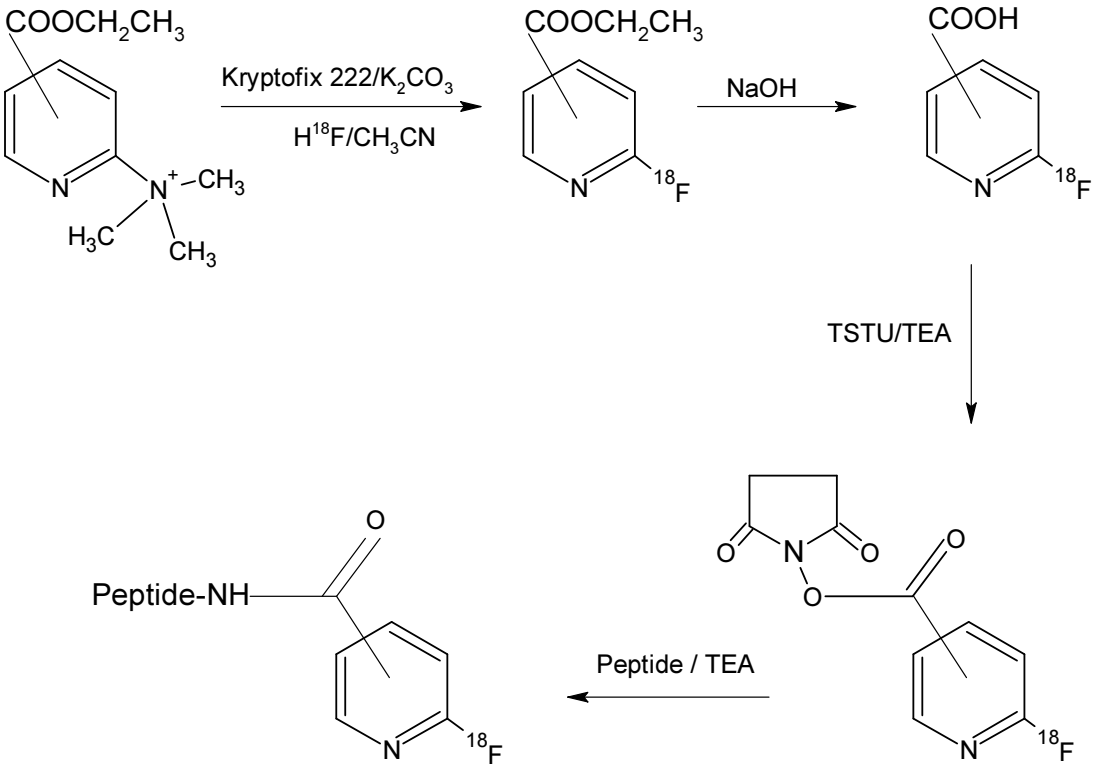

FIG. 1. Radiofluorination of [^{18}F]-fluoropyridine derivatives and bioactive molecules.

Labelling of some amino acids with [^{99m}Tc(CO)3(H2O)3]+ precursor: Comparison of chemical and biological behaviour

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Recent approaches to development of new radiopharmaceuticals for diagnosis are centred on the use of ^{99m}Tc-labelled peptides. Possible targets for labelling of peptides are amino acids: tyrosine, lysine and histidine. Labelled artificial amino acids has more advantages, as they are entered into cells *via* single mechanisms and increased uptake into malignity cells in comparison with normal cells. The labelled amino acids with technetium-99m are expected to facilitate research on tumours and cerebral function and to provide a clue to the research for diagnosis of membrane transport of amino acids[1]. Besides this direct labelling approach, the use of bifunctional chelating agent (BFCA) has been more fruitful[2]. Nowadays, hydrophilic organometallic $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor allows the forming of Tc(I) radiopharmaceuticals based on the tricarbonyltechnetium (I) core[3,4]. The results of labelling of some amino acids with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and examination of their chemical, pharmacological and biological behaviour were presented.

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was prepared according to the literature method (IsoLink^{TM}, Mallinckrodt Medical B.V., The Netherlands). Amino acids: tyrosine (Tyr) and lysine (Lys), were labelled with ^{99m}Tc (I), without and with hitting (30 min in boiling water bath). The dependance of labelling efficiency on pH was investigated too.

The quality control of the obtained $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was performed by gradient HPLC (Liquid Chromatograph, Hewlett Packard 1050, S/N with UV and Raytest gamma flow detector) and RP C18 column (150x4.6 mm). The solutions of 0.05 mol dm^{-3} triethylammonium phosphate (TEAP) with pH=2.25 and methanol were used like mobile phases. All other investigations were performed as isocratic HPLC with 80%H_2O:20% TEAP, pH=3.0 as mobile phase and flow rate 0.7 ml/min.

*T*CA precipitation method for determining the percentage of labelled amino acids bound to proteins (12% human albumin, incubation at 37^0C for different time intervals) was very useful. All lipophilicity measurements for labelled amino acids were done by solvent extraction method with n-butanol equilibrated with 0.15 M phosphate buffers. Organ biodistribution studies of ^{99m}Tc-labelled compounds were carried out on healthy white male Wistar rats (four weeks old). The animals were sacrificed one hour after application of 0.1 ml of ^{99m}Tc-labelled compound (~74kBq). The radioactivity per organ of interest (or per g) was measured in a NaI(Tl) well type detector and the percentage of radioactivity related to administrated dose was determined.

HPLC quality control results of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor have shown that the radiochemical purity of precursor was higher than 95 %. The values of retention times together with the labelling yield for each of Tc-carbonyl coordinated amino acid were presented in Tables 1 and 2. The labelling conditions like pH or heating of samples influenced at labelling yields.

TABLE I. DEPENDENCE OF RETENTION TIMES AND LABELLING YIELD FOR $[^{99m}Tc(CO)_3]^+$-TYR AND $[^{99m}Tc(CO)_3]^+$-LYS ON PH AND HEATING

Samples	pH	3.5		5.5		7.5	
	^{99m}Tc-species	R_t (min)	Labelling yield (%)	R_t (min)	Labelling yield (%)	R_t (min)	Labelling yield (%)
$^{99m}Tc(CO)_3$–Tyr	$^{99m}Tc(CO)_3$	3.141	100	3.358	60.20	3.287	22.37
	$^{99m}Tc(CO)_3$-Tyr	-	-	6.941	38.32	8.341	77.63
	$^{99m}TcO_4^-$	-	-	15.063	1.48	-	-
$^{99m}Tc(CO)_3$–Tyr*	$^{99m}Tc(CO)_3$	3.189	72.58	3.356	47.68	3.260	7.49
	$^{99m}Tc(CO)_3$-Tyr	8.048	27.42	5.267	52.32	8.242	92.51
	$^{99m}TcO_4^-$	-	-	-	-	-	-
$^{99m}Tc(CO)_3$-Lys	$^{99m}Tc(CO)_3$	3.140	100	3.454	100	4.997	92.90
	$^{99m}Tc(CO)_3$-Lys	-	-	-	-	7.298	1.45
	$^{99m}TcO_4^-$	-	-	-	-	14.923	2.74
						15.866	2.91
$^{99m}Tc(CO)_3$-Lys*	$^{99m}Tc(CO)_3$	3.189	100	3.308	13.63	4.889	0.48
	$^{99m}Tc(CO)_3$-Lys	-	-	5.930	79.63	7.189	99.00
	$^{99m}TcO_4^-$	-	-	12.911	6.73	14.453	0.52

TABLE II. BIODISTRIBUTION RESULTS FOR ^{99M}TC $(CO)_3$, ^{99M}TC $(CO)_3$ -TYR AND ^{99M}TC $(CO)_3$ LYS

Organs ^{99m}Tc-species	Blood*	Lung	Liver	Kidneys	Spleen	Stomach	Intestine
$^{99m}Tc(CO)_3$	0.9±0.1	5.5±1.3	90.3±3.9	0.9±0.3	0.6±0.1	0.4±0.1	1.0±0.2
$^{99m}Tc(CO)_3$-Tyr	2.1±0.3	1.7±0.2	23.7±0.6	11.4±0.5	0.6±0.2	1.2±0.3	10.8±0.6
$^{99m}Tc(CO)_3$-Lys	1.5±0.4	1.3±0.4	11.7±0.6	6.4±0.4	0.3±0.2	1.0±0.2	6.6±0.4

- Values represent the means ±SD (n=6) of the percent injected dose per organ or * per g.

The best labelling yield with ^{99m}Tc (I) carbonyl precursor was obtained at pH=7.5 with heating. The obtained-labelled compound had hydrophilic character. The percentage of protein binding for amino acids, for example labelled Tyr (67.2±0.5) and Lys (61.1±0.6) were lower than for ^{99m}Tc (I) carbonyl precursor (85.0±1.2). The biodistribution results (% ID/organ) obtained in health Wistar rats have shown the dependence of accumulation in organ of interest on the labelled amino acid. These observations suggested that the oxidation state of technetium-99m used for labelling, has important influence on *in vitro* and *in vivo* behaviour of ^{99m}Tc-labelled amino acid.

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Regulatory aspects - the prerequisite of the future applications of radiopharmaceuticals in Montenegro

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After major constitutional changes in 2003, which redefined Serbia and Montenegro as (de facto) a loose confederation of the two constituent states, all nuclear related issues went separately to the portfolios of the Republic of Serbia and of the Republic of Montenegro. Within the establishing of radiation protection regulatory framework (both legal and institutional) in Montenegro, and towards meeting the international requirements (like IAEA's Basic Safety Standards and the Code of Conduct on the Safety and Security of Radioactive Sources), the need for setting appropriate legaly binding requirements for the safe use of radiopharmaceuticals, as the basic step towards their proper application, is discussed.

1. ESTABLISHMENT OF THE NUCLEAR REGULATORY FRAMEWORK IN THE REPUBLIC OF MONTENEGRO

Following the decision of its Assembly from 4 February 2003, former Federal Republic of Yugoslavia (FRY) transformed into a new entity, called State Union of Serbia and Montenegro - a loose confederation, in effect, of the two constituent states: the Republic of Serbia and the Republic of Montenegro.

Only very few competences remained at the level of the Union, including mainly defence and foreign affairs&trading issues. These competences are conducted on the parity-of-the-two principle. It is agreed that within three years the constituent states will decide whether to go on together or to continue separately.

Among the vast majority of competences which passed from ex-federal level to the constituent republics, as a consequence of the above political change, are radiation&nuclear related issues - to start with the creation of the regulatory framework for nuclear, radiation, radioactive waste and transport safety.

While in Serbia there is quite a long tradition, as well as the experience and expertize in the field (originating mainly from "Vinca" nuclear research institute in Belgrade), that is not the case with Montenegro. Therefore, the transition of competences is not being felt so drastically in Serbia, as it is the case in Montenegro.

In Serbia, ex-federal legal and governmental nuclear safety infrastructure will likely continue to operate without much change, just under the new administrative umbrella (i.e. within the Ministry od Science and Environment).

In Montenegro, however, a similar transition was not possible. Therefore, an informal group of professionals in the field, being aware of the legal and institutional vacuum created after the above constitutional changes, initiated formation of an adequate framework for the radiation protection and for the security and safety of radiation sources. With the help of IAEA experts, a draft of the law [1] was written. With minor changes, the draft is

subsequently passed to the Government and is now in the regular procedure which would lead to its promulgation [2].

2. NUCLEAR MEDICINE IN MONTENEGRO: PAST, PRESENT AND PLANS FOR THE FUTURE

Past. Until the year 1993, a small nuclear medicine department was active within Clinical Centre of Montenegro in Podgorica. Basic Technetium Tc-99m and Iodine I-131 diagnostic and therapeutic applications were performed. The supply of radiopharmaceuticals was predominantly from the "Vinca" Institute, Belgrade. Regulatory aspects were covered by a federal radiation protection law, and effectuated by a federal regulatory authority in Belgrade.

Present. Following the difficulties caused by political instability and economic collapse in the country, nuclear medicine department in Podgorica ceased its activity by 1993. Up to date, it has not been re-established and re-activated, although the premisses are still existing and it could be put in functioning with a relatively small investment and effort.

Future. Before considering the plans for re-activating the department of nuclear medicine in Podgorica, the most fundamental issue should be fixed - regulatory aspects of the practice, both legal and institutional. Namely, there is no radiation protection regulatory authority in the country, while provisions of the old radiation protection law from the Federal Republic of Yugoslavia [5] and subsequent medical practice regulations (still in force), are obsolete and not in accordance with international standards (e.g. IAEA's Basic safety Standards and the Code of Conduct on the Safety and Security of Radioactive Sources).

3. THE NEED FOR SETTING A REGULATORY FRAMEWORK FOR THE SAFE USE OF RADIOPHARMACEUTICALS

Obviously, re-starting with nuclear medicine in Montenegro before setting the appropriate radiation protection regulatory framework is unacceptable. Working without e.g. licencing procedures and/or regular inspections - to mention just the most notorious regulatory aspects - could lead to serious harmful happenings to all: patients, staff, public and environment. Following the promulgation of the new radiation protecion law [2], regulatory authority should be urgently established and adequte regulations brought into force. In this respect, the assistance of international bodies, in particular that of the IAEA, will be of the utmost importance.

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Supported liquid membrane extraction of ^{99m}Tc(VII) in a hollow fibre contactor

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Supported liquid membrane (SLM) extraction of ^{99m}Tc(VII) in a hollow fiber contactor was investigated in this study. Technetium in a form of pertechnetate was extracted in the three phase system, from the acidic donor solution through the organic phase placed in the membrane pores and then to the alkaline acceptor solution. Tri-n-octylamine as a well known selective extractant for the separation of technetium from molybdenum was applied for this purpose [1]. The aim of this work was to characterize SLM extraction of ^{99m}Tc in order to investigate the applicability of this technique for the alternative, cyclotron production of ^{99m}Tc [2] and other positron emitting isotopes of Tc.

SLM extraction of ^{99m}Tc (VII) was performed in a membrane contactor consisting of a glass housing in a form of U-shaped glass tube and a single hollow fiber. The microporous hydrophobic polypropylene hollow fiber membrane (Hoechst-Celanese, USA) was used in this study. The inner diameter of the hollow fiber was 280 μm, the thickness of the wall was 190 μm, and the length of the hollow fiber was 120 mm. The donor solution was initially containing 13 kBq ^{99m}Tc (VII) in 1 dm^3 0.9 % NaCl at pH=3-4 and fed along the lumen of the hollow fiber, either in continuous or recirculated mode of operation, by a peristaltic pump. The membrane was impregnated by soaking in the organic phase (50% tri-n-octylamine in hexane) for 1h, which was followed by washing in a water-bath in order to remove the excess of the solvent. The acceptor solution containing 0.1 mol·dm^{-3} NaOH was fed along the shell side in continuous mode of operation. The activity concentration of ^{99m}Tc in the donor and acceptor solution was determined using an automated γ-counter.

The transport of ^{99m}Tc(VII) across the tri-n-octylamine-hexane based SLM as a function of the donor flow rate under continuous and recirculation mode of operation was investigated. The obtained results are discussed in terms of extraction efficiency, mass transfer coefficient and technetium flux through the interfacial area.

A typical time variation of ^{99m}Tc(VII) in the aqueous donor and acceptor phase at the donor and acceptor outlet of the contactor under the continuous mode of operation is given in Fig.1. One can see that the extraction equilibrium in the donor phase was established within a few minutes after the beginning of the extraction, while the time needed for reaching the stationary state in the acceptor phase is much longer (about 2 h).

The extraction efficiency of ^{99m}Tc(VII) based on the removal of technetium from donor solution under continuous mode of operation was calculated using the following equation: $E = 1 - C_d^{out} / C_d^{in}$, where C_d^{in} is the initial technetium concentration in the donor phase and C_d^{out} is the steady-state outlet concentration of ^{99m}Tc (VII) in the donor phase. Figure 2 presents a plot of the extraction efficiency, E, vs. the donor flow rate. It can be seen that E decreases with increasing of the donor flow rate, which is a typical behavior of donor controlled extraction. The SLM extraction under recirculated mode of operation gives very high percentage of extracted ^{99m}Tc (VII), exceeding 90 %, depending on the feed flow rate.

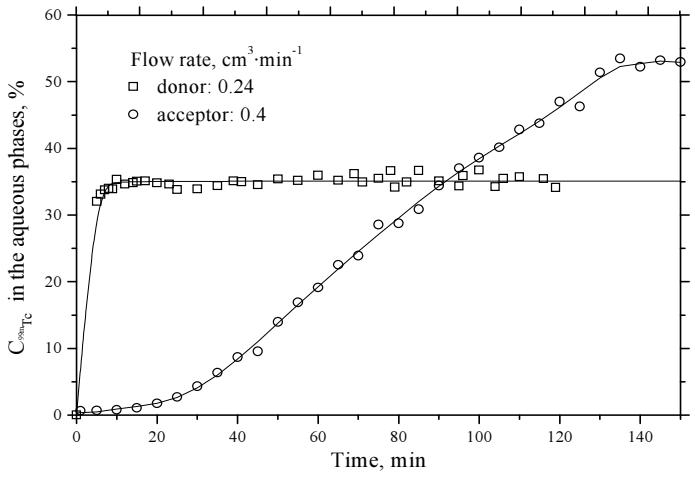

FIG. 1. Concentration profile of ^{99m}Tc (VII) in the outlet of donor and acceptor phases as a function of time of extraction.

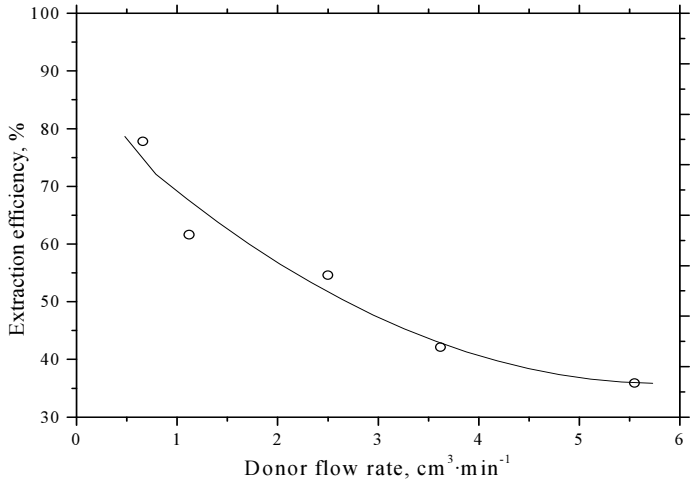

FIG. 2. The extraction efficiency of ^{99m}Tc (VII) as a function of the donor flow rate.

From the presented results it is evident that technetium might be efficiently removed from the donor phase, i.e. to be separated from molybdenum, however its re-extraction into the acceptor phase is not optimal under these conditions. SLM extraction based production of Tc radionuclides seems to be possible, but the process needs further optimization.

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Status of radiopharmaceuticals in Sudan

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There are two centers for Nuclear Medicine (NM) in Sudan:

(1) RICK: Radiation and Isotope Center, Khartoum (Rick). It is the oldest radioisotope center in the country, started functioning in 1970. The Nuclear medicine department functioning with two planar gamma cameras and there is no SPECT. Radioimmunoassay (RIA) section is not under NM department. There are two NM specialists, 6 technologists, and there is NO Radiopharmacist.

(2) INMO: Institute of Nuclear Medicine, Molecular Biology, and Oncology (INMO), Wad Medani – Sudan. It is started working in 1993 as Radioimmunoassay (RIA) department. The Gamma camera started working in August 1995; Later on the Molecular biology department was added. In July 1999 the Radiotherapy Department was opened, all the development steps with the help of International Atomic Energy Agency (IAEA). There is only one planar gamma camera, and there is No SPECT. There are two NM specialists, only ONE Radiopharmacist, 10 technologists.

Nuclear Medicine Studies Carried in Sudan are listed below:

Routine tests done are: Thyroid, Bone, Renal (Dynamic / Static), Liver (SC / IDA), Brain (DTPA), and Lung. Two new studies are added later (e.g. Parathyroid imaging ($^{201}Tl^{+}$ and ^{99m}Tc) and Lymphoscintigraphy. In addition to the above studies there is hormone RIA section (e.g. Thyroid hormone profile, Reproductive hormones, Tumour markers)

Radiopharmaceuticals used in in NM are $Na^{99m}TcO_4$, MDP, DTPA, DMSA(III), MEBROFENIN (IDA'S), NonoColloid, MAA, etc.

Radioisotopes used are ^{99m}Tc, ^{131}I, ^{201}Tl and ^{125}I. Some Isotopes used for calibration of Dose calibrator & Gamma Camera are ^{133}Ba, ^{57}Co, ^{60}Co, ^{137}Cs etc).

Source of the Radioisotopes & Radiopharmaceuticals: The two centers had started importing from Amersham – UK (free from IAEA). Then diverted to CIS Bio International – FRANCE, there was no direct flight from Paris to Khartoum (this may cause sometime inconvenience for the patients). They were returned to the first box in 2001 up to now (i.e. Amersham – UK).

Academic activity: Radiopharmacist participate in the teaching for undergraduate students on radiopharmacy subject and also participate in conducted national training in collaboration with IAEA.

Equipments in the Radiopharmacy Lab.: There are some equipments available such as Lyophilizer, Laminar Air-flow unit, Dose calibrator, Bench lead glass window, Lead bricks.

There are some equipments required to the Radiopharmacy Lab., they are: pH meters, TLC scanner with the provision of measuring radioactivity for quality control.

Deep Freezer (-70^{o}C) with indicator + set temperature, Laminar flow unit with appropriate filters, Lead-lined waste container (foot operated), Pharmacopoeias (USP, BP, and EP), Centrifuge-speed up to 12000 RPM, Micropipettes with tip ejector different sizes (1.0ml - 20ml], [100ml – 100ml], and [0.5 – 5.0 ml].

Future Planning include Labelling of WBC , Labelling for RBC (GIT bleeding), Labelling of different compounds (Organic/Inorganic) with different radioisotopes (e.g. ^{99m}Tc, ^{131}I, ^{188}Re, etc), labelling of Peptides and Antibody for different studies, Therapeutic application., Study of pharmacokinetics of prepared compounds etc.

After installation of a SPECT, new studies will be added (e.g. Myocardial, MUGA, Brain and cardiac perfusion…etc). Mammary Lymphoscintigraphy (sentinel node identification), Help in Cancer patients (pain palliation), Central radiopharmacy (transportation?) etc are also planned.

Problems: There is No SPECT, Lack of understanding of NM by some Doctors, Difficulties in procuring short-lived radiopharmaceuticals, The high cost for kits, reagents, radioisotopes, etc., There are no company representatives and spare parts, There is a financial problems to conduct (projects, workshop, seminars, etc.), and Collaboration with internationally similar Institutes.

Current status of radiopharmaceuticals production at AECS laboratories

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Radioisotopes have essential role in the field of nuclear medicine for diagnosis and treatment purposes, where technetium – 99m have been occupied more than 80% of the total application in this field. The current nuclear medicine activities reflect an expanding demands on radiopharmaceuticals frome the points of quantity and types.

AECS has started large project for development and production of radiopharmaceuticals in cooperation with IAEA through several TCPs.

The aim of this project was to support the development process of nuclear medicine in the country and region.

Several facilities were installed for production of ^{99m}Tc genereators, ^{99m}Tc cold kits and iodinated compounds and recently a cyclotron facility (cyclone-30 IBA) was added.

The current production provide more than 20 products (table 1) which cover more than 90% of the demands in the region. The production process is carried out according to severe quality assurance programme in consistence with GMP requirements. A new facility is now under construction to meet the expanding demands of radiopharmaceuticals in the region and to satisfy the new GMP requirements. This facility is planned to have hot laboratories for ^{99m}Tc generator production with capacity of about 100 generators per batch, high class clean room (class A-) for cold kit production and iodination laboratory.

TABLE .I. RADIOPHARMACEUTICALS PRODUCED AT AECS LABORATORIES

-SYRTEC	^{99m}Tc generator	Weekly production
-SYRTCK-01	MDP kit	For bone scintigraphy
-SYRTCK-02	DTPA kit	For renal studies
-SYRTCK-03	Phytate kit	For liver scintigraphy
-SYRTCK-04	Sn-PYP	For in vivo RBC labelling
-SYRTCK-06	Glucoheptonate kit	for kidney image
-SYRTCK-07	DMSA(III) kit	For renal scintigraphy
-SYRTCK-08	DMSA(V) kit	For tumour study
-SYRTCK-09	Sulfur colloid	For liver

-SYRTCK-10	MAA kit	For lung study
-SYRTCK-13	Bromo IDA	For hepatobiliary
SYRTCK-14 -	nano colloid	For lymphoscintography
- SYRIK-01	Iodine-131 solution	For therapy
-SYRIK-02	^{131}I-MIBG	For neuroblastoma, phaeochromocytoma
- SYRIK-03	Iodine-131 capsules	For diagnostics
- SYRIK-04	Iodine-131 capsules	For therapy
-SYRGa-01	^{67}Ga-citrate	For tumour diagnosis
-SYRTl-01	Tl chloride^{201}	For cardiac study
-SYRF-01	^{18}F-FDG	For PET diagnosis

Establishment of a central quality control laboratory for radiopharmaceuticals

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The need for radiopharmaceuticals are increasing in the world and as well as in Turkey. Today there are 170 nuclear medicine laboratories using the current radiopharmaceuticals in Turkey . It is known that during the preparation and application of them the strict regulations should be used not to give any harm to the patients. For this reason the standarts of radiopharmaceuticals should be strictly followed. In most of the countries, production and application of them are arranged by regulations.

The application of radiopharmaceuticals should be inspected by the government. Hospital radiopharmacy laboratories should be inspected for the production of radiopharmaceuticals. Distribution of radiopharmaceuticals should be inspected by the authorities and enduser should be able to performe some of the quality tests.

Radiopharmaceuticals should be produced according to GMP,GLP and GRP rules. To fulfil all these necessaties at the Turkish Atomic Energy Authority- Cekmece Nuclear Research & Training Center a Central Quality Control Laboratory has been established with the cooperation of Ministry of Health of Turkey. The IAEA has also given full support to this programme with the project TUR/2/014 " Establishment of a National Radioisotope and Radiopharmaceutical Quality Control Laboratory." Under this project expert mission, scientific visit, on-the- job training and some equipment supply were given by the IAEA.

Now the laboratory is succesfully performing the quality control tests of cold kits and radiopharmaceuticals imported to Turkey or produced in Turkey. These tests are radionuclidic, radiochemical, chemical and biological and performed according to the Eur Ph, USP monographs or according to the protocols supplied by the producers.

Labelling of risedronate with ^{99m}Tc for bone scanning

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Bisphosphonate-coupled radiopharmaceuticals are widely used in the clinical setting for imaging and metastatic bone pain [1]. Risedronate is a pyridinyl bisphosphonate. It binds to hydroxyapatite crystals in bone and inhibits osteoclast-dependent bone resorption [2, 3].

The aim of the present study is to label risedronate with ^{99m}Tc and to evaluate its *in vitro* stability and its biodistribution in rabbits.

Risedronate was labelled with ^{99m}Tc by a direct method. Labelling was achieved in the presence of stannous chloride as a reducing agent. After labelling with ^{99m}Tc, the product was analysed for impurities of Tc-99m pertechnetate and hydrolyzed-reduced technetium. This was accomplished using the 0.9 % saline and acetone. The labelling efficiency was determined using ascending paper chromatography on Whatman 3 MM paper. For *in vivo* biodistribution studies, ^{99m}Tc-risedronate was intravenously administered to rabbits and images were obtained by using a gamma camera.

According to the chromatographic results, the labelling efficiency was found 99 % without significant changes until 6 h post labelling at room temperature. The *in vivo* distribution of ^{99m}Tc-risedronate was studied during a 6 h period of time. Results showed that the bone/soft tissue ratio at 2.5 h after injection was 3 (Fig. 1).

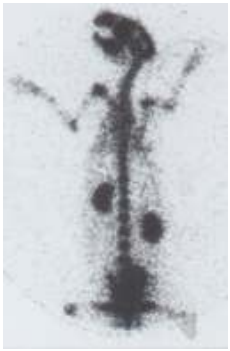

FIG. 1. The whole body image taken at 2.5 h after injection of ^{99m}Tc-risedronate in rabbit.

These preliminary findings show that, ^{99m}Tc-risedronate has promising characteristics for bone scanning in nuclear medicine.

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^{99m}Tc labelling of alendronate: a new potent biphosphonate

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Alendronate (4-Amino-1–Hydroxy Butylidene-1,1-bisphosphonic Acid) is one of the bisphosphonates. Alendronate may be more successful than medronate and etidronate on diagnosis of pure lytic metastases because of a indirect marker of bone resorption mediated by osteoclasts and more antiresorptif powerful. Also it could be a new radiopharmaceutical ajan on evaluation of the biphosphonates treatment efficiency by scintigraphy. The main objective of this study was to made ^{99m}Tc alendronate kit and investigate its biodistribution in an animals.

^{99m}Tc-ABP kit containing alendronate was prepared with reducing agent and a stabilizer. The kit was formulated with 5 mg ABP,0.36 mg stannous chloride and 0.025 mg gentisic acid at pH 3.5. Five mCi pertechnetate was used for labelling process. Labelling efficiency was measured by ITLC and was over 90% at room temperature. 0.1-0.2 mCi ^{99m}Tc ABP was injected to 7 anesthetized wistar rats (250-300 g). Scintingraphic images were obtained at 120 and 150 min. post injection.

High bone uptake and low background activity was observed in scintigraphic images. ^{99m}Tc ABP uptakes were 45% and 37% in bone tissues and kidneys respectively. There were little uptake and excretion in liver and intestines. The percentage was 2% and 3%, respectively.

The high bone uptake and less soft tissue retention of this new developed radiopharmaceutical for bone scanning was promising. Studies on ^{99m}Tc–alendronate biodistribution were completed in animals. Also the results had been encouraged us about labelling with rhenium for bone pallation. Studies on alendronate labeled with technetium and rhenium are going to be continued.

Preparation and characterization of ^{99m}Tc-RGD-yK-HYNIC peptide as potential radiopharmaceutical for nuclear oncology

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This paper presents the preparation and characterization of ^{99m}Tc-RGD-yK-HYNIC. This peptide [c(Arg-Gly-Asp-D-Tyr-Lys)]contains the RGD sequence, binding to αvβ3-Integrin receptors expressed during neoangeogenesis in tumours and surrounding blood vessels. The lysine residue was used to introduce Hydracinonicotinic acid (HYNIC) as binding moiety for technetium.

^{99m}Tc labelling was performed either using ethylenediamine diacetic acid, sodium salt (EDDA) (method 1) or tricine (method 2) as coligands.

Method 1 consisted in the addition of ^{99m}Tc-sodium pertechnetate (370-740 MBq), and peptide (5 μL, 5 μg) to a kit formulation (10 mg EDDA, 20 mg tricine, 50 mg manitol and 15 μg $SnCl_2$). After pH was adjusted to 6-6.5, the mixture was incubated at 70ºC for 30 minutes. Labelling by method 2 was performed by mixing tricine (35 mg, 0.5 mL) with the peptide (5 μL, 5 μg), followed by ^{99m}Tc- sodium pertechnetate (370-740 MBq) and $SnCl_2$ (1μg/mL in 0.1N HCl, 20 μL), at room temperature for 15-45 minutes.

Radiochemical purity up to 6 h post-labelling was evaluated by RP-HPLC using a binary gradient system (0.1% trifluoroacetic acid in water; 60% acetonitrile in water containing 0.1 % trifluoroacetic acid).

High specific activity (750-1500 Ci/mmol) was obtained by both methods. Radiochemical purity greater 91.0% and stability of at least 6 h was achieved when EDDA was used as coligand. Sep-pack purification using C18 light cartridges eluted with ethanol increased the radiochemical purity to >96 %, but only 40-50% of the activity was recovered. On the other hand, 2 different species (25 and 75 %, respectively) were formed when the coligand was tricine. However, only 0.9% of the activity corresponded to a low retention time species, probably oxidation products. No significant change was achieved by Sep-pack purification. Instability was significant, with an increase of oxidation products from 0.9% (t=0) to 8.6% (2 h) and 13.9% (4 h).

In vitro stability in plasma, kidney and liver homogenates was also studied. The Sep-pack purified radiolabelled peptides (100 μL) were incubated either with human plasma, or fresh liver or kidney homogenates (1 mL) for up to 120 min. After incubation times (10. 30, 60 and 120 min.) samples were precipitated with ethanol, centrifuged (1750g, 5min) and analysed by HPLC as described above. No evidences of metabolic degradation were observed.

Cystein challenge with 1000:1 molar excess of histidine was also performed. Radiolabelled peptides (40 μL), were incubated with cystein solution (40 μL) at 37 ºC and analysed by HPLC at 1, 2, 3 and 4 h. No evidence of ligand exchange was observed up to 4 h.

Binding to plasma protein at 30 min, 1, 2 and 3 h was also studied using size exclusion chromatography. Sep-pack purified peptides (25μL) were incubated with human plasma (450 μl) at 37°C, 25 μL added to Microspin G-50 columns (Pharmacia Biotech), and centrifuged at 2000 g for 2min. Protein bound peptide was calculated as the percentage eluted from the column. A very low protein binding of about 2% was obtained when EDDA was the coligand, indicating low lipophilicity and correlating with a high “in vitro” stability. Protein binding was not modified significantly by incubation times. A high protein bind of 44% resulted for the peptide having tricine as coligand. This result might be explained by the high “in vitro” instability of labelled compounds formed by method 2.

Biodistribution in normal CD1 mice (female, 25-30 g, 3 animals per time point) at 1 and 4 h post-injection was studied only for the labelled peptide obtained by method 1. Blood, lung and liver uptake at 1 hour post injection were low (1.6 ± 0.8%, 0.34±0.05% and 5.5±0.6%, respectively), as expected from a product with low lipophilicity and protein binding. Clearance from blood and lung after 4 h was almost complete. Excretion occurs mainly through the urinary tract (70 ± 6% and 78 ± 2 % at 1 and 4 h, respectively). Stomach and thyroid values were within acceptable levels (0.8 ± 0.1 % and 0.04.0 ± 0.01 %, respectively at 4 h.), indicating minimal "in vivo" reoxidation.

Tumour uptake was evaluated in an animal model obtained by subcutaneous inoculation of B16F1 murine melanoma cells ($0.5x10^6$ cells/animal) in C57B16 mice (female, 25-30 g, 3 animals per time point) at 1 and 4 h post injection. Tumour uptake at 1 hr post-injection was significant (2.1±0.7%,) but the clearance after 4 h was very important (0.7 ± 0.3%). Specificity of the uptake could not be proven.

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Clinical experience with ^{99m}Tc hynic-octreotide and ^{99m}Tc hynic-octreotate in patients with somatostatin expressing tumours

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In-111 labelled octreotide has been widely used for imaging of somatostatin expressing tumours. The drawbacks of In-111 labelling, such as high radiation burden, high cost and limited availability have led to the attempts to label octreotide with technetium. Recently octreotide and octreotate has been labelled with ^{99m}Tc using hynic as a conjugate. The aim of the present study was to evaluate the value of technetium labelled octreotide (TOC) and octreotate (TATE) in diagnosis of somatostatin positive tumours.

The study was composed of 83 patients ,55 female and 28 male. The ages were ranged from 3 to 82 with a mean of 44.5±39.3.Among 55 patients there were 19 carsinoid patients, 18 neuroendocrine tumour, 10 medullary thyroid cancer, 5 papillary thyroid cancer, 8 patients with thyroid eye disease, 6 ectopic Cushing disease, 4 gastrinoma and insulinoma, 3 pheochromacitoma, 1 paraganglioma, osteosarcoma.lymphoma and somatostatinoma. In 61 patients ^{99m}Tc hynic/TOC and in 22 patients ^{99m}Tc hynic/TATE were studied. All patient underwent a whole body scanning 1 and 4 h after injection and SPECT imaging was performed from the sites of suspicion.

In 60 patients studies were evaluated as positive and in 23 patient studies were evaluated as negative. There was no difference between early (1 h) and late (4 h) images in terms of diagnostic outcome. However the lession to background ratios were higher in early images as compared to that of late images. Lesion to background ratios fot TOC images were 7.44 ±8.42 and 9.20 ±10.96 at 1 h and 4 h respectively. Same ratio for TATE images were 5.20 ±5.29 and 10.76 ±10.37 at 1 h and 4 h respectively. SPECT images were extremely helpful in dedecting and localizing lesions.

Technetium labelled somatostatin receptor analogs octreotide and octreotate are excellent alternative to In-111 labelled octreotide for imaging somatostatin receptor positive tumours.Radiolabelling is easy, they are readily available for routine use and they give excellent images for clinical diagnosis.

The production of PET radiopharmaceuticals and generator-produced PET radionuclidles by iThemba LABS (South Africa)

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The most effective and sensitive method of imaging primary tumours and even very small metastases in cancer suffers, uses PET cameras. The iThemba LABS (South Africa) will produce and introduce PET (Positron Emission Tomogaphy) radiopharmaceuticals and radionuclides for the first time in the South African in the near future. Here we describe the progress that has been made at iThemba LABS with the production of PET radiopharmaceuticals such as ^{18}F-FDG and generator-produced positron emitting radionuclides such as ^{68}Ge/^{68}Ga and ^{82}Sr/^{82}Rb generators.

In order to establish state-of-the-art PET facilities in South Africa, it is essential to make available, PET radionuclides for the local nuclear medicine community. iThemba LABS as a production facility for accelerated based radionuclides, therefore, has played an important role in the establishment of PET in South Africa.

^{18}F-FDG (^{18}F: $t_{1/2}$ = 109 m) is regarded as the "workhorse" of PET, with over a million scans being carried out per year worldwide. Experience worldwide has shown that the use of ^{18}F-FDG imaging modifies the treatment recommended in 45% of cases [1]. We will describe here how the proton beam of the accelerator (66 MeV) was adapted for use with an IBA Stand Alone Target (SAT), first generation water targetry, to produce ^{18}F. In addition, it will be shown how the TRACERlab MX_{FDG} automated radiolabeling modules of GE Medical Systems will be used to produce the labelled ^{18}F-FDG.

^{68}Ga ($t_{1/2}$ = 68.3 m) is a positron emitter and can be utilized for diagnosis and tumour localisation with a PET camera, especially when labelled with peptides [2]. We describe a procedure for the production of the ^{68}Ge by solvent extraction (purification step) and the manufacture of the ^{68}Ge/^{68}Ga generator. The generator is manufactured in two stages: (1) preparation of the column generator using inorganic ion exchange SnO_2 and (2) the loading of the generator with ^{68}Ge. The design and the manufacture of the components making up the generator is also described.

^{82}Rb ($t_{1/2}$ = 75 s), is a positron emitter used for myocardial imaging with PET [3]. The demand for ^{82}Sr/^{82}Rb generator to produce sterile and non-pyrogenic ^{82}RbCl for injection with no significant breakthrough of ^{82}Sr/^{85}Sr is regarded as essential in the PET market. Here we describe a procedure for the production of ^{82}Sr by ion exchange chromatography (purification step) and the manufacture of the ^{82}Sr/^{82}Rb generator to produce the sterile ^{82}RbCl.

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Calcium phytate: A promising lung perfusion agent without risk of biological contamination

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Lung ventilation and perfusion imaging has evolved over the years and its routine use has become widespread, where more than 70% of pulmonary thromboembolism can be diagnosed through the ventilation-perfusion scintigraphy. This non-invasive diagnostic tool offers information on the lung functional vascular bed [1].

Historically the human Albumin macroaggregates and/or microspheres have been the selection radiopharmaceuticals to carry out the lung perfusion studies, where different formulations for diagnostic kits have been developed. However, due to the potential danger that represents the use of haemoderivates in the transmission of viral illnesses, specifically the Hepatitis C and the VIH, our country suspended at the beginning of the last decade the import of these kits.

As a result of this decision we tried to obtain a reagent kit based on non-derived blood particles that were big enough to be caught by pulmonary capillaries and to allow its use in the lung perfusion imaging. For this purpose was used phytate, because of the Inositol hexaphosphoric acid (phytic acid) forms complex with numerous cations, many of which show a markedly lower solubility to that of the sodium phytate in biological systems, being the chosen cation Ca^{2+} [2].

The experiments were carried out starting from a complete factorial design of the type 2^2, where the employed variables were:

Ca^{2+} concentration varied ranging from 5 to 15 mg of Ca^{2+} for a Ca^{2+}**:**Phytate molar ratio of 4.6:1 and 13.8:1, respectively.

Addition order of reagents, pre-supposing that this last influenced in the size of the particles:

Variant 1: Ca-Tc/Phytate; Variant 2: Tc-Phytate/Ca.

Some preliminary results on labelling of 1-hydroxyethylene-diphosphonic acid (HEDPA) with ^{90}Y

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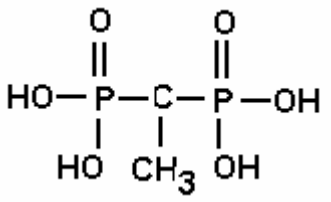
The interest for radiopharmaceuticals for direct management of serious illness and specially cancer and rheumatism has increased during the last decade. Such radioisotopes as ^{89}Sr, ^{186}Re, ^{153}Sm, ^{90}Y and ^{166}Ho are now used routinely in the practice of medical clinics. The tendency is to concentrate the studies in designing radioparmaceuticals that fulfill these important requirements: a) realize a high absorbing dose in malign cells in the shortest time and b) not damage the healthy cells.

Radioisotopes that emit α and β particles generally fulfill these requests. ^{90}Y is one of the radioisotopes of the choice. ^{90}Y has a LET useful for therapy. $E_{\beta max}$ = 2.3 MeV, $T_{1/2}$=64.1 h with no gamma emissions [1].

^{90}Y is produced from the homemade ^{90}Sr-^{90}Y generator. ^{90}Sr was fixed in Aminex-5 ion-exchange resin of Bio-Rad Company. The ^{90}Sr-^{90}Y generator consist of three chromatographic columns, the first was loaded ^{90}Sr, the second is for safety reason, with aim to fix breakthrough of ^{90}Sr from first column and the third column transforming ^{90}Y from organic complex form (α-hydroxyisobutyrate) in inorganic (cationic) form. The solution of ^{90}Y produced is of high purity and useful for labeling sensitive molecules[2]. This ^{90}Y solution is used for labeling 1-hydroxyethylenediphosphonic acid (HEDPA). The structural formula of HEDPA is:



Following reaction home makes HEDPA:

$$PCl_3 + CH_3COOH \rightarrow \text{HEDPA}$$

The aim of the work was to study the conditions of labeling, investigate yield of labeling, and the stability of constitute complex.

1. Initially the capability of complexion of HEDPA with ^{90}Y was studied. For this purpose it is used HPLC method to check formation of HEDPA-Y complex. It is mixed 1 ml of HEDPA solution (concentration 1mg/ml) with 0.1 ml solution of YCl_3 at pH≈5 (concentration 0.1mg/ml). It is compared RT of pure HEDPA with potentially formed complex. The reaction mixture has been studied by using HPLC system of KNUER Company and NucleosilmC18 5μm, as a column. As elute has been used a mixture of 0.05M acetate buffer at pH6 and methanol (ratio 93:7). The flux of the liquid was 0.2 ml/min and pressure 2–4 Mpa. 30 min

were sufficient for this process investigation. There exist evidently differences in RT between pure HEDPA and mixture HEDPA-Y.

2. Investigation of condition of labeling HEDPA-^{90}Y.

It is prepared stock solution of HEDPA with concentration 1mg/ml. This solution is used for further experiments. Preliminary experiments have indicated the importance of quality water that is used for experiments. Normal distilled water cannot be used, because of content trace of cationic ion as impurities. All experiments are done with solution prepared with three-distillation water. Solution of ^{90}Y is prepared in 1M ammonium acetate at pH≈5 It is studied the yield of labeling.

The investigation is performed in these conditions:

- 0.5 ml of HEDPA solution is mixed with 0.5 ml solution of 90Y +0.5 ml three distilled water at pH4.9 (A)
- 0.5 ml of HEDPA solution is mixed with 0.5 ml solution of 90Y +0.5 ml three distilled water at pH 5.4 (B).

Yield of labeling is checked by PC, Whatman No.1, and developer was 0.1M TRIS solution at pH7. Yield of labeling is studied 5, 60 and 180 min after mixing. Table I shows experimental data obtained.

TABLE I. EXPERIMENTAL DATA

Time, min	Yield of labeling (A) %	Yield of labeling (B) %
5	79.5	91.0
60	80.0	90.0
180	80.3	91.5
24 h	81.0	92.0

It is clear that yield of labeling is higher in pH 5.4. Also one can not observe significant differences between 5 min, 60 min and 180 min reaction time.

The product is also stable after 24 h.

Our task now is to continue improving the yield of labeling and evaluation complex behaviour in sera medium to follow later with animal experiments.

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Labelling of bombesin analogue by asymmetrical technetium-99m nitrido core

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Bombesin (BBN) analogue has been shown to bind selectively and avidly to GRP receptors on prostate, breast, pancreatic and colon cancer cells. A radiolabeled agent capable of identifying these tumors could benefit early diagnosis and therapy for patients. Recent developments in the preparation of Tc compounds based on the incorporation of the nitrogen donor in the metal coordination sphere for labeling small biomolecules led to the synthesis of complexes containing nitrido cores. The nitrido moiety $[Tc(N)]^{+2}$ is a powerful pi-electron donor and shows a high capacity to stabilize the Tc(V) oxidation state.

The aim was the synthesis of bombesin analog Cys-β-Ala-BBN (7-14)NH_2 (Cys-β-Ala-Q-W-A-V-G-H-L-M-NH_2) and the evaluation of the labeling using Tc-nitrido core by an asymmetrical method.

The labeling was conducted in two steps. The first step was the preparation of the precursor ^{99m}TcN species. To a ready lyophilized vial (CIS Bio International, Schering) containing as main reagents succinic acid dihydrazide (SDH) as nitride-donor ligand and stannous chloride as reduction agent for the pertechnetate, 0.5 mL of Na$^{99m}TcO_4$ eluted from a ^{99}Mo/^{99m}Tc in saline 0.9% (370 MBq) was added plus 0.5 mL of ethanol. The vial was left standing during 30 min at room temperature for reaction time. For the second step, 50 μL of a 0.9 mM water solution of Cys-BBN and 3 ug of a diphosphine (PNP6) dissolved in 200 μL ethanol were simultaneously added to the precursor vial. Reaction was carried out for one hour at 100 °C. Radiochemical evaluation of the TcN kit was done using ITLC-SG with a solvent system Ethanol/Chloroform/Toluene/Ammonium Acetate 0.5M (E/C/T/AA) (5;3;3;0.5) to detect TcO_4 and TcN and ethanol/water (1:1) for TcO_2. Radiochemical purity of the complex ^{99m}TcN-PNP-CysBBN was checked by ITLC-SG with the first solvent mixture indicated above, and also by HPLC.

The TcN yield obtained was 97.54 ± 1.14% and radiochemical purity for ^{99m}TcN(PNP)Cys-BBN was also high (96.29 ± 1.25%). In TLC analysis the Rf for TcN and for TcO4 obtained with the first solvent association was 0-0.1 and 1.0 respectively. To check TcO2 the second solvent (Ethanol/water) was used, because in this solvent TcN goes to solvent front. The Rf of ^{99m}TcN-PNP-CysBBN in (E/C/T/AA) was 1.0. Two peaks were observed in HPLC, a small one with retention time of 21.97 min and a bigger one at 22.76 min.

Even though the labeling had to be carried out in two steps, the procedure was simple and convenient, and radiochemical purity was high. These results recommend further studies of the complex in biological models .

Human polyclonal IgG labelled with three different Beta-emitter radionuclides: Labelling yields and in vitro stability studies

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The aim of the present work was to label human polyclonal immunoglobulin (IgG) with ^{153}Sm, ^{177}Lu and ^{188}Re and to evaluate labelling yields of the different procedures and the *in vitro* stability of the labelled products. a) Indirect labelling of IgG with ^{153}Sm and ^{177}Lu. 1st) Step: conjugation of bicyclic anhydride of diethylenetriamine-pentaacetic acid (cDTPA) (molar ratio 20:1 and 10:1) to IgG (c = 5 mg/ml) in CO_3HNa 0.1M pH 8.3. Incubation for 1h and purification by Shepadex G-50 column. 2nd) Step: labelling of conjugated IgG-DTPA (m = 1-2 mg) with ^{153}Sm (specific activity = 22.5 GBq/mg of Sm) and ^{177}Lu (specific activity = 3.47 GBq/mg of Lu) in the chemical form of acetate with activities near to 92.5 MBq. b) Indirect labelling of IgG with ^{188}Re-MAG_3. 1st) Step: labelling of the bifunctional chelating agent. 1.5 mg of $SnCl_2.2H_2O$ in 0.25 ml of citrate buffer 0.1M pH 5 and 0.75 mg of S-benzoyl-MAG_3 disolved in 0.25 ml of $CNCH_3$:H_2O (6:4) were added to the perrhenate (370 MBq) eluted from a ^{188}W/ ^{188}Re generator and incubated for 30 min at 90 °C under a nitrogen atmosphere. 2nd) Step: esterification of ^{188}Re-MAG_3 with tetrafluorophenol (molar ratio 35:1) using 25 mg of ethyl-3-(3-dimethylaminopropyl)-carbodiimide. After 40 min incubation the active ester was purified by Sep-Pak C18 and eluted with CH_3CN. 3rd) Step: Conjugation of the active ester to IgG (molar ratio 200:1). 0.5-6.3 mg of IgG (c = 10 mg/ml in buffer Na_2CO_3 0.1M pH 10) was added to the purified ester and incubated for 30 min. The ^{188}Re-MAG_3-IgG was purified by centrifugal filtration. c) Direct labelling of IgG with ^{188}Re. 1st) Step: IgG (c = 10 mg/mL) reduction with 2-mercaptoethanol (molar ratio 1000:1). Incubation for 30 minutes and purification by Shepadex G-50 column. 2nd) Step: $SnCl_2.2H_2O$, sodium tartrate and gentisic acid were added to the reduced IgG (1 mg), labelled with 2.2 mL of perrhenate (421.8 MBq) and incubated for 90 min. Quality control: The radiochemical purity of all labelled IgG was assayed by GP HPLC using a Protein Pack SW300 column with phosphate buffer 0.02 M pH 7.2 as eluent at 1 ml/min. In vitro stability: aliquots (100 µl) of each labelled IgG were incubated in saline solution at room temperature. The stability was evaluated by HPLC as described in quality control.

Results: a) ^{153}Sm-DTPA-IgG and ^{177}Lu-DTPA-IgG: the best labelling yields for the both radionuclides were obtained for conjugation ratios of 20:1 and were 86.9 % (incubation time 70 min) and 87.7 % respectively. Specific activities were 30.7 and 6.66 MBq/mg respectively. b) ^{188}Re-MAG_3-IgG: the labelling yields for each reaction step were 99 %, 75 % and 82.8 %. The maximum yield for the complete procedure was 62.1 % with a specific activity of 61.42 MBq/mg of protein. c) ^{188}Re-IgG was 98.7 % with a specific activity of 421.8 MBq/mg. *In vitro* stability in saline: the percentage of total activity bound to protein for samples of: indirectly labelled IgG (non scavenger added) with ^{153}Sm, ^{177}Lu and ^{188}Re, and directly labelled IgG with ^{188}Re (gentisic acid added) were 72.2 %, 67.7 % (both at 2 h), 94.0 % (at 21 h) and 12.5 % (at 22 h) respectively. Conclusions: The direct labelling method with ^{188}Re showed the highest specific activity (421.8 MBq/mg). The best *in vitro* stability (94.0 % in

saline at 21 h) correspond to ^{188}Re-MAG$_3$-IgG although this reaction showed the lowest labelling yield. The higher specific activity obtained when labelling with ^{188}Re eluted from a generator improves the application of labelled biomolecules in targeted radiotherapy. New experiences must be done with ^{153}Sm and ^{177}Lu obtained from irradiation of isotopically enriched targets.

Evaluation of a ^{32}P patch for therapy of squamous cell carcinoma in cats

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Brachytherapy, that is, the placement of radioactive sources into or near the tumour, has been a big challenge in nuclear medicine for the therapy of cancer. PirocarbotratTM, is a gelatin-protected charcoal suspension labeled with chromic [^{32}P] pyrophosphate that behaves very much like a sealed B- radiation source for the treatment of solid tumours. The aim of this work was to make use of these properties in order to design a silicon patch coated with PirocarbotratTM for topical application in skin cancer lesions. Materials and methods: we selected four adult cats with nasal, state II squamous cell carcinoma (SCC). Measurements of the lesions sizes were taken and the patches were specially designed for its application on the lesion surface with minimal contact with the normal surrounding tissue. Animals were then anesthetized to get immobilized to both facilitate patches application and to prevent their remotion. Dosimetric calculations were done in each case taking into account the time of exposure and the activity contained in the patch. Total surface of the patches was 4.5 cm^2 and the activity per surface varied between 30.7 - 32.2 MBq (871.3 - 830.1 uCi / cm^2). The patches were applied on the surface of the nose SCC lesions for 3.5 - 4.0 h for a total radiation dose of 28 - 33 Gy (2800 - 3300 rads). During the time of exposition, the animals were isolated in cages specially conditioned for this purpose. When exposition time finished, the patches were removed and the animals were returned to their owners.

Clinical evaluation after fifteen days of the treatment showed that in one case tumour disappeared and an erythema with alopecia and hypopigmentation developed in the treated site. In the other three cases lesion reductions were about 50% of their original size with concomitant development of peritumoural fibrosis as well as central necrosis in the treated site. The shared feature in the four cases was the great local inflammatory response after brachytherapy at the site where patches were applied. All these responses are in accordance with those expected after radiation therapy. Furthermore, as total radiation dose (28-33 Gy) was delivered in only one session of 3.5-4.0 h, tissue damage such as fibrosis, necrosis and erythema of the treated site is also indicative of the effectiveness of the treatment. However, the histopathological results of the follow-up biopsies, showed that total remission was not achieved in none of the four cats. Conclusions: Surface applicators are used for superficial tumours as the maximum dose is at the surface and falls off rapidly with depth. On the other hand, dose fractionation is related to tissue repair which depends on the cell turnover rate of the tissue nature (normal or neoplasic). Therefore, the treatment planning should take into account both lesion depth and tissue rate of cell turnover in order to achieve the local control of the tumour for a longer period and to prolong survival. Although total remission was not achieved, invasion and dissemination of the tumours were prevented after brachytherapy. Therefore, this clinical experience allow us to confirm treatment efficacy of the ^{32}P patch for skin cancer but signalling the importance of the planning dose scheme since until now, only

partial remission was achieved. Future directions will lie on finding optimal dose fractionation or extending exposure time with patches of minor activity per surface in order to achieve total remission.

Inhibitors of adrenal P-450c11 hydroxylase characterized by radioligand displacement and steroid hormone secretion

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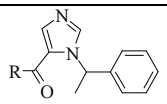
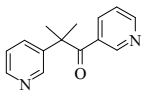
Inhibitors of steroid 11β-hydroxylation have been evaluated as radiotracers for adrenal scintigraphy. Derivatives of metyrapone had been synthesized for labelling with iodine [1-5] and PET radionuclides [6-7]. Etomidate (ETO), a potent inhibitor of cortisol biosynthesis [8] was derivatized in the course of the development of 4-^{131}I-iodophenyl-metomidate (^{131}I-IMTO), recently introduced for SPECT [9]. Functional derivatives of ETO have contributed to radiotracer design by identifying the structural requirements of specific radioligand binding to P450c11 hydroxylase. The interaction of ETO with cytochrome P-450c11 is highly selective and differs from metyrapone, which is bound to adrenocortical mitochondria and also to liver microsomes. Here, inhibitors were evaluated as displacers of specific radioligand binding to the membrane-bound mitochondrial enzyme system using 4-^{131}I-iodophenyl-metomidate (^{131}I-IMTO) and ^{3}H-metyrapol as radioligands, respectively. Binding inhibition of derivatives will be correlated with IC_{50}-values obtained by the inhibition of cortisol production in a cell culture [10].

Membranes prepared from rat adrenals were incubated with 20.000-40.000 cpm of ^{131}I-IMTO together with 2 nM carrier (resulting in a specific activity of 330-660 GBq/mmol) at 23°C for 20-30 minutes. Bound radioligand was isolated by filtration through glass fiber filters. For displacement studies test compounds were added at 0.1–100 nM. Non-specific binding was determined with ETO (10 μM). IC_{50} values were evaluated by non-linear, least squares regression analysis. In case of ^{3}H-metyrapol binding membranes were incubated with 10 nM radioligand (12 Ci/mmol) for 15 minutes on ice. Bound radioligand was separated by centrifugation (15 min at 40.000 x g). Imidazole phenylmetyrapone (10 μM) was used to determine non-specific binding.

ETO and MTO derivatives were chemically characterized as inhibitors of specific radioligand binding (Tab. 1). ETO showed the highest binding affinity (IC_{50} 1.08 nM). A comparison of the IC_{50}-values obtained with each radioligand shows that ^{3}H-metyrapol binding shares several key SARs with ^{131}I-IMTO binding: At both binding sites, (R)-MTO is preferred to (S)-MTO, the intact ester function (ETO, MTO, FETO) is essential for binding, since the free carboxylic acid is inactive. Modification of the ethyl ester (ETO) is accepted, offering a synthetic route to PET radiotracers; compounds with pyridyl residues (Py, BiPy) in place of phenyl act more potently with the ^{3}H-metyrapol binding site, just like metyrapone and metyrapol. The higher potency of the precursor phenylmetyrapone has been verified at both binding sites.

Specific binding to P-450c11 hydroxylase depends on an intact ester, the potency resides exclusively in the (R)-isomer. MTO inhibits both binding sites with high potency. Interaction sites involved in the binding of metyrapol are also involved in the binding of MTO, however, only a fraction of these sites contribute to metyrapol binding. The different affinities of metyrapol and metyrapone for ^{131}I-IMTO binding sites are expressed by their IC_{50}-values. Results presented in Table I suggest a common binding site on adrenal 11β-hydroxylase shared by MTO and metyrapol as interaction site.

TABLE I. COMPARISON OF THE INHIBITORY POTENCY OF ETO AND METYRAPONE DERIVATIVES BY THE DISPLACEMENT OF ^{131}I-IMTO AND ^{3}H-METYRAPOL

 ETO-derivatives	 Metyrapone	Displacement of ^{131}I-iodo-MTO	Displacement of ^{3}H-metyrapol
Inhibitors of P-450c11	R	IC_{50} (nM)	IC_{50} (nM)
(R)-ETO	C_2H_5O-	1.08 ± 0.42	1.72 ± 0.19
(R)-MTO	CH_3O-	3.69 ± 1.92	2.65 ± 0.69
(S)-MTO	CH_3O-	492 ± 2.81	179.5 ± 3.53
FETO	FC_2H_4O-	2.9 ± 0.5	2.19 ± 0.28
(R)-Pyridinyl-MTO (Py)		20.7 ± 3.8	5.51 ± 0.12
(R)-Bipyridinyl-MTO (BiPy)		179.7 ± 66.1	10.8 ± 3.4
(R)-MTO-acid		123.000 ± 41.000	34.300 ± 10.900
Metyrapone		1.160 ± 790	38.9 ± 0.56
Metyrapol		1.020 ± 390	99 ± 30
Phenylmetyrapone		183 ± 72	14.0 ± 4.7
Ketoconazole		710 ± 490	63 ± 20
11-Deoxycorticosterone (DOC)		890 ± 330	1.140 ± 290

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Use of Sr-90 beta emitter as an antifungal agent - an innovative dimension in therapeutic nuclear medicine

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The use of ionizing radiation in dermatological practice has been well recognized for many years[1]. However, its routine practice has markedly decreased owing to either the development of more efficient medications and / or to the increased awareness of potential genetic and somatic hazard of radiation.

In treating onychomycosis, the therapeutic limitations of conventional antimycotic agents (in respect of low cure rates, high relapse rate, inherent side effects, long duration of treatment and high expense) have provided clear incentives to explore alternative therapy procedures [2.3].

Next to ^{131}I, ^{90}Sr is being considered to be one of the most important β emitting therapeutic agents in current practice of nuclear medicine. In this present study, ^{90}Sr has been used for treatment of onychomycosis.

The objectives of the present research work were:

To use Sr-90 source (beta radiation) as a curative therapy for Onychomycosis, optimisation of its dosages and to promote an innovative clinical development in the field of therapeutic application of nuclear medicine.

To assess the efficacy of beta radiation either alone or in combination with conventional antifungal drugs, and

To reduce the duration of drug exposure and cost of treatment for onychomycosis.

Using the appropriate statistical formula, sample size of the study population was determined and in each group 92 patients were assigned. With an assumption of patients drop out and for better statistical analysis, a total of 330 patients were randomly allocated to enter in therapeutic regimen. They had all been clinically and mycologically diagnosed to have onychomycosis.

The study population was then divided into three groups:

Group – A (n =110) received griseofulvin orally 500mg once daily for 12-16 weeks; Group – B (n=110) received beta radiation, 500 rads twice in a week for 3 weeks (total 2500 rads); and Group – C (n=110) received combined beta radiation (total 2500 rads in 3 weeks) and

griseofulvin (500 mg daily for 6 weeks). Patients were followed up for 24 weeks. Efficacy of the treatment was evaluated in all 287 patients while 43 (13.03%) cases were dropped out from the initial allocation.

At the end of the follow up period (6 months after discontinuation of treatment) mycological cure rate was achieved 41 (42.70%), 36 (38.70%) and 65 (66.33%) in Group-A, Group-B and Group-C respectively. The mycological cure rate was highly significant (P=0.000) and considered to be the acceptable outcome of treatment. Clinical cure rate was considered as another way of assessment. Percentage of clinical cure rate was similar as mycological cure rate and equally significant (P=0.000). Recurrence rate of the disease was highest in griseofulvin-induced patients 21 (21.88%) and in beta radiation exposed patients was 14 (15.06%). This rate was least in combination therapy group of griseofulvin and beta radiation 4 (4.08%). Cure rate in Group – C is significantly higher than Group – A and B as well (P=0.000).

Several known side effects causing systemic involvement of oral drugs are already being experienced, side effects like blackening of surrounding soft tissue of nail were observed which were transient and self limiting [4]. Further to this, all sample population underwent for biochemical and haematological tests pre and post radiation application. No significant change of tests results were observed excluding any observable radiation side affects to this particular type of radiation application.

It can be concluded that the proposed new beta radiation treatment modality using Sr-90 for onychomycosis exhibited a low risk- benefit ratio. It is well tolerated and efficacious method to treat onychomycosis. From the observations of the present study it may be considered worthy to comment that in Group – C as the cure rate is highest, recurrent rate is the lowest, duration and cost of treatment are significantly less, this modality of treatment can be considered as the more acceptable procedure for management of onychomycosis in a developing country like Bangladesh. Group – B (beta radiation only) can also be accepted in special occasions to replace Group – A (Antifungal).

This innovative procedure of treatment could be introduced in other Nuclear Medicine Centres across the country benefiting a larger number of patients.

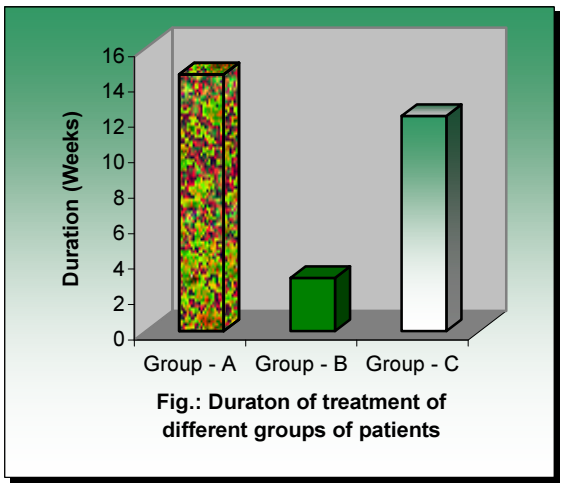

Fig.: Duraton of treatment of different groups of patients

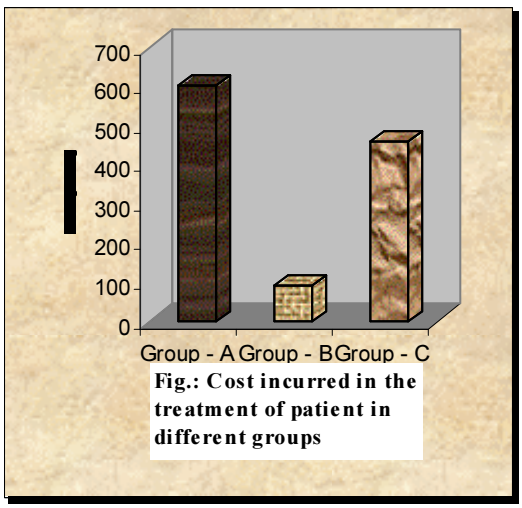

Fig.: Cost incurred in the treatment of patient in different groups

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^{131}I treatment response in thyroid neoplasms and evaluation of radiation dose complications. A review of ten years of experience

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Thyroid cancer (TC) is the most common endocrine malignancy , but its management is still a controversial issue that included an interdisciplinary approach who compounds surgery, radioiodine (RAI) therapy and sometimes external radiation therapy. The aim of our work is to evaluate the role of the RAI treatment in well differenciated thyroid carcinoma and its implication in terms of survival and complications. Methods and Material: we collected data from medical records of 215 patients with confirmed TC, registered from 1990 -2004 at the Nuclear Medicine Unit, Hospital Temuco, Chile, who received 50 to 150 mCi RAI therapy four to six weeks after lobectomy, partial or total thyroidectomy. Clinical and laboratory examinations were analyzed including blood count, thorax X Ray, whole body I-131 scans, and serum thyroglobulin. Results: the median age at the diagnosis was 51 years, there were 89 % females. All patients were treated surgically and recived postoperative RAI treatment. Total thyroidectomy, subtotal thyroidectomy and nodule excision was done in 69%, 31% and 37% of patients respectively. The histopathologic results included papillary (62 %), follicular (24%) and papillary-follicular carcinoma (14 %). Regional lymph nodes were positive in 36 % and distant metastases were detected in 20 %, located in the lungs in all of those patients. Additional RAI doses between 100-200 mCi were administrated in 35% patients after to wait a period longer than 1 year. The overall survival rate at 14 years was 93 %, and recurrent disease was detected in 15%. at 5 years and 20% at 14 years from the diagnosis. There were no major complications and minimal alterations in the blood count was observed. We conclude that total or near total thyroidectomy followed by RAI treatment appears to benefit for better survival and lower recurrence disease. Favorable prognostic management included radioiodine therapy at the primary treatment using high actvities (more than 100 mCi) after total or near total thyroidectomy. Additional pulmonary metastases did not influence prognosis, and secondary effects were mild and related to the symptoms of hypothyroidism.

^{188}Re labelled anti-EGFr humanized monoclonal antibody h-R3 for radioimmunotherapy of glyomas

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Locally administered monoclonal antibodies labelled with radioisotopes like ^{90}Y, ^{131}I, ^{186}Re, ^{212}Bi, ^{211}At, ^{177}Lu and others, constitute a viable and promising alternative for management different kind of malignancies. The development of ^{188}W/^{188}Re generator has given the possibility of having a radionuclide showing satisfactory features for radioimmunotherapy (E_β=2.12 MeV, E_γ=155 keV, $T_{1/2}$=16.9 h and easy to make labelling approaches, similar to used for ^{99m}Tc).

Neuroepithelial-derived tumours have an overexpression of the EGF receptor with regard to adjacent normal tissue. It could be related with the autocrine stimulation of the neoplasm by EGF and TGFα. Humanized monoclonal antibody h-R3 has shown high affinity for this EGF receptor, blocking the binding of EGF to it receptor and inducing apoptosis. Thus, it could be a good candidate for radioimmunotherapy of neuroepithelial malignancies. The aim of the present work was to label monoclonal antibody h-R3 with ^{188}Re, to assess it an animal model and evaluate its internal dosimetry in patients with grade III-IV glyomas.

Direct labelling method was employed, using 2-mercaptoethanol as a reducing agent. The amount of sodium glucoheptonate, ascorbic acid and stannous fluoride were varied to achieve optimum labelling yield. ^{188}Re-labeling yield was proportional to the volume of stannous glucoheptonate solution added to the formulation. Radiochemical purity of ^{188}Re-h-R3 was 98.0±0.4%. Challenge against 300-fold molar excess of L-cysteine was made to assess the stability of the tracer. There was not found significant difference between stability of ^{188}Re-h-R3 and ^{99m}Tc-h-R3 against cysteine challenge up to 24 h.

Animal biodistribution study was performed at 3 and 24 h after intravenous administration of ^{188}Re-h-R3 through tale vein of Male Wistar rats. The results were compared with those attained using ^{99m}Tc-h-R3 as a control. Biodistribution study showed high radiopharmaceutical uptake in kidneys and small intestine. Urinary excretion was similar for the antibody labelled with ^{188}Re as well as ^{99m}Tc.

A Phase I dose escalation trial was performed by administering into the post-operative cavity through an indwelling catheter a single dose ^{188}Re-h-R3. The study was reviewed and approved by the ethics Committees of the all involved institutions. Five patients with partial tumour resections have been included. Immunihistochemical study of tumours showed an overexpression of EGFr. SPECT and planar images as well as multiple blood and urine samples were collected up to 24 h after administration of 3 mg of MAb labelled with 10 or 15

mCi of ^{188}Re. Biodistribution was computed from scintigraphic images and the absorbed dose were estimated using the MIRD methodology at organ and voxel level. Data processing and statistical analyses were performed using the SPSS and Microcal Origin v6.0 software packages.

The effective half-life of the ^{188}Re-h-R3 in the tumoural bed were ranged 7,3-14,4 h (mean value 8,4±2,8 h). The liver, kidneys and urinary bladder showed the highest uptakes of the compound leaving the tumoural bed. The mean absorbed dose in the tumour ranged 13,9 Gy-68,4 Gy and the maximum doses were ranged 26,9 Gy-136,2 Gy. The maximum absorbed dose for liver, kidneys and urinary bladder was lower than 2 Gy in all patients. Transitory acute side effects following treatment were headache, seizures, and worsening of pre-existing neurological symptoms. Two patients developed stable disease during 3 months, 2 patients with multiform glyoblastoma are practically asymptomatic and in complete remission after one year of treatment. The other patient with glyoblastoma multiform is not yet evaluable after one month of treatment.

Proposed procedure allowed the stable efficient labelling of h-R3 with ^{188}Re. Preliminary results of this study strongly suggest that loco-regional radioimmunotherapy of high grade glyoma using the anti-EGFr humanized monoclonal antibody h-R3 labelled with ^{188}Re may be safe and constitute a promising therapeutic approach for these patients.

Dimeric scFv antibody construct of ior-CEA1 as potential agent for therapeutic application

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The classic radioimmunotherapy is based on transporting to the tumour, the necessary activity of a therapeutic isotope using a monoclonal antibody (MAb) against tumour-associated antigen, which constitutes an ideal vehicle given its inherent specificity. In this sense, the whole immunoglobulins (Ig) have some severe practical limitations due to their unhappy pharmacokinetics, their relatively high molecular weight (150 kDa) and slowed clearance. Likewise the Ig molecule presents a poor diffusion through the tumour mass and a relative high immunogenicity.

The solution to these problems has been encountered by genetic engineering, methods where the multivalent recombinant fragments have become the paradigm of design of constructed molecules. They are able to retain the the parental antibodies specificity and affinity, with reduced immunogenicity and improved pharmacokinetics.

The monoclonal antibody ior-CEA1 has been employed for the diagnosis of primary tumours, recurrences and metastasis of colorectal tumours and has also been used extensively in the daily clinical practice as well [1,2]. Inserted in this rebirth of the antibodies, this report explores the use of a new smaller molecular weight multivalent analogue of the parent ior-CEA1 antibody to be used for the diagnosis and therapy of CEA-expressing cancer tumours such as colorectal, lung, ovary among others.

The dimeric scFv-ior-CEA1 construct was produced in the Centre for Genetic Engineering and Biotechnology and displayed to bind CEA epitope with a similar binding affinity to that of the murine IgG. Labelling method was achieved using Chloramine T, with a molar ration of Chloramine T**:**tyrosine of 2,5. The methodology proposed for the radioiodation attained incorporation over 90% of radioiodine to the protein. Although it is a relative high labelling yield, for the immunoreactivity studies and biodistribution the fragment was purified by means of FPLC.

For the comparison of the immunoreactivity of labelled and unlabelled diabody, was plotted the specific union vs the antibody concentration for both molecules and adjusted to a straight line. The results showed that the ^{131}I-$(scFv)_2$ retain 85% of its immunoreactivity after labelling. This decreasing in the immunoreactive capacity could be attributable to the iodine/tyrosine binding in the region of recognition of the molecule. That is, from the 16 tyrosine present in each scFv, four of them are in the complementarity determinant regions (1 in V_L CDR1 and 3 en V_L CDR3), and due to the voluminous size of the iodine atom, it could produce steric impediments that hinder the formation of the antigen-antibody complex. The non-specific union in the study was of 6%. The Scatchard analysis was used to calculate the apparent affinity constant. The binding affinity was of $3{,}5 \times 10^7$ M^{-1}, similar to other biomolecules of this construct [3].

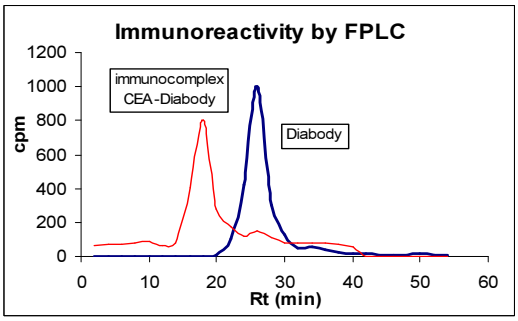

FIG 1. ^{131}I-Diabody and immunoconjugate CEA/^{131}I-(scFv)$_2$ analized by size exclusion FPLC.

The immunoreactivity was also assayed using size-exclusion FPLC to evaluate their antigen-binding capacities after radiolabelling. Seventy-five nanograms of radiolabelled ^{131}I-(scFv)$_2$ were incubated with 15-fold excess of CEA for 1 h at room temperature and the sample was analysed by size-exclusion FPLC. Figure 1 shows the radiochromatograms of Diabody and immunoconjugate CEA/(scFv)$_2$, where a radioactive bulk is eluted with a lower retention time suggesting the immunocomplex formation. The immunoreactive fraction of the radioiodide fragment can be calculated from radiochromatogram by means of the integration of the area under the curve for the corresponding fractions to the complex antigen-antibody and the ^{131}I-diabody. The obtained result was 87%, similar to 85% obtained by means of the classic technique.

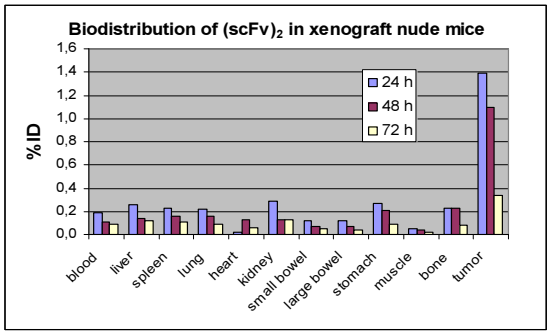

FIG. 2. Biodistribution the ^{125}I-ior CEA1-(scFv)$_2$ in CEA xenograft nude mice at 24, 48 and 72h.

In order to study the "in vivo" stability and immunoreactivity of the diabody, as well as to check the possible cross-reaction with the antigens NCA-95 expressed in granulocytes, whose previous variant of the scFv exhibited [4]. It was carried out a study of biodistribution of the ^{131}I ior-CEA1-(scFv)$_2$ in MNRI healthy and nude mice bearing LS174T human CEA-positive tumours.

The results (Fig. 2) showed a typical behaviour for this kind of biomolecules, without significant localisation in any critical organ. Being this, an advantage for its possible use as radioimmunotherapeutic agent, since the exposition levels in healthy tissues will be low. Both biodistribution exhibited a low uptake in bone marrow suggested the absence of crossed reactions with the NCA-95 antigen. In the figure can be also observed a high uptake in tumour. The excellent tumour**:**blood ratios reflect the specific retention in the target tumour and avidity of the biomolecule for the CEA antigen.

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Development of a new ^{131}I-labelled bisphosphonic acid for palliative therapy of metastatic bone pain

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Approximately, 80% of all patients with cancer in advance stage could develop bone metastases. Nowadays, the management of metastatic bone pain remains controversial. Analgesics are widely described according to the World Health Organization analgesic ladder supplemented by local field, external beam irradiation for persisting skeletal symptoms. In the context of advancing malignancy, however, many patients progress to multi-site, poorly localized or migratory pain, which is less amenable to local field irradiation. Wide field, hemibody radiotherapy is useful in this setting, but it is limited by significant morbility, particularly gastrointestinal disturbance, when the abdomen and pelvis are included in the treatment field.

The use of bone seeking radiopharmaceuticals represents a major advance in the management of refractory metastatic bone pain. Acting systemically, this approach allows multiple sites to be treated simultaneously with relatively sparing of healthy surrounding tissues. As a result, toxicity is low in comparison with other systemic therapies and treatment is well tolerated. Used isotopes for pain palliation by bone metastases are very expensive, with exception of ^{32}P, which could cause bone-marrow depression. The aim of the present work was to develop a new bisphosphonic acid labeled with ^{131}I for metastatic bone-pain palliation.

An aryl-substituted bisphosphonic acid (PICIC-1), which would easily accept the ^{131}I, was synthesized starting from DL-tyrosine. Reaction was made into three phases: 1. protection of amine group with methylchloroformiate; 2. phosphonation of carboxyl group by reaction with a mixture of phosphorous acid and phosphorous pentachloride and 3. deprotection of amine group by hydrolysis. The reaction yield was 60%. Purity of obtained compound was tested by reverse-phase HPLC using a RP18 column (4.6 mm x 100 mm) and a gradient from 0% to 100% of methanol in water as mobile phase. The chemical purity was higher 97%.

The structure of the synthesized compound was analyzed by IR and NMR spectroscopy and by electrospray mass-spectrometry. The IR spectrum showed a wide peak due to the absorbed water, because the compound was highly hygroscopic. Despite, this peak hindered the interpretation of the spectrum, there were observed characteristic peaks of phosphonic groups and aromatic ring. NMR spectrum showed characteristic peaks corresponding to main functional groups of the molecule. Mass-spectrum showed two main peaks: one at 308.9 Da related with the loss of one OH group and the other at 325.1 Da corresponding to the molecular weight of attained bisphosphonic acid.

Compound was labeled with ^{131}I by using Chloramine-T method. Then, it was purified through AgCl filters. Labeling yield was higher 95%. The stability of the label was assessed up to 72 h in PBS 0.05 M pH = 7.0-7.2 and HSA. Just after 72 h incubation, the dehalogenation was significantly higher in HSA (≈15%) than in PBS.

Biodistribution was studied in male Sprague Dawly rats (190-210g). Two hundred μCi (7.4 MBq) of ^{131}I-PICIC-1 (0.1 mg) were injected through a lateral tail vein and organs were removed at 2 h, 24 h, 48 h. Scintigraphic images of the rats were performed up to 48h after intravenous administration of the radiopharmaceutical.

Synthesized compound showed a bone uptake of 1.2% of administered dose per gram of tissue. Significative uptake was observed in kidneys and bowels, suggesting an excretion pathway by these organs. Skeleton of the rat was visualized in scintigraphic images, with an uptake proportional to metabolic activity.

^{131}I-labeled-PICIC-1 showed satisfactory bone affinity and could be a promising new radiopharmaceutical for metastatic bone-pain palliation.

The role of somatostatin receptors in normal tissues in pharmacokinetics of radiolabelled octreotates

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Radiolabelled somatostatin analogues have great potential for the visualization and treatment of somatostatin receptor-positive tumours and their metastases. But receptors for somatostatin can also be expressed with different density in normal organs and tissues. In this report we studied the effect of somatostatin receptor blockade on distribution profiles and elimination characteristics of DOTA-Tyr^3-octreotate (DOTATATE) labelled with ^{90}Y, ^{111}In, and ^{153}Sm in rats.

Animal studies were carried out using male Wistar rats. Radiolabelled DOTATATEs we administered to animals intravenously in the dose of 1 μg/kg. To determine the effect of somatostatin receptor blockade to distribution and elimination of the agents, some groups of rats were pretreated by intravenous injection of 0.1 mg/kg unlabelled octreotide (Sandostatin) 15 min before the radiolabelled DOTATATE administration.

Somatostatin receptor blockade substantially descreased radioactivity uptake in the adrenals and pancreas of animals. This finding is in agreement with the fact that specific binding sites for somatostatin are present in both the exocrine and endocrine pancreas and also in the adrenals of rats. A partial reduction of radioactivity accumulation in the thyroid, skin and bone of somatostatin receptor-blocked animals was also found. Moreover, a significant decrease of radioactivity in gastrointestinal tract (both stomach and bowels) in pretreated groups was also determined. Radioactivity uptake in the gastrointestinal tract may be partly due to hepatobiliary excretion of the peptides and/or their metabolites and partly due to excretory mechanisms involving specific binding of the agents to somatostatin receptors localized in the gastric wall and a consequent secretion of the radiolabel to the gastric content. According to our results, bile excretion of radiolabelled DOTATATEs determined in the perfused rat liver was very low and negligible. The results suggest that receptor-specific binding plays in important role in the accumulation of the peptides in the gastrointestinal wall of rats. In agreement with these results, a substantial decrease of radioactivity excretion by feces and a higher and more rapid urine elimination of radioactivity after receptor blockade in comparison with control groups were determined. High and long-term radioactivity uptake in the kidneys for all peptides under study was found. Kidney accumulation of radioactivity expressed in the percents of the administered dose was surprisingly slightly higher after somatostatin receptor blockade in comparison with that of control animals. In case the renal uptake of the peptide may be partly due to the somatostatin receptor-mediated process, an opposite results could be expected. However, it is necessary to have in mind that radioactivity uptake in the kidney would be related rather to the radioactivity passed trough the kidney (i.e., the kidney + urine) than to the administered dose. In case such calculation was made, a mild decrease in the renal uptake of radioactivity was determined after somatostatin receptor blockade.

In conclusion, receptor-specific binding of radiolabelled somatostin analogues alters their distribution profile in normal organs, tissues and systems and also changes the elimination pathways of radioactivity in rats. In interspecies comparison of these results, species

differences in expressing of somatostatin receptor subtypes in individual organs have to be considered.

The study originated within a Coordinated Research Project of the IAEA - Research Agreement No. 12125 and was financially supported by the Grant Agency of Charles University - grant No. 146/2004.

Preclinical study of radioiodinated glucose-Tyr3-octreotate: Comparison with ^{111}In-DOTA-Tyr3-octreotate

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Targeted radionuclide imaging and treatment are based upon the interaction of radioligands with receptors in the target tissue (namely high density receptor specific tumours). As receptors for somatostatin (mainly somatostatin receptor subtype-2 /$sstr_2$/) are over-expressed in several human tumours of endocrine origin, a number of radiolabeled somatostatin analogs have been recently introduced as the vectors for targeted imaging and therapy; commercially available ^{111}In-DTPA-octreotide being a gold standard in this field. Several publications demonstrate that a substitution of Tyr instead of Phe in the peptide position 3 and oxidation of carboxyl end of octreotide significantly increased the affinity of the peptide to $sstr_2$. More recently it has been shown that NH_2-terminal carbohydration leads to a further improvement of the peptide receptor affinity and its retention in the tumour (1).

In this study we present preclinical analysis of distribution profile and elimination pathways of radioiodinated glucose-Tyr^3-octreotate (^{125}I-gluc-TOCA) in comparison with that of another promising targeted radiopharmaceutical, namely ^{111}In-DOTA-Tyr^3-octreotate (^{111}In-DOTA-TOCA).

Gluc-TOCA was radioiodinated using chloramine-T method. For radiolabeling of DOTA-TOCA with radiometal, to 200 μl of 0.4 M acetate buffer with 0.24 M gentisic acid pH 5, 10μl of peptide solution in 0.1% TFA (1mg/ml) were added together with 0.5-1 mCi of $^{111}InCl_3$ in 0.04 M HCl. Reaction mixture was heated 25 minutes to 90-95°C. Pharmacokinetic studies were performed on male Wistar rats.

Results confirmed that specific internalization of ^{125}I-gluc-TOCA by $sstr_2$ – expressing AR423 rat pancreatic carcinoma cells in vitro was about twice of that for ^{111}In-DOTA-TOCA. In rats, ^{125}I-gluc-TOCA exhibited prolonged plasma clearance in comparison with the other peptide. Slower decrease of plasma radioactivity after ^{125}I-gluc-TOCA was due to its higher lipophilicity and thus higher plasma protein binding resulting in slower elimination rate in the kidney (the main elimination pathway being glomerular filtration). Radioactivity accumulation of ^{125}I-gluc-TOCA in organs with high density of somatostatin receptors (the pancreas and adrenals) was significantly higher and radioactivity uptakes in the kidney significantly lower in comparison with ^{111}In-DOTA-TOCA at 1 hr and 2 hrs after dosing. 24 and 48 hrs after administration, high and long term radioactivity retention in the pancreas, adrenals and the kidney residualized after ^{111}In-DOTA-TOCA whereas in case of ^{125}I-gluc-TOCA a substantial decrease in radioactivity concentrations in all organs and tissues were determined. In somatostatin receptor reach organs the peptides under study were internalized, transferred to lysosomes and digested by proteolytic enzymes. Simultaneously, in the kidney the peptides were eliminated by glomerular filtration and subsequently partially reabsorbed in the proximal tubular cells (probably by receptor-mediated endocytosis via megalin/cubilin system). The peptides were consequently transported to lysosomes and also digested. Whereas

resulting breakdown products remained in the lysosomes for a long post-injection period after ^{111}In-DOTA-TOCA, degradation products of ^{125}I-gluc-TOCA diffused from the site of peptide degradation and turned to the blood stream. Consequently, they were partly eliminated by urine and partly trapped by specific organs (namely the thyroid). HPLC analysis of radioactivity in the urine confirmed predominant elimination of the parent peptide after ^{111}In-DOTA-TOCA. In case of ^{125}I-gluc-TOCA the intact peptide was found in urine 2 hrs after dosing whereas at 24 and 48 hrs after administration the eliminated radioactivity was mostly in the form of ^{125}I-gluc-TOCA - metabolites. Rat liver perfusion experiments showed that bile clearance of ^{111}In-DOTA-TOCA was negligible. Even if bile clearance of ^{125}I-gluc-TOCA was substantially higher (0.253 ± 0.118 ml/min in comparison with 0.0008 ± 0.0003 ml/min for ^{111}In-DOTA-TATE), this value was still very low in comparison with perfusate flow (25.0 ml/min). Radioactivity in the bile was mostly in the form of the parent peptide.

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Bio-evaluation of ^{90}Y phosphate for radiation synoviorthesis in animal models of arthritis

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It is estimated that about 3% of the general population is affected by rheumatoid arthritis which manifests mainly as synovial inflammation of joints. Radiation synoviorthesis has been shown to be an effective method of treatment in certain types, resulting in improved quality of life for the patient by reducing pain, improving mobility and preserving the functioning of the joint. We have earlier reported the preparation of ^{90}Y phosphate particles and their evaluation in normal rabbits/rats. In order to determine the therapeutic efficacy, the radiolabelled preparation was tested in animal models of arthritis.

In brief, ~ 8 mCi (296 MBq) of $^{90}YCl_3$ was mixed with 2.5 mL of 5 mg/mL Yttrium chloride followed by addition of 1.25 mL of 10 mg/mL orthophosphoric acid to precipitate ^{90}Y phosphate. The reaction mixture was centrifuged at 3000 rpm for 10 minutes. The precipitate containing the ^{90}Y phosphate particles was washed and reconstituted in 2 % gelatin. The radiochemical yield was determined as the % of the total radioactivity associated with the particles. The radiochemical purity of the ^{90}Y phosphate particles was determined with Instant Thin Layer Chromatography (ITLC) using Water:Methanol:Acetic acid (48:48:4) as the developing system in which $^{90}YCl_3$ moves towards the solvent front (R_f=1) while ^{90}Y phosphate particles remains at the point of application (R_f=0).

Arthritis was induced in rats and rabbit using Complete Freund's adjuvant (CFA). Rats (~weight 300 g) / rabbit (weight ~2 Kgs.) were anaesthesised using ketamine and xylazine. CFA was injected ~50µl (rat) /~350 µl (rabbit) into the left fore limb. Measurement of the joint circumference was taken before induction of arthritis as well as post CFA injections. The animals were also monitored daily with respect to movement/swelling of the joints. Nearly ten days after CFA injection, joint swelling was observed both in rats and the rabbit and the animals had difficulty in movement. Arthritis was confirmed by X-ray of the affected joint, which was significantly different from the normal joint. Intra articular injection of ^{90}Y phosphate (~200 µCi in rabbits; ~50 µCi in rats) was given into the inflamed joint**.** The effectiveness of the treatment was evaluated by measurement of the joint size (circumference) and restoration of the normal movement of the animal. After ^{90}Y phosphate injection, joints became almost normal and the movement of the animal was also restored in about one week. The potential of ^{90}Y phosphate as a radiation synoviorthesis agent could thus be successfully evaluated using animal models.

[$^{186/188}$Re]rhenium sulphur colloid/lipiodol suspension: radiolabelling and biodistribution following intrahepatic arterial injection in HCC bearing rats

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The aim of this study was to prepare $^{186/188}$Re-sulphur colloid/lipiodol suspension and perform biodistribution studies following its intrahepatic arterial injection in hepatocellular carcinoma (HCC) bearing rats, to assess its potential as a radioembolizing agent for the treatment of HCC.

$^{186/188}$Re-sulphur colloid was prepared by acid reduction of sodium thiosulfate containing potassium perrhenate and $^{186/188}ReO_4^-$ solution [1]. The precipitate was washed, dried and ultrasonically dispersed in 1–2 ml of lipiodol for 10 min (at 40 KHz). The radiochemical purity of the $^{186/188}$Re-sulphur colloid/lipiodol suspension was >99%, as determined by ITLC developed with acetone and physiological saline. In vitro stability studies showed that the radiochemical purity of the $^{186/188}$Re-sulphur colloid/lipiodol suspension remained >99% even at 72 h, suggesting that the radiocolloid remained in the lipiodol phase. Particle size analysis of the colloid by laser diffraction technique revealed that about 12.5% of the particles was between 1.7–3.4μ, 61.5% between 0.5–1.7μ and 26% between 0.1–0.5μ.

Carcinogen induced HCC bearing Wistar rat models were developed for biodistribution studies, using a reported protocol [2]. Biodistribution studies were performed in these HCC bearing rats after injection of approximately 7.4–11.1 MBq of the $^{186/188}$Re-sulphur colloid/lipiodol suspension (0.2–0.3 ml) via the hepatic artery. Results of the tissue distribution studies (Table I) showed 98.80 ± 0.31%, 98.39 ± 0.77%, 97.74 ± 0.96% and 92.55 ± 3.43% retention of radioactivity in the liver at 1 h, 24 h, 48 h and 72 h, respectively. Lung uptake was insignificant till 24 h and increased to 1.00±0.39% at 72 h. A slow but steady excretion of the radiocolloid from the lipiodol phase was observed over a period of 72 h and the excretion was mainly through kidneys.

Scintigraphic images of the rat liver showed focal concentration of the radiopharmaceutical even on the seventh day post injection (p.i). Histological studies with the rat liver (seventh day p.i.) showed necrosis (Fig. 1) suggesting therapeutic response to the radiopharmaceutical.

This preliminary results suggest that $^{186/188}$Re-sulphur colloid/lipiodol suspension may be a potential radioembolizing agent for the possible treatment of HCC.

TABLE I. BIODISTRIBUTION OF $^{186/188}$RE-SULPHIDE COLLOID/LIPIODOL SUSPENSION IN HCC BEARING WISTAR RATS FOLLOWING INTRAHEPATIC ARTERIAL INJECTION

Hours post.inj.	Percent injected activity per organ					
	Lungs	Liver	Spleen	Stomach	Kidneys	Excreta (cage)
1	n.d.	98.8 (0.31)	n.d.	n.d.	n.d.	n.d.
24	0.03 (0.06)	98.39 (0.77)	0.08 (0.06)	n.d.	0.02 (0.03)	0.57 (0.19)
48	0. 16 (0.09)	97.74 (0.96)	0.03 (0.03)	n.d.	0.08 (0.04)	1.15 (0.11)
72	1.00 (0.39)	92.55 (3.43)	0.17 (0.22)	0.05 (0.07)	0.15 (0.09)	4.56 (1.51)

n.d: not detected. Each value is mean (±S.D), n = 5. Radioactive concentration in blood, bone, muscle, heart and whole body were insignificant throughout the study.

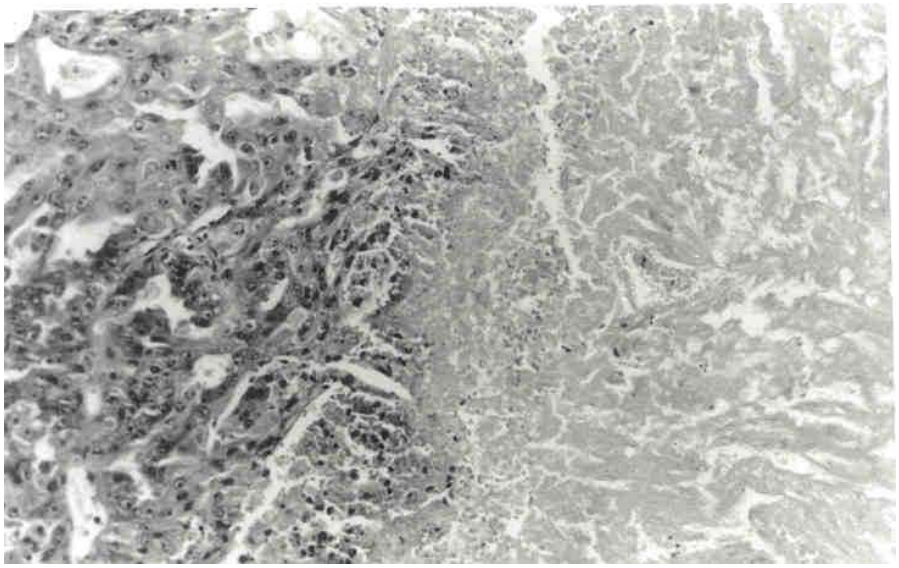

FIG. 1. Section from the representative area of the rat liver tumour showing necrosis in tumour (right half) and residual viable tumour (left half) following hepatic arterial administration of $^{186/188}$Re-sulphur colloid/lipiodol suspension (7th day post injection).

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Preparation and evaluation of ^{177}Lu-DOTA-Tyr3-Octreotate for use as a therapeutic radiopharmaceutical

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Several peptide receptor-specific somatostatin (SST) analogs have been extensively investigated for *in-vivo* application in peptide receptor radionuclide therapy (PRRT). These studies exploit the over-expression of SST receptors in tumour cells compared to normal tissues. Recently, ^{177}Lu with ideal nuclear properties [$E_{\beta(max)}$ = 497 keV, $T_{1/2}$ = 6.73 d] is being considered as a radioisotope for the development of new agents for PRRT. The use of ^{177}Lu as the radionuclide provides an additional advantage that of emitting gamma photons of 113 keV (6.4%) and 208 keV (11%) which are suitable for carrying out imaging studies simultaneously. While the high thermal neutron capture cross-section of ^{176}Lu (2100 b) makes it quite convenient to produce high specific activity ^{177}Lu using moderate flux reactors, the comparatively longer half-life of ^{177}Lu provides logistics advantages over the use of other therapeutic isotopes normally used for PRRT. The possibility of efficient targeting of neuroendocrine tumours, intestinal adenocarcinomas and lymphomas with radiolabeled peptides which are somatostatin analogues provided impetus for the present study. In the present study, in an attempt to prepare a therapeutic agent for targeted delivery, tyrosine-3-octreotate has been chosen as the peptide vector and its conjugate with a polyazamacrocycle viz. DOTA is radiolabeled with ^{177}Lu.

^{177}Lu was produced by thermal neutron irradiation isotopically enriched Lu_2O_3 target (60.6% ^{176}Lu) in Dhruva reactor at a flux of ~5×10^{13} n/cm^2/s for 14 days. The irradiated target was dissolved in 1 N aqueous HCl, evaporated to near-dryness and reconstituted in deionized water. About 5×10^3 Ci/g (~185 TBq/g) of ^{177}Lu activity was obtained at 6 h post end of bombardment. It is worthwhile to note there is a possibility of the formation of ^{177m}Lu ($T_{1/2}$ = 160.5 d) on thermal neutron bombardment of Lu_2O_3 target. However, the gamma ray spectrum did not show any significant peak corresponding to ^{177m}Lu. This is expected as the radioactivity due to ^{177m}Lu produced will be too insignificant on 7 d irradiation owing to its long half life and comparatively low cross section (7 b) for its formation. Assay of trace level of ^{177m}Lu activity present in ^{177}Lu activity produced was carried out by recording gamma ray spectrum of a sample aliquot, initially having high radioactive concentration, after complete decay of ^{177}Lu activity (45-65 d EOB). The average level of radionuclidic impurity burden in ^{177}Lu due to ^{177m}Lu was found to be 150 nCi of ^{177m}Lu / 1 mCi of ^{177}Lu (5.5 kBq /37 MBq) at EOB. This implies that the radionuclidic purity of ^{177}Lu produced was ~99.985% at EOB.

The ^{177}Lu labeling of DOTA-TATE was effected by adding 20 μL $^{177}LuCl_3$ (1.13 nmole Lu) solution to a 200 μL solution of the conjugate (17.4 nmole) in 0.1 M ammonium acetate buffer of pH 5. Optimization studies of the reaction time and temperature indicated an incubation time of 1 h at a temperature of 80°C was required for obtaining maximum complexation of ~98%. The radiolabeled conjugate was characterized by paper chromatography using 50% aqueous acetonitrile as the eluting solvent and it was observed that while ^{177}Lu-DOTA-TATE moved towards the solvent front (R_f = 0.7-0.9), the uncomplexed ^{177}Lu remained at the point of spotting (R_f = 0). The complex was further

characterized by HPLC using water (A)-acetonitrile (B) with 0.1% trifluoroacetic acid as the mobile phase and employing a gradient elution technique (0-4 min: 95% A, 4-15 min: 95% A to 5% A, 15-20 min: 5% A, 20-25 min: 5% A to 95% A, 25-30 min: 95% A). The retention of the radiochemical purity to the extent of >90% after 10 days at room temperature indicated the adequate stability of the complex. With a view to minimizing the peptide/metal ratio, extensive optimization studies were carried out wherein >90% complexation could be achieved at peptide/metal ratio of 4:1.

Biodistribution studies carried out in normal mice showed no uptake in any of the major organs and tissues with rapid clearance of the activity via the renal route within 3 h post-injection (p.i.). The specificity of the radiolabeled conjugate has been ascertained by carrying out *in-vivo* studies in nude mice bearing prostate carcinoma (PC-3), which is not documented to over-express somatostatin receptors. In this study, the excretion was found to be completely through the renal route within 20 min p.i. without any accumulation of activity in the tumour as well as in any other organs/tissues. To determine the target specificity of the radiolabeled agent, *in-vitro* cell-uptake studies as well as *in-vivo* studies in xenographted nude mice models bearing AR42J rat pancreatic cell lines are planned in the near future.

Radioimmunotargetting in India with special reference to hepatic metastases: Recent advances

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Over the last five years there has been a renaissance in the use of therapeutic antibodies (1).and more than 25% of pharmacologic agents that are currently under development are based on antibodies ,the total income generated in 2002 exceeded $ 3 billion, with a prediction to rise to $ 10-20 billion by 2010. Combination therapy using antibodies together with chemo or radiotherapy has been shown to be advantageous with anti EGFR as well as with rituximab.,since the toxicity profiles of antibodies and the other modalities have little overlap,improving therapeutic outcome without increased toxicity. Attatching a toxic radiopayload to the antibody for cancer therapy is of interest to nuclear medicine .Radiolabelling confers a 'force multiplier' effect since the crossfire effect mediates the elimination of cancer cells other than those directly targeted. Further the action at a distance mode allows the use of surface located antibodies that do not intenalize as well as antibodies targeted at tumour stroma or vasculature. In the US radiolabelled antibodiea for the therapy of lymphomas have now become part of the routine armamentarium.

For logistic and financial reasons we have advocated the use of pancarcinoma antibodies as particularly advantageous for developing countries.(2) We have in experimental work utilized Gold-199 and Silver 199(3),but human studies have been with Technetium- 99m and Iodine-131,but Rhenium-186/188, Lutecium and Samarium are now under study.

This communication describes our use of a pancarcinoma antibody M3 directed against the Tissue Polypeptide Specific Antigen for radioimmunotargetting both in experimental animals and in human patients, and in particular hepatic metastases. The Iodogen technique was adapted to high dose labeling with Iodine-131.While observing the temporal course of biodistribution of Iodine-131 antibodies it was observed that initially there is a massive localization in the Reticuloendothelial system (RES) particularly the liver and spleen apart from tumour localization.Aftter two weeks there is an intriguing late accumulation in the spleen even though the liver counts have declined, and in some cases a late localization in the testes even though not clinically involved by the tumour.

We observed that in both experimental mice as well as in human patients the nonspecific wasteful accumulation of the antibodies in the liver could be sharply reduced 40-60% by blocking the RES, using the inexpensive modality of nonspecific polyclonal human gammaglobulin from 12 days prior to the radiolabelled antibody (4).

As multiple liver metastases quite commonly seal the prognosis in patients in whom the primary has been successfully surgically ablated,and are very often not amenable to surgical excision, external radiotherapy and radiofrequency ablation, we explored the possible use of radiolabelled antibodies in such patients. (We reasoned that even the nonspecific localization of such antibodies in the liver could have some advantages in this clinical situation!)(5)

Initial experience (6) showed that such radiolabelled antibodies in doses of 25-50 mg antibody bearing 50-150 mCi radioiodine-131 were well tolerated with no deterioration of haematological(red cell,white cell and platelet count) or hepatic(Serum Glutamic Pyruvic Transaminase levels) or renal(Serum creatinine) function parameters. Dramatic improvement in pain was observed with size stabilization of the liver metastases for periods as long as a year in 4 out of 6 patients, but metastases did not disappear. In some cases some metastases shrank but new lesions appeared, suggesting thre selection of resistant cell populations. Repeated administration of these murine antibodies was limited by the appearance of serological and/or clinical evidence of Human Anti Mouse Antibody reactions.

Possible solutions to this problem that we are evaluating are humanization of the anticancer antibodies and plasmapheresis apart from immunosuppression with corticosteroids.

It also appears logical to now suggest the use of radiolabelled antibodies in the immediate post surgical primary ablation scenario to eliminate or forestall micrometastases rather than in patients in whom massive hepatic metastases have already developed.

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Radiobioconjugate targetting in cancer in developing countries: Regulatory issues

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The radiobioharmaceuticals for targeting cancers include radiolabelled monoclonal antibodies, peptides, anti sense nucleotides as well as intermediate molecules in pretargetting strategies. Scientists in developing countries that wish to use these agents whether for diagnosis or for therapy face formidable barriers, and this communication proposes pragmatic strategies to overcome these hurdles.

Firstly such agents prior to radiolabellling have to be prepared according to Good Manufacturing Practice standards,suggested criteria for which have been formulated (1).

Secondly, for therapy use, since centralized distribution of a high dose labeled radiopharmaceutical such as an Iodine labeled antibody is not available from a source in the country or region, and may not be feasible for logistic and economic reasons, compounded by the distances between the radiopharmaceutical manufacturing centers and the user hospitals,high dose radiolabelling has to be undertaken in house in the user centre. This necessitates understanding of the hazards especially with volatile isotopes such as radioiodine, and adequate precautions to limit these. For the addition of radioiodine remote controlled pipetting has been proposed. Another strategy is the use of resins to mop up excess radiolabel.

With other radiolabels such as Rhenium, Lutecium and Gold, the problem of volatility is avoided but obtaining these at adequate specific activity and purity is sometimes difficult resulting in the need for concentration techniques. Rhenium-188 from Tungsten generators is superior to the reactor produced Rhenium-186/188 mixtures.

This problem does not exist as regards diagnostic targeting where Technetium-99m is adequate and is available sterile from column generators.

The use of pretargetting strategies with the final agent being a radiolabelled biotin or radiolabelled chelate may enable centralized preparation and distribution of these.

Thirdly the availability of biological agents is severely hedged in by patents.Developed countries manufacturers in order to obtain local regulatory approval are chary of sharing raw material with the developing county users at any stage prior to FDA approval.After FDA approval they are exported at exorbitant costs to recover the costs of regulatory approval which are not only expensive but delay the availability of these to cancer patients in the developing countries by almost a decade!.

It therefore is apparent that the developing countries need to manufacture their own therapeutic molecules,and a governmental agency is often needed to catalyse this as pharmaceutical industry is reluctant to undertake this unless the molecule is already approved and clinically successful,a Catch 22 situation!An individual research laboratory can only manufacture milligrams of an agent, and to allow investigator multicentre trials of an agent quantities of the order of 50 grams are required using biofermenters; mega kidney dialysis

cartridge type systems. Individual patients need antibodies in quantitites of 15-100 mg per therapy. Eventual commercial manufacture of an agent involves making kilogram quantities in air lift fermenters. Manufacture of these 50 gram quantities is estimated to cost Rs 50,000 per gram which is substantially cheaper than the Rs 10,00,000 per gram at which such agents are imported,It is therefore emphasized that developing countries need to emulate the UK story where even in a developed country the Medical Research Council set up a Therapeutic Antibody Centre initially at Cambridge and now at Oxford.Cooperation amongst developing countries is also obviously advantageous!

Fourthly developing countries need to have their own regulatory standards in force and not blindly adopt those extant in developing countries. For example the costs of testing a product for minute quantities of mouse viruses or DNA is tremendous and it appears unnecessary to undertake for each batch, as multiple chromatography steps used for chemical purification eliminates these biodangers as well.

It is urged that when dealing with an aggressive lethal cancer, the risk of imminent death from this is far greater than the infinitesmal risk of contracting a mouse virus infection! Already for non radiolabelled conventional chemotherapy fast track regulatory approval procedures exist as compared to noncancer drugs!

Fifthly some newer manufacturing techniques for biomolecules such as those using yeast in alcoholic media for antibodies may eliminate the biohazards associated with conventional mammalian cell or bacterial routes for manufacture.

Sixthly there is an alarming patent trend where not only monoclonal antibodies but also the sites against which these are directed are sought to be restricted!

In conclusion, it is salutary to remember that even in the developed countries the tremendous cost of the regulatory approval process has led to available drugs being frozen in outmoded tehnology e.g. with a murine rather than human antibody because obtaining approval for the better molecule is considered costly and time taking!The developing countries when setting up their regulatory procedures need to keep it low cost and fast to avoid such pitfalls.

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Estimation of radiation dose to Indian reference man due to indigenously produced renal and liver radiopharmaceuticals

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Board of Radiation and Isotope Technology (BRIT), Department of Atomic Energy, India has been commercially supplying cold kits for ^{99m}Tc based radiopharmaceuticals since the last few decades after necessary Quality Control including tests for sterility and biodistribution pattern. These kits are being regularly used by various nuclear medicine centres in India. Although the data on the doses received by the critical organs, on use of different radiopharmaceuticals are available in literature, it would be ideal to provide actual data for BRIT kits to the users. With this aim, we have carried out dosimetric studies for the various radiopharmaceuticals which are used as renal and liver scintigraphy agents.

These doses were estimated based on the animal studies. The various radiopharmaceuticals were prepared and administered into normal adult mice/rats and the biodistribution studies were carried out at different time points from 15 minutes to 24 h to know the percentage uptake by various organs. The organs of interest such as kidneys, liver, intestine, bladder, tail and the carcasses were collected and the percentage uptake was measured for each time point. Assuming that the radiopharmaceuticals undergo similar metabolism in human as the laboratory animals, the percentage uptake obtained in the animal were applied to the Indian reference man and the doses were calculated theoretically. The average activity of these radiopharmaceuticals administered for getting the diagnostic information in Indian Population has been taken from the available records. The Mean dose per unit cumulative activity, the average weights of the organs in Indian reference man [1,2] and the energies of all the radiations from ^{99m}Tc with their abundance [3] were considered while calculating the doses.

The cumulative radiation doses received from these agents in 24 h by kidney, liver, intestine, lungs, bladder and stomach, are given in the Table I. The doses calculated to the organs are not only due to the activity present in the organ at that moment of time but also due to the activity present in the other organs. The doses given in the table are the cumulative doses received in 24 h during which four physical half lives of ^{99m}Tc have elapsed, by which time nearly all the ^{99m}Tc activity decays out both physically and biologically. The doses at the various time points have also been calculated. These doses have been compared with the doses published by ICRP. In case of Indian Reference Man, the dose estimates due to EC and Mebrofenin radiopharmaceuticals are compared with MAG3 and IDA of ICRP reference man respectively.

It is well realized that both height and weight for the reference Indian are lower than ICRP values (25% for weight and 4% for height). The doses calculated for Indian reference man are higher than values given by ICRP. This could primarily be due to the reason that Indians differ significantly in many parameters- anatomical, physiological, and metabolic from the Caucassian, the latter exemplified by the ICRP reference man. The other reasons may be due to the method of preparation of these radiopharmaceuticals and the theoretical model considered in this study. It would be worthwhile for hospital to use the values of Indian Reference men instead of using the ICRP values by default.

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TABLE I: RADIATION DOSE IN MGY/MBQ

Organs	GHA		EC		DTPA		Sulphur Colloid		Mebrofenin		Phytate	
	Indian Reference Man	ICRP Reference Man	Indian Reference Man	ICRP Reference Man (MAG3)	Indian Reference Man	ICRP Reference Man	Indian Reference Man	ICRP Reference Man	Indian Reference Man	ICRP Reference Man (IDA)	Indian Reference Man	ICRP Reference Man
Kidneys	1.71E-01	4.9E-02	1.18E-02	3.4E-03	3.01E-03	3.9E-03	5.03E-02	9.5E-03	1.53E-02	6.1E-03	9.49E-03	9.5E-03
Liver	9.27E-03	2.7E-03	4.37E-03	3.1E-04	7.11E-04	1.2E-03	3.29E-01	7.1E-02	7.51E-03	1.4E-02	1.05E-01	7.1E-02
Intestine	2.30E-02	1.14E-02	1.05E-02	1.3E-02	3.50E-03	11.9E-03	28.4E-03	14.9E-03	1.34E-01	26.3E-02	9.11E-03	14.9E-03
Lungs	1.80E-03	1.7E-03	9.80E-04	1.5E-04	1.94E-04	9.9E-04	1.78E-02	5.9E-03	1.05E-03	1.3E-03	5.02E-03	5.9E-03
Stomach	4.64E-03	2.7E-03 (w)*	1.87E-02	3.9E-04	3.72E-04	1.3E-03	1.20E-02	6.4E-03	7.95E-03	5.6E-03	3.86E-03	6.4E-03
Bladder	3.07E-03	5.69E-02 (w)*	2.07E-02	1.1E-01	7.64E-03	6.2E-02	2.75E-03	1.1E-03	1.55E-02	2.2E-02	7.70E-04	1.1E-03

(w)* indicates the value given for wall.

In vitro cell toxicity of 4'-epi-iodo[I-125]-4'-deoxy-doxorubicin on human colon cancer

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Doxorubicin is an anthracycline antibiotic that is one of the most effective and wide spectrum antineoplastic agents currently available. Auger electron-emitting radioisotopes, such as I-125, are known to be highly cytotoxic when localized in cell nuclei. In the present study, we investigated the *in vitro* cytoyoxic potential of auger electron-emitting radionuclide (I-125) delivered to DNA via doxorubicin.

4'-epi-iodo-4'-deoxy-doxorubicin (IDX) and 4'-epi-iodo[I-125]-4'-deoxy-doxorubicin (^{125}IDX) were synthesized [1,2]. Drug activity was tested on HT-29 cell line (colon cancer). Cell toxicity was quantitated by viable cell measurement [3].

comparative cell toxicity of IDX and ^{125}IDX showed a great different activity between them. The response rates with ^{125}IDX were consistently higher than those with IDX at all concentrations. The median minimal dose required to induce a significant antimetabolic effect was at least 7 times greater for IDX than for ^{125}IDX.

Our data show that it may be possible to use antineoplastic agents to deliver radioactivity to cancer cells. Such conjugated radioisotopes may be useful for attacking tumour cells *in vivo*, particularly for single cell micrometastases. The use of doxorubicin has been limited by incidence of cumulative dose-dependent cardiomyopathy and loss of effeciacy due to multidrug resistance. ^{125}IDX may improve the therapeutic index and decrease cardiac toxicity.

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Preparation of [^{66}Ga]bleomycin complex as a possible PET radiopharmaceutical

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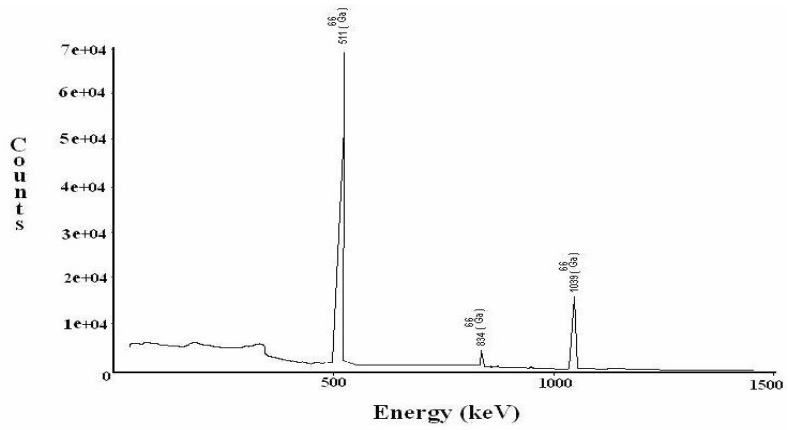
Several radiolabeled bleomycin derivatives have been developed for imaging and/or therapy of neoplastic tissues. The most important imaging compounds contain indium-111 (1), cobalt-57 (2), and rhodium-105 (3). In our previous studies we have prepared radiolabeled bleomycin complexes such as gallium-67(4) as therapuetic and/or imaging agents. Our recent studies on the preparation and tumour imaging properties of [^{67}Ga]bleomycin in normal and tumour-bearing mice showed a good tumour/blood and tumour/muscle ratio suggesting that it is an appropriate diagnostic agent (6). Due to the interesting properties and increasing importance of positron emission tomography, we investigated the possibility of incorporating ^{66}Ga as a positron emitter with an antineoplastic compound, bleomycin, for use in tumour imaging. We optimized ^{66}Ga complex formation conditions with bleomycin, to develop [^{66}Ga]BLM. We hereby report the production of ^{66}Ga, preparation, optimization, stability, and formulation studies of [^{66}Ga]-bleomycin complex.

Targetry: An electroplated ^{66}Zn target on a copper backing plate was irradiated at an angle of 6 degrees toward the proton beam in order to achieve higher production yield. The target was cooled by a flow of 18°C distilled water with a rate of 50 Lit/min.

Chemical Separation: The irradiated target was dissolved by 10 N HCl (15 ml, H_2O_2 added) and the solution was passed through a cation exchange resin (Dowex 50 W×8, H^+ form) (h:10 cm, Ø:1 cm). The resulting high-purity [^{66}Ga]$GaCl_3$ solution was used directly in the labeling step.

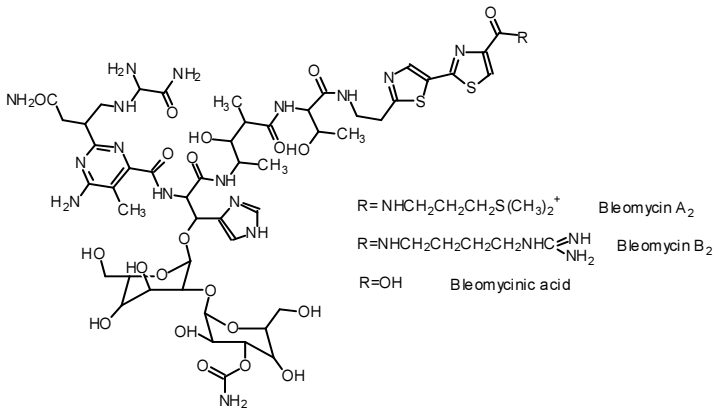
Labeling of bleomycin with [^{66}Ga]$GaCl_3$: [^{66}Ga]$GaCl_3$ (0.25-2.5 mCi) dissolved in acidic medium obtained above (0.5-2 ml) was transferred to a 2 ml-vial and pH was adjusted to various pHs (1-7) using 1M HCl and/or 1M NaOH. The mixture was evaporated by slight warming under a nitrogen flow. A mixture of BLM (0.25-2.5 mg) in normal saline (0.1 ml) was then added and was heated at different temperatures (25, 50, 80, and 100°C) and was cooled in an ice bath, and rapidly sent for use. The active solution was checked for radiochemical purity by polymer-backed silica gel layer chromatography using a 1:1 mixture of 10% ammonium acetate and methanol as mobile phase. The final solution was then passed through a 0.22 μm filter and pH was adjusted to 5-7 by the addition of 1 M sodium acetate buffer.

Radionuclide purity: The gamma spectroscopy of the final sample was carried out by HPGe detector, and showed a radionuclidic purity higher than 99 % showing the presence of 511, 834, and 1039 keV gamma energies, all of which are resulted from ^{66}Ga.



[^{66}Ga]BLM complex in final product: Stability studies were based on the previous studies performed for other radiolabeled bleomycins (5). A sample of [^{66}Ga]BLM (0.5 mCi) was kept at room temperature for 5 hrs while checked by RTLC every half an hour. A micropipet sample (50 μl) was taken from the shaking mixture and the ratio of free radiogallium to [^{66}Ga]BLM was checked by radio thin layer chromatography (eluent: 10% NH_4OAc buffer and methanol (1:1).

Total labeling and formulation of [^{66}Ga]BLM took about 60 min, with a yield of 97%. A suitable specific activity product was formed *via* insertion of [^{66}Ga]gallium cation. No unlabelled and/or labeled by-products were observed upon RTLC analysis of the final preparations. The radio-labeled complex was stable in aqueous solutions for at least 24 h and no significant amount of other radioactive species were detected by HPLC 24 h after labeling. Trace amounts of [^{66}Ga]gallium chloride (≈2%) were detected by TLC. RTLC showed that radiochemical purity of the [^{66}Ga]labeled components was higher than 98%. In contrast to other labeled bleomycins, [^{66}Ga]bleomycin, is a PET radiotracer with a rather long half life, and the high chemical stability of this radiopharmaceutical makes it a very suitable diagnostic agent.



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Production, quality control and initial imaging studies of [^{82m}Rb]Rb injection for PET studies

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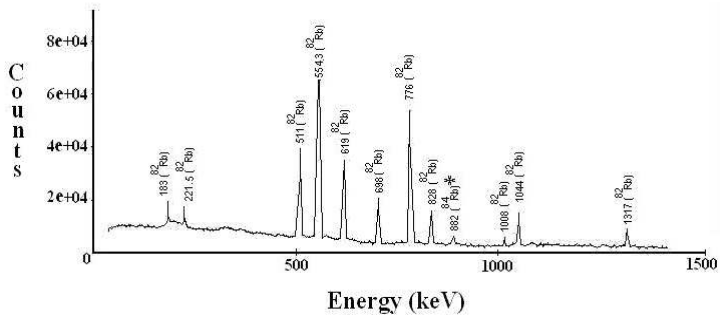
Rubidium-82m (HL: 6.5 h, $E_{\beta+}$=0.80 MeV, E.C.=74%) is a rather long-half life PET radioisotope with a potential use in myocardial studies by PET which has not been regularly reported in nuclear medicine studies. Due to our recent works on the producion and quality control of some PET radiotracers in the country, we were interested in the production, formulation and administration of Rb-82m solution for future clinical PET applications. In this study, following production and formulation of Rb-82m as a PET radiopharmaceutical, the preliminary imaging studies was acquired by a dual head SPECT system, equipped with a co-incidence camera. Also, due to the production of ^{81}Rb/^{81m}Kr generator for country use in our institution, biodistribution of radiorubidium was studied in normal rats using Rb-81 radioisotope.

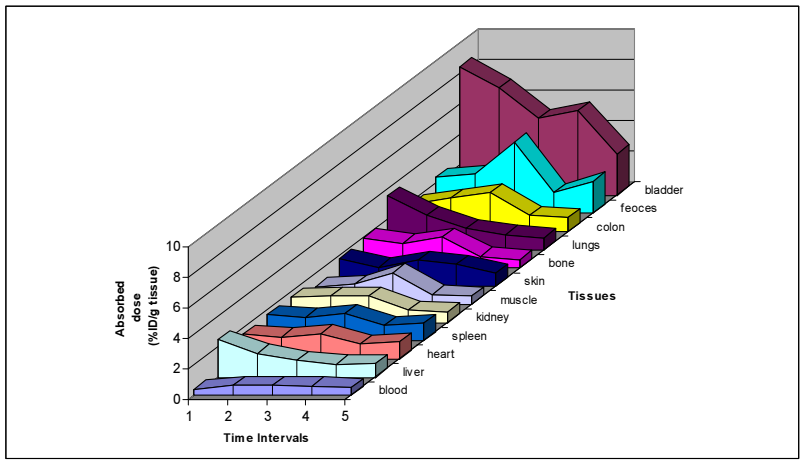
Production of rubidium-82m for imaging studies: Gasseous Krypton-82 with 30% enrichment and 251 mm thickness was irradiated under a pressure of 2.7 bar at 15.4 MeV energy in a stainless steel chamber. The target was cooled using 18ºC water while a current of 10 μA with a total current of 5.5 μAh was employed (maximum chamber pressure 3.6 bars). Details of the production system are summerized in Figure 2. After irradiation, the walls were rinsed with milli-Q sterile hot water in order to yield rubidium-82m in hydroxide form with an activity of about 35.0 ±2 mCi (production yield: 6.37 mCi/μAh). The bombardment was performed with a proton current of 10 μA and a total proton dose of 5.5 μAh. After washing the compartment and extraction of ^{82}Rb^{m}, the activity of the final product was 35.04 mCi.

Quality controls- Radionuclide purity: The gamma spectroscopy of the final sample was carried out by a HPGe detector.



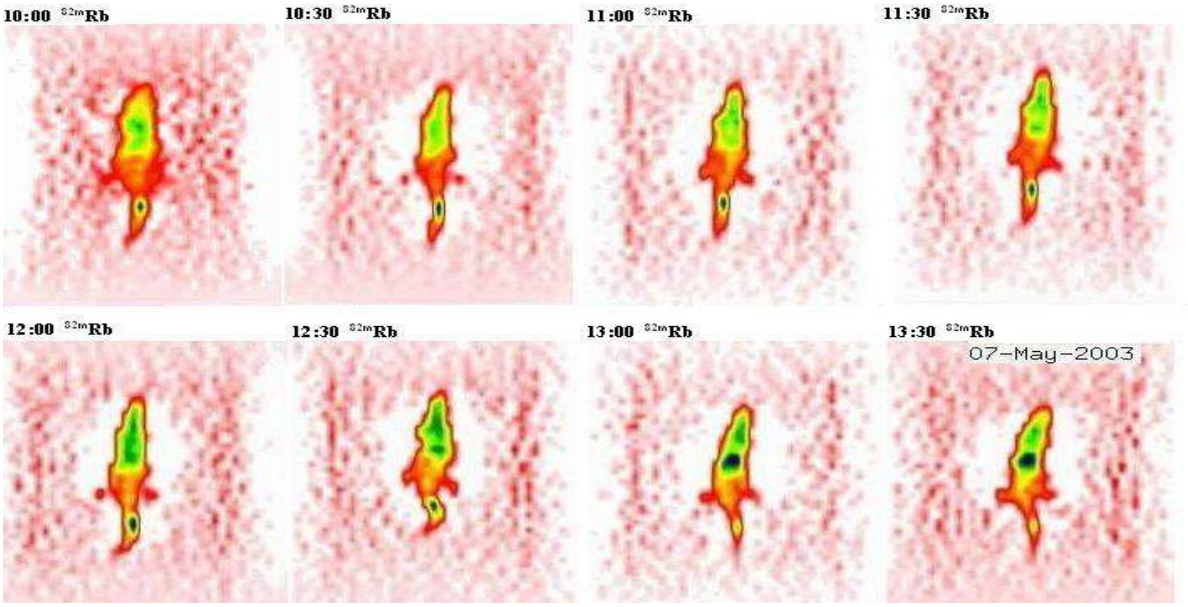
Biodistribution

The distribution of [^{82m}Rb]Rb among tissues was determined in rats. A volume (0.1 ml) of final [^{82m}Rb]Rb solution containing 20-40 μCi .



Imaging studies

0.1 ml volumes of the final [^{82m}Rb]Rb solution containing 300 μCi radioactivity (≤ 6 ng rubidium in in 50 μL) were injected into the dorsal tail vein of healthy rats.



As a result of the bombardment of ^{82}Kr with 15 MeV protons, 35.04 mCi activity was finally achieved. The nuclear reaction efficiency was 6.37 mCi/μAh. Total production and formulation of [^{82m}Rb]Rb took about 60 min. TLC showed that the radiochemical purity of [^{82m}Rb]Rb was >95%. In contrast to other PET radiotracers for cardial studies, [^{82m}Rb]Rb has a rather long half life that causes long data acquisition times by PET or co-incidence systems. High chemical stability of the radiopharmaceutical, makes it easier for autoclaving process. Biodistribution studies performed by Rb-81 isotope was performed in normal rats

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Preparation, distribution, stability and tumour imaging properties of [^{62}Zn] bleomycin complex in normal and tumour-bearing mice as a molecular pet generator

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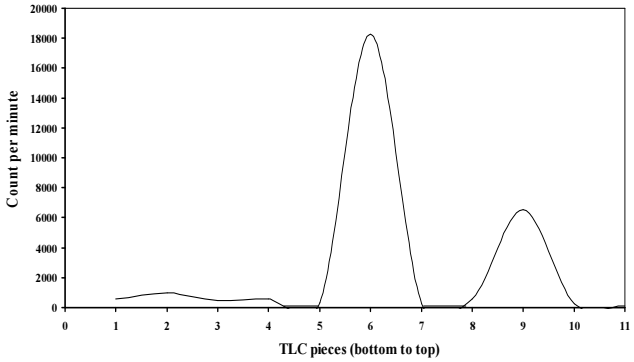
PET is a powerful imaging technique with many advantages over single photon imaging. Nowadays, several positron emitter radioisotopes and their radio-labeled complexes have been developed for imaging. ^{62}Zn/^{62}Cu positron generator system has been widely proposed as a source of perfusion tracers (Green, 1987 and Green et al, 1988). In this study we have developed a ^{62}Zn/^{62}Cu-bleomycin tracer system as a biological generator for PET.

The radiochemical separation of the ^{62}Zinc (Neirinckx 1977) from natural copper target (electroplated over Ni layer) was carried out with a method described by (Green et al, 1990 and Okazawa et al, 1994) with slight modification. After dissolving of the irradiated target, solution was heated under a flow of nitrogen to dry up until a precipitate was formed. The precipitate was rinsed 2 times by distilled water (10 ml) and a portion of 2N HCl was added and mixed gently. The solution was pumped through a column 10x100mm filled with BioRad AG-1X8 resin and preconditioned with 2N HCl. For the elution of ^{62}Zn in anion exchange resin, 0.005 N HCl was adopted instead of water used by (Green et al, 1990, Qaim 2001), because [^{62}Zn] chloride form is suitable for the BLM labeling The samples were taken for the gamma spectroscopy analysis, using HPGe Canberra detector, in each steps.

[^{62}Zn]Zinc chloride (0.25-2.5 mCi) dissolved in acidic media obtained above (0.5-2 ml) was transferred to a 2 ml-vial and pH was adjusted using HCl 1M and/or NaOH 1M (pH=1-7). The mixture was evaporated by slight warming, under a nitrogen flow. A mixture of BLM (0.25-2.5 mg,) in normal saline (0.1 mL) was then added. This mixture was heated at different temperatures (25, 50, 80 and 100°C). The mixture was cooled in an ice bath and rapidly sent for use. The active solution was checked for radiochemical purity by polymer-backed silica gel layer using a mixture of ammonium acetate 10%-methanol as the mobile phase. These analyses were carried out every 30 min after labeling step. The final solution was then passed through a 0.22 m filter and pH adjusted to 5-7 by the addition of sodium acetate (1M) buffer. Pyrogen test was performed using a commercial LAL kit.

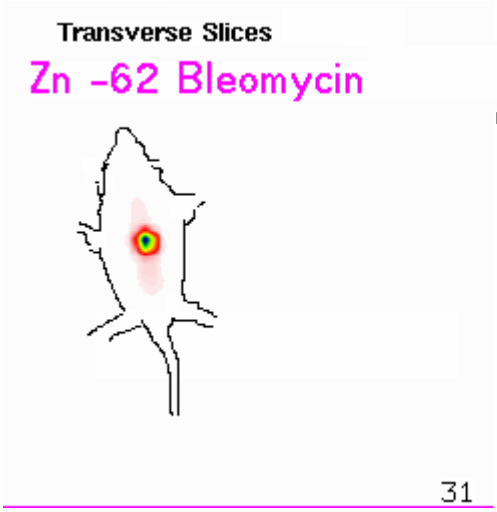


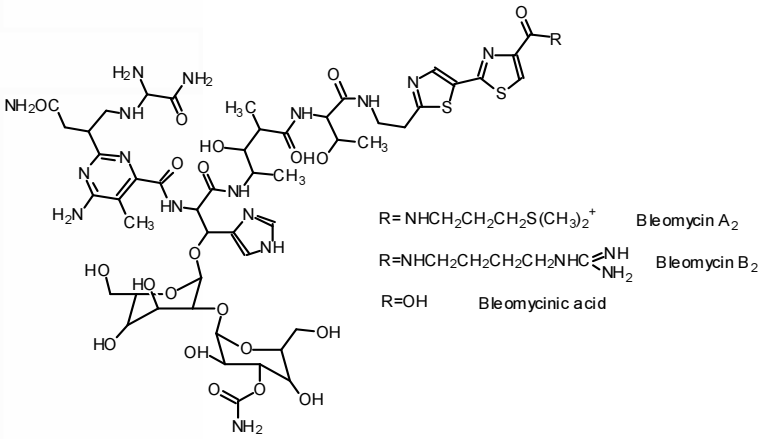
Stability of [^{62}Zn]BLM complex in final product and human/mice serum *in vitro.*

A sample of [^{62}Zn]BLM (0.5 mCi) was kept at room temperature for 48 hrs while checked by RTLC at various time intervals (2, 4, 8, 12 and 24). A micropipette sample (50 mL) was taken from the shaking mixture and the ratio of free radiozinc to [^{62}Zn]BLM was checked by radio thin layer chromatography (eluent: 10% NH_4OAc and methanol 1:1). The patterns for [^{62}Zn]$ZnCl_2$ and [^{62}Zn]BLM were not changed in 24 hrs.

Animal studies: Fibrosarcoma cells (about 10^4) of were injected SC to the dorsal area of Balb C mice weighing 20-25 g. After 14 days the tumour weighed 0.7 g and was not grossly necrotic. The distribution of [^{62}Zn]-$ZnCl_2$ and [^{62}Zn]-BLM among tissues were determined for untreated mice and for mice with fibrosarcoma. A volume (0.1 ml) of final [^{62}Zn]-BLM solution containing 20-40 uCi radioactivity (6 mg bleomycin in 50 mL) was injected into the dorsal tail vein. The total amout of radioactivity injected into each mouse was measured by counting the 1-ml syringe before and after injection in a radiometer with a fixed geometry. The animals were sacrificed by ether asyxphycation at selected times after injection, the tissues weighed and their specific activities determined with a gamma-ray scintillation as percentage of injected dose per gram of tissues (data not shown here)

No unlabelled and/or labeled by-products were observed upon RTLC analysis of the final preparations. In contrast to other labeled bleomycins, [^{62}Zn]bleomycin, has a lower half life causing less undesirable irradiation and it also benefits from PET radiopharmacutical advantages. Its rather higher half-life in contrast to other PET radioisotopes and high chemical stability of radiopharmaceutical form makes it a suitable possible PET tracer for use in neighborhood PET centers. [^{62}Zn]-BLM can be used in PET oncology studies due to its suitable physical and chemical properties as a PET radiopharmaceutical.





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Production of ^{123}I (NaI) radiopharmaceutical via xenon gas target, using NRCAM cyclotron

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Iodine-123 is one of the most famous radioisotopes, used in nuclear medicine. Among the various types of nuclear reactions for ^{123}I production the following reactions is favored due to absence of ^{124}I and ^{125}I impurities:

$$^{124}Xe(P,2n)^{123}_{55}Cs\xrightarrow[\beta^+]{5.9\,\min}{}^{123}_{54}Xe\xrightarrow[\beta^+]{2.08\,hr}{}^{123}_{53}I\ldots\ldots\ldots and\ldots\ldots\ldots I^{124}Xe(P,pn)^{123}_{54}Xe\xrightarrow[\beta^+]{2.08\ hr}{}^{123}_{53}I$$

In this project we produced ^{123}I using above reactions. ^{124}Xe enriched gas target was employed. Some important issues must be taken into account when using gas targets, such as:

1) Released energy from the proton beam during penetration through the gas target produces the heat followed by increase the average temperature and pressure of the gas.

2) The multiple scattering produced by proton collision with Xe molecules causes scattering through various angles.

3) Cross-section investigation is essential for nuclear reactions. Energy and range of optimized energy must be determined to obtain the best yield with lowest impurities.

Reduction of proton energy, temperature and pressure increase, multiple scattering, optimized bombardment energy determination and non-uniformity of density gradient, all must be taken into account when a suitable target is designed in this project. Investigation of gaseous target behavior during proton irradiation at high currents is performed. As a result, an optimized target for I-123 production has been manufactured.

Fast preparation of ^{131}I mIBG at NRCAM

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The diagnostic value of meta-iodobenzyl-guanidine (MIBG) labeled with I-123 and I-131 in the detection and treatment of neuroblastoma and pheochromocytoma is well known. Currently studies of certain myocardial dysfunction as well as drenomedullary abnormalities have performed [1,2,3]. In this work labeling with ^{123}I or ^{131}I has been optimized by modifying literature method s that involve on the nucleophilic exchange in presence of Cu (I) and an excess of reducing agents [4,5,6,7].

MIBG was purchased from Sigma chemical Co and $CuSO_4.5H_2O$, tin sulfate, 2,5dihydroxy benzoic acid and sodium citrate from Merck. ^{131}I solution in the form of 0.01 N, NaOH were supplied from Nuclear research center in Tehran. Activity in the vial was measured with an ionization chamber dosimeter until, and a pre-calculated volume of sterile water for injection was added to adjust the radioactivity concentration of 25mCi/ml at reference time.The production run proceeded as follows:

a- 30µl copper sulfate solution containing 32.5 mg $CuSO_4.5H_2O$ in 10mldemineralized water, was mixed with 500µl stock solution containing 1.5mg $SnSO_4$, 20mg 2,5 dihydroxy benzoic acid, 45mg sodium citrate.

b- The above solution was added to 1mg MIBG in a 10 ml glass vial. The solution on the vial was crystallized by liophilization and sealed.

c- 1ml ^{131}I solution containing 25 mCi ^{131}I was purged with N2 for 5 min, ^{131}I solution added to the lyophilized vial. Vial was transferred in an aluminum container then suspended in boiling water for 30 min. where the boiling water with container inside it was monitored with a detector that was installed inside the laminar. Upon cooling, the contents of the vial were made isotonic by 2ml oxygen free citrate buffer solution.

Table.I shows the amount of buffer for deferent patients.An acceptable radiochemical yield 90 % is obtained at 100°C within 30 min. Chemical and radiochemical purity of ^{131}I -MIBG were determined by TLC [8,9] Table II. The developed kit followed by a simple radiochemical manipulation allows preparing ^{131}I-MIBG at medical centers

TABLE I. THE AMOUNT OF BUFFER FOR DIFFERENT PATIENT

	1 patient	2 patient	3 patient	4 patient
Gentisic acid	0mg	2mg	4mg	6mg
Citric acid	9mg	17mg	25mg	33mg
Sodium solfate	5mg	6.5mg	8mg	9.5mg
Sodium Citrate	62mg	87mg	112mg	136mg
WFI	2ml	3ml	4ml	5ml

TABLE II. THIN LAYER CHROMATOGRAPHY (TLC) METHOD FOR QUALITY CONTROL

Solid phase	Mobil phase	Retention parameters
Silica gel glass	n-propanol , 10%ammoniumhydroxide	R_f: MIIBG 0.75 R_f: iodide 0.15
Silica gel glass	Ethanol, ethyl acetate (1/1)	R_f: MIIBG 0.15 R_f: iodide 0.75

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^{166}Ho labelled DTPA derivative for radiation brachytherapy using catheter balloons

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Brachytherapy is one of the effective treatments for in-stent restenosis. Filling the dilatation catheter balloon with radioactive solutions has the advantages of an accurate source positioning and uniform dose delivery to the vessel walls. In addition, it can be used easily with an existing catheter. Moreover, a solution based beta ray source allows for the treatment of large vessels. Of the variety of radioisotopes prepared in a soluble form for use as a liquid radiation source, Ho-166 is a good radioisotope, because it can be readily produced by irradiating a natural Ho target using a low or medium flux research reactor (^{165}Ho has 100% natural abundance).

For X ray imaging, various iodinated X ray contrast agents having the 1,3,5-triiodobenzoic acid platform are used, mainly for computed tomography (CT) and angiographic applications.

In the present study, we prepared a new DTPA bisamide derivative, DTPA-BTIPA (3-amino-2,4,6-triiodoisophthalic acid) containing iodine in the structure.

^{166}Ho was labeled with DTPA-BTIPA as a possible agent for IVRT for the prevention of restenisis. The optimum condition of the radiolabeling of DTPA-BTIPA with Ho-166 was achieved by varying different reaction parameters.

To estimate the ^{166}Ho-complex as a liquid radiation source for a potential clinical application of IVRT, which is readily excreted through the urinary system in the event of a balloon rupture, a dynamic imaging was acquired.

^{166}Ho-(DTPA- BTIPA) was prepared by a simple mixing at room temperature. High radiochemical stability (>98%) was maintained over a period of 6 h at room temperature. The radioactivity curve in the kidneys of the rabbit administered with ^{166}Ho-(DTPA-BTIPA) via an ear vein showed that the ^{166}Ho-(DTPA- BTIPA) was rapidly cleared through the kidneys. The average of T_{max} and $T_{1/2}$ of ^{166}Ho-(DTPA-BTIPA) in the kidneys were 2.26 ± 0.78 min and 7.80 ± 1.16 min, respectively.

The serial static image scans of the rabbit administered with ^{166}Ho-complex revealed that none of the tissues except for the urinary system had radioactivity concentrations. Both the radiochemical and biological studies revealed that the ^{166}Ho labeled DTPA-BTIPA can be further investigated as a potential agent for vascular brachytherapy having the characteristic of a CT contrast.

The use of the ^{166}Ho-DTPA-BTIPA for IVRT is a good alternative to see if the balloon has close contact with the blood vessel wall for the delivery of a sufficient radiation dose to the stenotic artery.

In-house prepared diagnostic radiopharmaceuticals for nuclear oncology - step towards development of the radionuclide therapy

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In this paper we present our experience with in-house prepared radiopharmaceuticals for diagnosis of malignant diseases as a step towards development the radionuclide therapy.

This work has a very important social and economic impact for our country, especially to improve the diagnosis of patient with malignant diseases, and to develop the treatment and therapy of this patients.

At the present we have a clinical application of :

Bone scan – [^{99m}Tc] methylenediphosphonate - for staging of bone disease particularly in prostate, breast and lung cancer,

MIBI scan - [^{99m}Tc] MIBI – localization of active disease in thyroid cancer, parathyroid and in brest cancer cases,

Pentavalent DMSA – [^{99m}Tc] DMSA(V) – localization of tumours (medullar thyroid cancer),

MIBG scan – [^{131}I] MIBG – localization of neuroendocrine tumours that take up norepinephrine, and in progress:

Octreotide scan – [^{99m}Tc]-DOTA TOC – localization of tumours with somatostatin receptors (pancreatic tumours, carcinoid tumours, medullar thyroid cancer, neuroblastoma),

Monoclonal antibodies scan - [^{99m}Tc] anti CEA – staging of tumour that express specific antigens (colorectal and prostate cancers).

The aim of our work is to establish the protocols of preparation and application of ^{99m}Tc DMSA (V) and ^{131}I MIBG.

The radiopharmaceutical DMSA(V) was in house prepared as asterile, pyrogen-free, freezed product under nitrogen.

Each vial contain DMSA-1.0 mg and Stannous chloride dehydrate 0.4 mg with the final PH 2.0. Before labelling the kit was reconstituied by the addition of 0.5 ml sterile, pyrogen-free 3.5% NaHCO3. Reconstitution and labelling was performed by addition of sterile, pirogen-free, isotonic sodium ^{99m}Tc pertechnetate – 6 ml final volume. The product contains no antimicrobial preservative. After incubation of 15 min. and before use, limpidity of the solution after preparation, pH (~8) and radioactivity was checked.

The quality control of this radiopharmaceutical was effected by:

1. Paper Chromatograpy (PC) using two solvents - acetone and 0.9% NaCl

2. Instant Thin Layer Chromatograpy (ITLC) using mixture of the n-butanol:acetic acid:water (3:2:3)

We evaluated the percentage of labeled complex technetium ^{99m}Tc-DMSA(V), hydrolized and free technetium ^{99m}Tc. In every samples the labeling efficiency resulted more than 95%.The normal *in vivo* biodistribution of this radiopharmaceutical was monitored in normal rats both scintigraphycaly as well as by counting of the dessected tissues, 2 and 4 h after application.

The ^{131}I MIBG was prepared by iodination of meta-iodo-benzylguanidinum-sulfarte. 3mg MIBG and 6mg ammonium sulfate were dissolved in ethanol-water 1:1 mixture and 1% CuSo4. Adding 2.5 – 10 mCi Iodine and evaporing the solution carefully, under infrared lamp for 45-60 minutes was performed radiolabeling procedure. After evaporation, the vial was placed into the furnace for a labeling reaction by heating for 40 minutes at temperature between 166-175 ^{o}C. After labeling the vial was cooled completely, measured the activity of the melt and dissolved with 3-4 ml of Walpole buffer pH-5.0 by vortexing.

The quality control of ^{131}I MIBG was effected by:

1. Paper Chromatograpy (Whatman- FN4-TLC) using abs.Ethanol:ethylacetate mixture 1:1 .

2. Polygram-Sil-NHR using mixture of the n-butanol:acetic acid:water (5:2:1)

We evaluated the percentage of labeled complex and in every samples the labeling efficiency resulted more than 95%.

The normal *in vivo* biodistribution of this radiopharmaceutical was monitored in normal rats both scintigraphycaly as well as by counting of the dessected tissues, after application.

MIBG was injected in the patient following the indications:

- conformation in suspected neuroectodermally derived tumours including neuroblastoma, phaeochromocytoma and medullar thyroid carcinoma;

- staging of the disease

- before and after surgery of the primary tumour

- follow-up after treatment to exclude a sub-clinical relapse, especially in the bone marrow and also in the case of any clinicalabnormality during follow-up, particulary bone pain.

Iodine-131 labelled monoclonal antibody in the diagnosis of colon cancer

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An anti-carcinoembryonic antigen (CEA) monoclonal antibody, KSB6H9CHI, was labelled with Indine-131 using Iodogen method. PD-10 column (Sephadex) was used to purify the free Iodine-131 from Indine-131-Monoclonal antibody.

Prior to PD-10 column elution, the radiolabeling yield obtained was 70%. However, the purity of the labeled antibody obtained had improved to more than 95% following with purification with the PD-10 column.

In vitro study using instant thin layer chromatography on the labeled antibody indicated that the preparation was stable up to 2 h. No microbial growth was observed on the preparation following 14-day incubation in growth media. This showed the radiolabeled antibody was sterile and suitable for clinical use.

A 39-year old patient with a known case of colorectal carcinoma was administered with the radiolabeled CEA, KSB6H9CHI antibody. The study showed accumulation of the radiolabeled antibody at the target site with high target to background ration after 48 h post injection. The image of the target site was still visible until the eight-day post administration of the radiopharmaceutical.

HEDP-^{188}Re kit as a bone therapeutic drug – formulation and biodistribution studies

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Poland

In this study, a lyophilized kit preparation of hydroxyethylidinediphosphonate (HEDP) for labelling with ^{188}Re has been developed for both palliative therapy and diagnosis of metastatic cancer to bone. A generator-produced rhenium-188 with a short physical half life of 16.9 h and a maximal beta-energy of 2.1 MeV is very attractive for therapeutic applications.

Carrier free $Na^{188}ReO_4^-$ was obtained from an in-house alumina-based $^{188}W/^{188}Re$ generator system by elution with 0.9% NaCl. To prepare ^{188}Re-HEDP up to 97 mCi in 2ml eluate of sodium perrhenate was added to a vial containing 5.0 mg HEDP, 4.9 mg $SnCl_2$, 2.9 mg ascorbic acid and 0.5 mg $KReO_4$. Then 200 µl of 3 M HCl was added to decreas pH to 0.5 – 1. The whole mixture was heated for 15 min at 95°C to 100°C, allowed to cool, whereafter 2.0 ml sodium acetate solution (39mg/ml) contains 50 µl 30% NaOH was added to adjust the pH to 5-6. The radiochemical purity was determined by ITLC-SG using 0.9% NaCl as solvent and paper chromatography using Whatman 1 and acetone as developing solution. Using saline as eluent, ^{188}Re-HEDP and perrhenate move with the solvent front and only reduced colloids or ReO_2 remain at the origin, while using acetone, ^{188}Re-HEDP stays at the origin and free perrhenate moves with the solvent front. Stability of ^{188}Re-HEDP was observed when stored at 20-25°C for 1, 2, 3, 24, and 48h. All the compounds were analysed by ITLC for RPC.The TLC scanner was used in the analytical work. We also analysed ^{188}Re-HEDP biodistibution characteristic and bone uptake following intravenous injection in rats to assess its potential for clinical use. The healthy Wisstar rats were injected with approximately 15.3 MBq labelled preparation in a volume of 0.2 ml intravenously and then sacrificed at 1 h, 3 h and 24 h (three rats at each time). Samples of different organs were weighed counted in a well-type gamma counter to calculate activity in various organs. Tissue concentrations were calculated and expressed as percent injected dose per gram tissue (% ID/g). Bone/muscle uptake ratio was detrmined from the % ID/g values of those organs.

Radiochemical analysis indicated high yields of labelling were above 99% after storage at 20-25°C for 24 h, and the labelling compounds being stable for 48 h (RCP>96%). The biodistribution study showed the radioactivity in the bone tissue to be as high as 2.68% ID/g at 1 h increasing to 3.32% ID/g at 3h. The activity level in the kidney was higest at 1 h but decreased through the study. Radioactivities in the blood, lung, liver, spleen, and muscle were all lower than 0.23% ID/g at 1 h and decreased rapidly. The bone/muscle ratio decreased to 55.71 at 24 h.

The results of this study showed the preparation ^{188}Re-HEDP is sufficiently stable, high level of deposition in bone was observed while injected intravenously to healthy rats. The kit formula for preparation ^{188}Re-HEDP provides an alternative promising radiopharmaceutical to treat and diagnosis bone metastases.

Problems in quality control of excipients in radiopharmaceutical kits. Validation of standard addition method in quality control of ascorbic acid assay in ^{188}Re-HEDP kit

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A Method of Standard Addition (MOSA) and HPLC analysis for quality control and determination of ascorbic acid (AA) in Re-HEDP lyophilized samples kit are presented. The chromatographic conditions are the following: Column - ODS, Microsorb, 250 x 4.6 mm, 100 Å, 5 µm; Detector - UV, 245 nm; sensitivity – 0.5 AU/1V; Eluent-1 ml 85% H_3PO_4 + NaOH to pH = 6.4 and distilled water up to 1 L; Flow - 1 ml/min; Isocratic. The chromatographic parameters describing separation process (in terms of validation – system suitability test) are the following: retention time 2.6 - 2.7 min. Resolution (R): 2.0 - 2.6; Symmetry factor (at 5% peak height): 1.46 -1.56; Separation factors: α_1 = 1.05 - 1.2, α_2 = 1.13 - 1.16; Number of theoretical plates/m (N/m): 24220–25863. Parameters R and N/m were calculated based on well known equations with using the peak width at half height.

Results of analysis of some samples are presented in Table I.

TABLE I. EXAMPLE OF DETERMINATION OF AA BY MOSA IN SAMPLES OF KIT

Series	01/04	01/04/2	02/04
Sample weight, mg	20.0	18.7	19.1
Assay, mg/g	116.2 ± 15.1	183.5 ± 10.1	150.1 ± 11.6
[1]RSD(x_s),%	6.45	2.37	3.83
[2]Recovery,% (mean value)	100.3	99.7	97.6
Linear range; µg/ml	0.00 – 12.00		
Number of independent measurements, n	7		
Slope, a	222368	192741	222960
RSD_a, %	2.64	1.57	1.77
Intercept, b	1033809	1327749	1278696
RSD_b, %	4.09	1.65	2.23
Mean Standard Deviation of Regression	62179	32083	41810
Correlation Coefficient, R	0.9983	0.9994	0.9992
F -Test for correlation	1432	4042	3185
$F_{1;\ 5;\ 0,05}$ (tabulated value for 95% significance level)	6.61		

[1]Relative standard deviations for the results obtained by MOSA method were calculated 0 as $RSD(x_s) = S_{xs}/x_s$ x 100%

$$S_{xs} = \frac{SD^{\$}}{a}\sqrt{\frac{1}{n} + \frac{\overline{Y}^2}{a^2\sum_1^n (X - \overline{X})^2}}$$

where

$SD^{\$}$ - mean standard deviation of regression,
n – number of spiked samples prepared for the construction of the standard addition curve,
a – slope of the standard addition curve,
$\overline{Y}$ - mean value of measured analytical signal,
X – concentration of added ascorbic acid,
$\overline{X}$ - mean value of added ascorbic acid concentrations.

Assay of ascorbic acid in analysed samples, x_s, was obtained by extrapolation the standard addition curve up to y = 0 (where y is measured analytical signal – peak area) or calculated as $x_s = b/a$ (where a and b are slope and intercept of regression line, respectively).

[2]Recovery Rec(%) was calculated according to 0:

Experimental values of F-test for correlations in all causes are much higher than tabulated value, which confirms significance of correlations. Slopes of MOSA curves are similar, what is characteristic for MOSA analysis of samples with the same matrix 0, 0

The precision, described by standard deviation, in MOSA analysis is lower than in typical analysis based on repeated measurements for the same sample and standard calibration graph. This comes from that standard deviation of assay in MOSA directly reflects error of curve (mean standard deviations of regression). When the standard deviations of measured concentrations in standard calibration is calculated based on mean standard deviations of regression – it value is usually similar to value obtained by MOSA 0.

The accuracy of a MOSA method expressed by recovery in all cases is satisfactory is better than for standard calibration method where recovery was 123.6%, 94.5% and 102.6% for samples 01/04, 01/04/1 and 02/04 respectively.

The calculated assays of AA in tested samples are lower than amount of AA declared (218 mg/g) for this series before processing. This fact reflects loss of AA caused by matrix effect (e.g. presence of tin(II) in kit as well as loss during processing (lyophilization etc.). It was confirmed in preliminary spectrophotometric and HPLC experiments that addition of tin (II) to AA causes meaningful decreasing of analytical signal for ascorbic acid.

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Comparative evaluation of DOTA-TATE radiolabelled with ^{177}Lu and ^{90}Y

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During the last decade, due to the significant progress in various related scientific fields (target, radio- peptide chemistry, monoclonal antibodies, biotechnology, etc.), a number of new radionuclides, tumour antigen binding, regulatory peptide analogues and bifunctional complexing agents are available. These are exploited for development of more effective radiopharmaceuticals suitable for receptor mediated therapy involving the beta- and beta-/gamma-emitters. The therapeutic efficacy of the new radiopharmaceutical depends on the ability of the carrier molecule to recognize the tumour cells receptors and on the physical properties of the selected radionuclide (emitted radiation, energy/range in the tissue, half-life). However, it should be remembered that the radionuclide attached to the biological molecule may affect its receptor affinity.

The goal of this work was to compare the biological properties of a somatostatin analogue, DOTATATE (DOTA-Phe^1,Tyr^3-octreotate) labelled with ^{177}Lu or with ^{90}Y. The criteria used in this evaluation were: labelling yield, specific activity of labelled preparations, stability of the obtained complexes, their stability in human serum and affinity to somatostatin receptors present at the rat pancreatic tumour cells AR42J.

The labeling was carried out in acetic buffer (0.4 M) or in ascorbic acid solution (50 mg/ml) at pH= 4.5 followed by 25 min incubation at 95^{o}C. The radiochemical purity of radiolabelled peptides was determined by TLC, HPLC and SepPack separation. Human serum stability was tested at 37^{o}C over the period of 24 h after labelling. The protein binding was determined using Minispin G-50 columns. The biological investigations including internalisation and receptor affinity were carried out on live AR42J cells at temperature 37^{o}C during 120 minutes. For the determination of non-specific binding the somatostatin receptors on the cells were blocked by Sandostatin or cold peptide.

The complexes of DOTATATE with ^{90}Y and ^{177}Lu were obtained with high radiochemical purity, over 99% in both investigated solutions. The obtained specific activity of the labelled preparation for carried free ^{90}Y was higher than 1,5 Ci/μmol peptide. We found that the labelling yield and in consequence obtained specific activity depend on concentration of the metalic impurities in labelling mixtures. When the concentration of Fe was higher then 0.1 μmol/Ci ^{90}Y and of Zn, Cu, Cd higher then 0.5 μmol/Ci ^{90}Y, the labelling yield decreased to about 20%. The complexes of DOTATATE with both lanthanides are stable at the room temperature over at least 24 h when labelling was carried out in ascorbic acid (the radiochemical purity values for ^{90}Y and ^{177}Lu were: after 4 h 99.91% and 99.40%, after 24 h 99.08% and 99.62% respectively), and decreased to about 6 h when labelling was carried out in acetic acid. Both complexes are stable in human serum for at least 4 h. The protein binding is low and for both preparations does not exceed 2%. The investigations of cell internalization indicate that the tracers are rapidly internalizing to the AR42J cells (about 80% for ^{90}Y-DOTATATE and ^{177}Lu-DOTATATE during 60 – 90 minutes, while the binding to the receptors on cell surface is about 20 %). The non-specific binding is low and equal to about 1%. The somatostatin receptor affinity of ^{177}Lu-DOTA defined as concentration of cold

peptide, at which half of the receptors is saturated with the radiolabelled peptide, IC_{50}, is equal to about 40 nM, which is indicating high receptor affinity of ^{177}Lu-DOTATATE.

$^{188}W/^{188}Re$ generator - investigation of long term stability

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The rhenium-188 ($T_{1/2}$ = 16,98 h) is a beta-gamma emitting radionuclide (average energy of β^- particles - 764 keV) suitable for therapeutical application. The gamma emission of ^{188}Re (155 keV) allows evaluation of biodistribution of radiopharmaceuticals and helps in radiation dosimetry study.

Radionuclide ^{188}Re is produced from ^{188}W as a daughter isotope. Tungsten-188 is obtained by neutron activation of ^{186}W in high flux nuclear reactor (^{186}W (n,γ) = ^{187}W (n,γ) = ^{188}W).

The production of rhenium-188 at the centralized laboratory needs handling of large amounts of activity to overcome the loss of activity during isotope production and transportation to the end user. The alternative is a portable generator in a system similar to $^{99}Mo/^{99m}Tc$ generator. In this paper we present results of the long term stability study of $^{188}W/^{188}Re$ generator.

The generator column containing 1 g of alumina was first activated using 0,9% NaCl solution (pH = 3.0). Then the 5,5 GBq of ^{188}W (195 GBq/g W) in the form of tungstenic acid was slowly loaded on the column (flow 0,1 ml/min). After ^{188}W deposition the alumina column was washed with 0,9% NaCl solution (pH = 5.0 – 6.0). The generator was eluted 2-3 times per week during 3 months. The yield of elution was measured as ^{188}Re activity calculated on the elution time. The radionuclidic purity of the eluates was checked by γ-spectroscopy. It covered the overall assessment of radionuclidic impurities related to the ^{188}Re activity. The radiochemical purity of eluted ^{188}Re-perrhenate solution was determined by TLC methods. Chemical purity of the eluate was determited using ICP-Optical Emission spectrometer (Optima 3300 XL, Perkin-Elmer).

The results of our study show that the elution yield of ^{188}Re calculated on the elution time was about 90% of the theoretical activity and more then 90 % of eluated activity was collected in first 4 ml of the eluate. We also observed low level of radionuclidic impurities in eluate (less then $5*10^{-2}$ %). The content of ^{188}W (^{188}W break-through) in eluate from generator was lower then $2*10^{-2}$ %. Only in first elution the content of ^{188}W was higher of about 1,2 % . Some of the chemical impurities were observed in the first eluate as well but then were coming to the negligible level. In first elution the Al concentration was about 120 ppm, but in next elution the Al concentration decreased to about 10 ppm.

Binding of ^{211}At- Rh(16-S4-diol) complexes to biomolecules — A new tool for preparation of astatine radiopharmaceuticals

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In the field of radioimmunotherapy, the radioisotope choice is related to the type of disease to be treated. The types of particles emitted are directly related to the tissue penetration and cell killing ability of the isotope. Many radiometals are under investigation for therapeutic applications, most notably the Auger emitter ^{67}Ga ($t_{1/2}$=3.3 d), α particle emitters ^{211}At ($t_{1/2}$=7.2 h), ^{212}Bi ($t_{1/2}$=1 h), ^{213}Bi ($t_{1/2}$=45.6 m), and β particle emitters ^{90}Y($t_{1/2}$=64 h), ^{188}Re($t_{1/2}$=16,7 h), ^{153}Sm(($t_{1/2}$=46,8 h, ^{177}Lu ($t_{1/2}$=6.7 d), ^{67}Cu ($t_{1/2}$=2.6 d), ^{105}Rh ($t_{1/2}$=36), ^{47}Sc ($t_{1/2}$=3.3 d), and ^{109}Pd ($t_{1/2}$=13.5 h,) [1]. Solid tumours have been successfully treated with 8 emitters including ^{90}Y, ^{177}Lu and ^{131}I, where the β particle from these isotopes has a tissue range of several millimeters. The tissue range of β particles is not optimal for treatment of small clusters of cells or single cells, micrometastatic disease, leukemias, and lymphomas. Treatment of these diseases may be more efficient with α-emitters, which combine high cytotoxicity and a short tissue range. Considerable effort has been placed in the development of the α-emitters ^{212}Bi, ^{213}Bi and ^{225}Ac and ^{211}At. Unfortunately, the half-life of both ^{212}Bi and ^{213}Bi is short, potentially limiting applications. ^{225}Ac has also limited applicability, due to decay to intermediate product ^{221}Fr which can easy diffuse from the biomolecule. Actually the most promising is ^{211}At.

^{211}At decays via two branches to the stable ^{207}Pb. High energy α particles with mean energy of 6.4 MeV are emitted in the decay, corresponding to a mean range in human tissue of 65μm. Therefore, this nuclide may be optimal for the treatment of micrometastases. Additionally EC decay give rise to high intensity Po X rays making ^{211}At easy to follow with γ camera [2].

Astatine-211 labeled immunoconjugates have been synthesized and evaluated for their therapeutic potential. Proteins labeled with ^{211}At by direct electrophilic astatination were unstable by virtue of rapid loss of ^{211}At following in vivo administration. Better stabilization, but not fully satisfactory exhibit biomolecules labeled by electrophilic astato-destannylation of *N*-succinimidyl- 3-(trimethyl stannyl) benzoate.

In this comunicate we present preliminary results of our studies on labeling biomolecules by utilization of metal cation bridge between ^{211}At and biomolecule. As promissing metal cation which should form strong and inert complexes with At^- ligand, Rh^{3+} were selected. In the first step the mixed complexes between Rh^{3+}(16-S4-diol)At^- were studied.

The ^{211}At was obtained at AIC-144 cyclotron in Institute of Nuclear Physic in Cracow and U-200 cyclotron in Joint Institute of Nuclear Research, Dubna The apparatus for separation of ^{211}At from the metallic target is a home-made one and generally follows the construction

described in Ref. [3]. The separation of ^{211}At is carried out under argon, at the gas flow of 120 cm^3 min^{-1}. After 15 minutes of flushing the apparatus with argon, the activated target is placed in the quartz tube inside the resistance furnace, and the heating is switched on. The desired temperature of 650 ^{o}C is achieved during 30 min., and kept constant during further 15 min. The evolved ^{211}At is collected in a cold trap consisting of a 1 mm I.D. polyethylene tube, immersed in ethanol cooled with liquid nitrogen down to the temperature between -55 ^{o}C and 50 ^{o}C. The obtained ^{211}At was dissolved in 100 μl of mixture Na_2SO_3 $NaNO_3$ solution.

The formation of Rh(16-S4-diol)ClI$^+$ and Rh(16-S4-diol)ClAt$^+$ complexes were studied using paper electrophoresis technique and ion exchange method. We found that after mixing of equivalent amounts of $RhCl_3$, tetrathioeter, trace amount of ^{131}I or ^{211}At the mixed complexes are formed. The probably structure of the complexes are presented in Fig.1. In next step we plan to study stability of the obtained complexes in various media and study of possibility to exchange of chloride anion in the complexes by biomolecule with thiol terminate group.

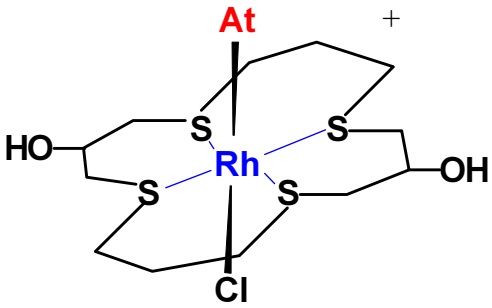

FIG.1. Structure of Rh(16-S4-diol)ClAt$^+$ complex.

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Biological evaluation of ^{153}Sm and ^{166}Ho complexes with macrocyclic ligands containing acetate pendant arms as potential agents for therapy

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For the development of therapeutic radiopharmaceuticals it is essential to choose the appropriate beta-emitter as well as the carrier biomolecule [1].

Different carrier biomolecules, namely antibodies and peptides, have been linked to different beta-emitters (^{153}Sm, ^{166}Ho and ^{177}Lu) using tetraaza macrocycles as bifunctional chelators [2, 3]. The cavity size of these chelators, the rigidity of the macrocyclic backbone and the nature of the pendant arms seems to play an important role on the thermodynamic stability and kinetic inertness of the radiocomplexes and on their biological behaviour [4, 5].

In our research group we have been exploring the possibility of using tetraazamacrocycles with different cavity size, pendant arms and rigidity for preparing ^{153}Sm and ^{166}Ho complexes useful for therapeutical applications and/or bone pain palliation [5-7].

In this communication we present the results obtained when we reacted trita and teta (Fig. 1) with ^{153}Sm and ^{166}Ho. ´

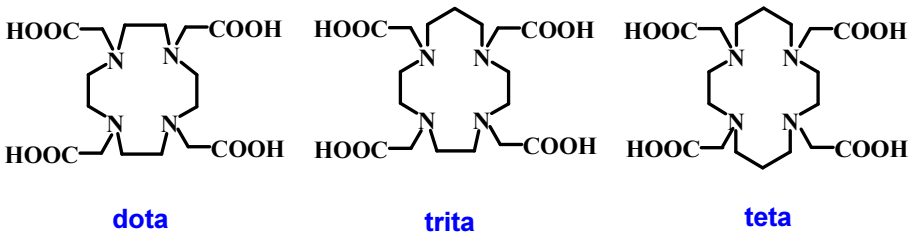

FIG. 1. Structure of the macrocyclic ligands dota, trita and teta used in this study

The complexes are formed in good yields (> 98%), are hydrophilic and present an overall negative charges, as well as low plasmatic protein binding. Good *in vitro* stability in physiological media and human serum was also found for all the complexes. The biodistribution studies in mice are also presented and have shown that ^{153}Sm/^{166}Ho-trita and

^{166}Ho-teta have rapid tissue clearance, comparably to the corresponding dota complexes (Fig. 2). In contrast, ^{153}Sm-teta has a significant lower total excretion and a significant liver and muscle uptake (Fig 2).

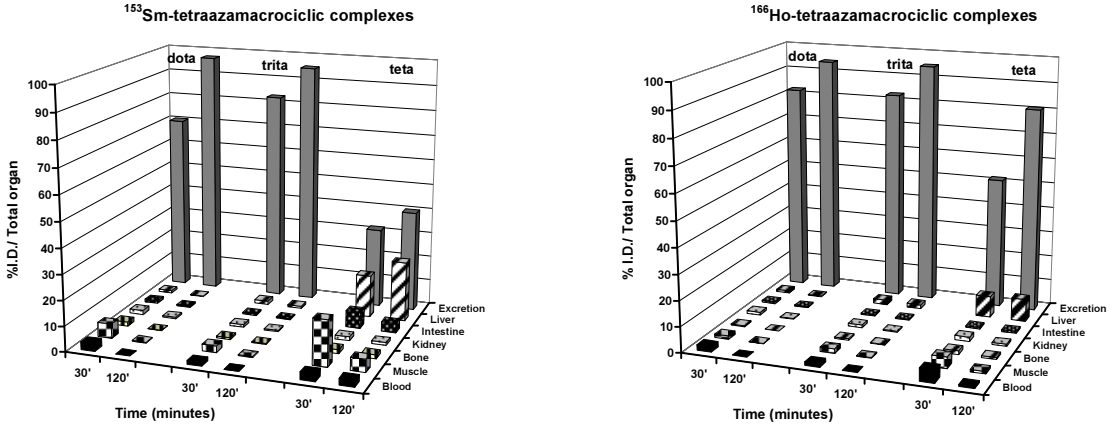

FIG. 2. Biodistribution data, expressed as percent of injected dose per total organ (% I.D.± SD) of ^{153}Sm/^{166}Ho-dota, -trita and -teta complexes, 30 minutes and 2 h after i.v. administration in female CD-1 mice (n = 3-4).

Our results indicate that ^{153}Sm/^{166}Ho-trita form very stable complexes *in vivo*. However, teta, which has a larger cavity size, forms less stable complexes with the larger ion Sm^{3+}. The biological profile of ^{153}Sm/^{166}Ho-trita is very interesting for the evaluation of these complexes as therapeutical agents when conjugated to biomolecules.

ACKNOWLEDGEMENTS

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Use of ^{99m}Tc HMPAO in demonstrating perilesional changes in intracerebral hemorrhage

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Spontaneous intracerebral hemorrhage has a poor prognosis and is considered the deadliest form of stroke. Many recent studies have shown that there are many mechanisms involved in the pathology of this disease: edema formation and extension, ischemia, inflammation, thrombin activation, rebleeding, reperfusion, metabolic changes, apoptosis. All of these factors (and maybe many others) are affecting brain tissue surrounding hematoma and are responsible of the progressive neurological deterioration and of the poor prognosis; most of these damages are not revealed by anatomical imaging techniques (CT or MRI). The aim of our study was to asses the role of brain perfusion SPECT with HMPAO in demonstrating perfusion changes in brain tissue surrounding hematoma.

We performed brain perfusion SPECT in 11 pts with primary intracerebral hemorrhage, within an interval of 24h to 5 days from the onset of stroke. Acquisition has been made 30 min after iv inj of 25 mCi of ^{99m}TcHMPAO, by using a dual head gamma camera. In all patients we compared the images with the CT scan, performed in the same day with brain SPECT.

8/11 patients showed a larger perfusion defect than expected after CT examination. In 2 patients hematoma maximum diameter was comparable on CT and on SPECT images; in 1 patient with intraventricular hemorrhage we found a quasinormal aspect of the perfusion study. In the group of patients with larger perfusion defect, SPECT revealed a large cold spot with a similar size compared with CT and a surrounding hypoperfused area. In 3 patients imaged at 36–72 h from onset, SPECT revealed an area of hyperperfusion in the peripheral cortex, adjacent to hypoperfused area and corresponding to a normal-appearing brain tissue on the CT scan. In 2 patients brain perfusion SPECT revealed crossed cerebellar diaskisis. In one patient we found a cortical hypoperfusion in the contralateral cortex, also corresponding to a normal appearing brain tissue on CT scan.

Brain perfusion SPECT revealed not only the irreversible cerebral lesions of the brain within the cold area of the hemorrhage, but also hypoperfused areas surrounding the hematoma. These areas contain viable brain tissue that may be a target for future neuroprotective therapeutic strategies. We can conclude that brain perfusion SPECT could play an important role in evaluating patients with hemorrhagic stroke, by early demonstrating severe functional changes responsible of clinical deterioration, thus allowing prompt dedicated therapeutic intervention.

In vitro and in vivo evaluation of phosphonates labelled with therapeutic radionuclides

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A great interest for radiotherapy of metastatic bone is represented by phosphonate ligands labeled with β-emitting therapeutic radionuclides such as ^{186,188}Re, ^{153}Sm, ^{177}Lu, ^{166}Ho. The radiolabeling of such ligands leads to complexes which present a synergic biological activity at manifest lesions due to both beta-radiolysis and chemotherapeutic effect of the ligands.

This study is focused on the chelating process of two phosphonates with biological activity and therapeutic potential, HEDP (1hydroxyethylidenediphosphonic acid) and TTHMP (triethylenetetraminehexamethylene phosphonic acid) with therapeutic radiometals ^{188}Re ($T_{1/2}$ = 17 hrs, $E_\beta$max = 2.12 MeV, E_γ= 155 keV) and ^{177}Lu ($T_{1/2}$ = 6.7 days, $E_\beta$max = 490 keV, E_γ= 208 keV). The ligand structure effect on the *in vitro* stability and on the biological affinity of the therapeutic agents was investigated.

The radiolabeling of HEDP with ^{188}Re was performed by carrier added method. Samples containing HEDP, stannous chloride, ascorbic acid; sodium hydrogen carbonate, sodium chloride; hydrochloric acid and water were labeled with $Na^{188}ReO_4$ (658.6 MBq) eluted from a ^{188}W/^{188}Re generator (MAP Medical, Finland). $KReO_4$, as carrier, was added to the eluate prior to the labeling process. The labeling was performed under inert N_2 atmosphere. The samples were sealed and incubated at 90ºC for 30 min., while the labeling pH was 1.5 – 1.7. After incubation and cooling at room temperature the pH of the samples was adjusted at 5.5 – 6.0. The labeling of TTHMP with ^{188}Re was done following the same protocol described for the HEDP labeling; the [PO_4^{3-}] to [Sn^{2+}] ratio was kept at the optimal value which was determined to be 3. Samples containing HEDP respectively TTHMP in sodium hydrogen carbonate / gentisic acid buffer were labeled with carrier free $^{177}LuCl_3$ in 0.05N HCl (1.66 GBq/μg) from Nordion, Canada. The samples were sealed under inert N_2 atmosphere and incubated at 90ºC for 30 min. The quality control was performed by TLC. The pharmacokinetic studies of the final products in rats were performed.

The radiochemical purity of the labeled compounds was higher than 95%, with high stability at the 48 h time interval. The in vivo biodistribution studies, in rats, show a rapid and quantitative accumulation in bone of labeled compounds and elimination via the urinary tract. The kinetic of bone accumulation processes of labeled phosphonates is determinated by the chelates structures. The maximum values of the bone uptake were ranged from 75.14% (injected dose/g organ) for ^{188}Re-TTHMP to 94.1 % for ^{177}Lu-TTHMP. ^{188}Re-HEDP and ^{177}Lu-HEDP showed a very similar bone accumulation pattern with a maximal value of 88.08% injected dose/g organ at 48 h p.i.

The bone accumulation and the blood clearance of ^{177}Lu-TTHMP were rapid, at 4 h after administration 93% of injected dose/gram organ was already uptaked; the high specificity and stability of this compound was maintained up to 48 h p.i.

The structure effect on biodistribution highliths that the luthetium, showing an easy chemistry, forms more stable chelates with poliphosphonates in spite to diphosphonates. On the other hand, the less reactive rhenium coupled with diphosphonates shows a better in vivo behaviour than Re-TTHMP.

The comparative evaluation encourages the use of ^{177}Lu-TTHMP and ^{188}Re-HEDP as a therapeutic agents as results from the Figure 1 and 2, presented below, due to their high and stable bone uptake, as well as a good ratio between the accumulated dose in the target organ to the accumulated dose in critical organs.

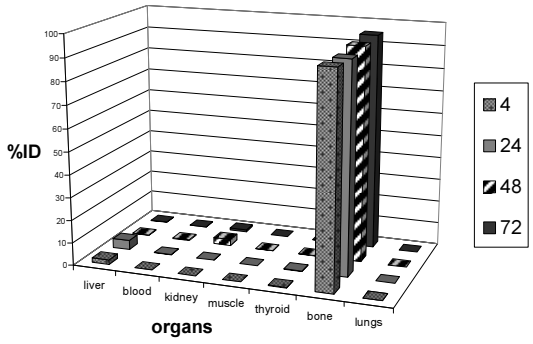

FIG. 1. Biodistribution of 177Lu-TTHMP

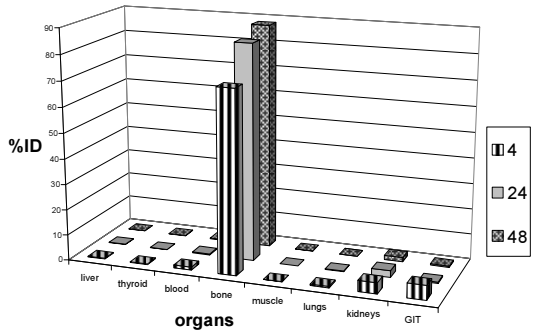

FIG.2. Biodistribution of 188Re-HEDP

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Gallium-68 solution for biomolecules labelling

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Nowadays positron emission tomography (PET) is the most powerful method of diagnostic medicine. One of the factors that limits applications of PET is its high costs, in particular, associated with the necessity to have a cyclotron and a radiochemical laboratory directly in a clinic. The usage of generator nuclides will make possible to decrease considerably the cost of radiopharmaceuticals (RPh), and, therefore, the cost for the diagnosis procedure as a whole; this will make PET more available for a wide circle of patients. The use of generators and kits for them is an alternative for the complete cycle of obtaining RPH directly in a hospital.

Among all generator PET nuclides, gallium-68 is the most promising one by the combination of its properties (nuclear-physical and chemical properties, availability and the price). Several years ago at our participation the generator based on titanium dioxide (modified with zirconium dioxide) sorbent and a dilute HCl solution (0,1N) as an eluent has been developed [1]. The gallium from such solution can be fairly easily transformed into a desired chemical form. At present "Cyclotron" (Obninsk, Russia) manufactures this generator.

The purpose of the presented work was study operating characteristics of commercial generator (efficiency and profile, parent Ge-68 breakthrough) during long-time operation, elaboration method of deep eluate purification and labeling of protein.

During more then 2-years operation period efficiency of Ga-68 elution was dropped with 78-81% up to 36-40%. Breakthrough of parent nuclide Ge-68 did not exceed $5*10^{-3}$ % in all time of operation. The elution profile of fresh generator is presented on Fig.1, in the course of time it becomes less sharp.

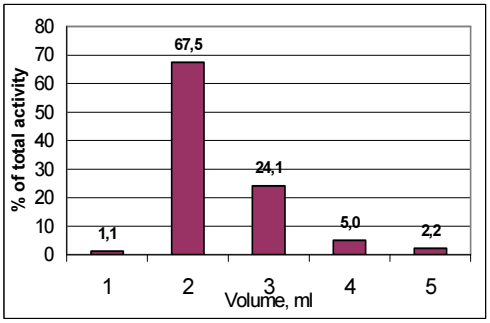

FIG 1. Elution profile of commercial generator.

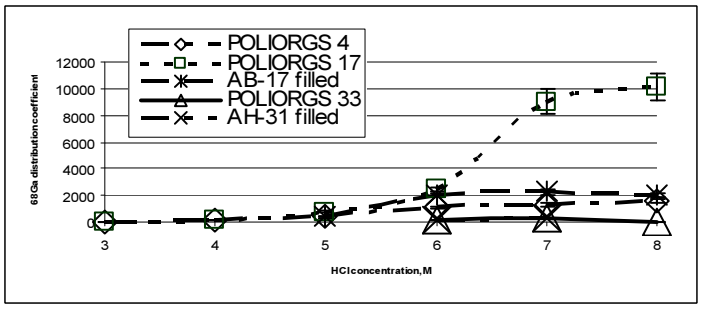

FIG 2. The distribution coefficient (K_D) ^{68}Ga on

We tried to purify and concentrate an eluate using a ion exchange technique on POLIORGS™ sorbents. These are "filled" sorbents - non-woven, polyacrylonitrile fibrous material filled with low size (10-30 mkm) granulated complexing sorbent. They are designed in GEOCHY (RAS, Moscow) and produced on RISF (Tver). It has been found, that Ga-68 adsorb practically quantitatively from from concentrated HCL solution (> 4M; Fig. 2). At the same time a sorption of Ge-68 considerably increases at more then 6 M HCL concentration. For safety work we have created semi-automatic module which is based on multi-head peristaltic

pump (Gilson) and chemically inert valves (Burkert). On this system different sorbent in static conditions (15 min) we received the next results: efficiency of ^{68}Ga washout 96.5 %, breakthrough 2.5 %, losses on a column ~ 1 % (not decay corrected). Practical yields are 78±8 % ^{68}Ga in 120 mkl of 0.01 M HCl for 10 minutes. Thus concentration of ^{68}Ge has decreased 20 times and about 10-100 times for chemical impurities (Fe, Zn, etc) as well. The microcolumn with a sorbent has undergone more than 40 concentration cycles without loss of working characteristics.

For labeling experiments we used well-known DTPA-Octreotide as model compound. Labelling of DTPA derivative is not so effective as DOTA one, but we fond condition where radiochemical yield was nearly 90%. After intravenous administration in tumour bearing mouse this tracer shown the biodistribution like ^{111}In-DTPA-Octreotide (Table 1)

TABLE I. BIODISTRIBUTION OF LABELLING DTPA-OCTREOTIDE IN MELANOMA-BEARING MICE (%ID/G)

Label on DTPA-Octreotide	In-111		Ga-68	
Organ/tissue	20 min	1 h	20 min	1 h
Blood	0,6	0,1	1,0	0,1
Lung	0,7	0,2	1,1	0,2
Liver	1,4	0,4	1,8	0,8
Kidneys	6,9	3,6	3,9	1,5
Stomach	0,3	0,1	0,5	0,1
Intestine	2,1	0,9	2,7	0,9
Urinary bladder	35,8	45,3	52,8	73,1
Tumour	**2,3**	**3,0**	**8,1**	**0,9**
Muscle	0,2	0,1	6,5	0,3
Bone	0,1	0,2	0,6	0,1
Tumour/ Muscle ratio	10,2	20,9	1,2	3,1
Tumour/ Blood ratio	4,0	28,1	7,8	9,4

Qualification of the GMP compliant FDG production and distribution unit

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Recently we installed in our laboratory a GMP-compliant compact combined FDG production and dose distribution unit. This unit, constructed by HWM, Rossendorf, consists of the mini-cell, housing the FDG synthesis module from GE, standing side-by-side with the class A shielded isolator containing remotelly controlled dose distribution module.

Use of the isolator technology combined with laminar flow HEPA filtered air inside the dose distribution hot-cell allows to achieve class A environment for the aseptic filing of FDG into the opened sterile vials. Synthesis of FDG and preparation of the mother batch solution is achieved inside the hot-cells providing class C clean room environement. The whole production unit is standing in the class D laboratory.

The complete set of IQ-OQ-PQ tests was performed to assure that the FDG production and distribution unit is performing accordingly to GMP requirements and is providing the product of consistent quality. The majour component in the qualification tests are the aseptic procedure validations, which consisted of the procedures recommended by GMP guidelines and adapted for the specificity of the FDG production. As a minimum for the aseptic validation we considered the Mediafil Test, Bioburden Test and test productions.

All the test results (Table I below) assure that the FDG production unit allows to produce FDG for central distribution in accordance with the requirements of European pharmacopoeia and GMP guidelines.

TABLE I: SUMMARY OF BIOLOGICAL TEST RESULTS (STERILITY)

Tested equipment		Number of tested vials, Test Method			Total vials	Number of cfu* found
		Sterility	BioBurden	MediaFill		
^{18}F-transfer lines		-	8	8	16	0
TRACERLAB-FX_FDG	FDG-1	-	5	3	8	0
	FDG-2	-	3	3	6	0
Product transfer lines	FDG-1	-	4	3	7	0
	FDG-2	-	3	3	6	0
Dose distribution module	KIT-1	-	10	10	20	0
	KIT-2	-	10	10	20	0
Complete production runs (validation batches)	GLUPET-1	23	-	14	37	0
	GLUPET-2	17	-	14	31	0
	TOTAL :	40	43	68	**151**	**0**
Total operators tested: 4						

* cfu – colony forming units

Syrian experience of ^{18}FDG production system setup and related quality control aspects

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In this study, analysis conditions and calibration curves of acetonitrile, ethanol, ethyl ether have been determined using gas chromarography. Then, the concentration of these solvents in ^{18}FDG produced by IBA module, have been determined. The cold FDG retention time and the D-glucose analysis considtions and calibration curves have been also determined using radio-ion chromatography containing amperometric cell and γ ray detector.

^{18}FDG contents of kryptofix 2.2.2. has been also determined by thin layer chromatrography method.

The results showed that ^{18}FDG content of acetonitril, ethanol, ethyl ether were 0.12-0.02.-0.07 ,g/ml, respectively, while the concentration of D-glucose ranged between 0.1 and 0.4 mg/ml and the kryptofx 2.2.2. was less than 0.02 mg/ml.

Keywords: ^{18}FDG quality control, Kryptofix 2.2.2., Ethanol, Ethyl Ether, Acetonitril.

Regulatory control of radiopharmaceuticals in Tanzania

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Millions of nuclear medicine procedures are performed each year worldwide and the demand for radioisotopes is increasing rapidly [1,2].The use of radiopharmaceuticals in Tanzania like in many other countries has also been increasing in recent years that necessitates the need for close regulatory control. Their applications in the country started in 1980`s in the areas of medical diagnosis and therapy and at preesent only one center provides such services. This paper describes how radiopharmaceuticals are regulated (radiological point of view) from importation, their use and the management of the resulting radioactive waste. The regulatory framework for nuclear safety, radiation protection and security of radioactive materials was established following the national interest in the area of peaceful applications of nuclear technology [3]. The regulations require that any person intending to import, export or transport any apparatus, article, plant, installation or other material or substance which is a source or intended to be used for the purposes of an undertaking involving the emission of radiation, must apply for a license from the regulatory authority.Radiopharmaceuticals are not produced in the country therefore whoever intends to import, possess and use has to obtain an authorization from the regulatory authority. Licensing requirements inlude the presence of : -

- Qualified personnel to administer radiopharmaceuticals
- Quality Assuarance and Quality Control Programs
- Radiation protection measures including emergency planning ,preparedness and response
- Radioactive waste management program
- Written laboratory procedures and safety guidelines

With regard to radioactive waste generated, it is a requirement that waste be managed properly for the protection of personnel, general public and the environment [3,4]. While liquid wastes are diluted and disposed in a normal sewage system after reaching the respective exemption levels, solids and biological materials are incinerated after sufficient radioactive decay. There are few drawbacks in the regulatory process that include:

- Inadequate number of qualified nuclear medicine personnel
- Lack of periodical calibration of dose calibrators which is an essential component of quality assurance.
- License to import radiopharmaceuticals is valid for one year allowing multiple importation without verification by the regulatory authority. This may give the licensee a room to import more radiopharmaceuticals than declared in the license application form

At present nearly all aspects of peaceful applications of nuclear technology, including the use of radiopharmaceuticals are being regulated satisfactorily. Based on the available data on the amount imported in the past three years, and the annual frequency of procedures carried out, it can be concluded that there is an incerease of radiopharmaceutical importation and application in the country.

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Regulatory aspects on the management of waste generated from radiopharmaceuticals application in Tanzania

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The application of radiopharmaceuticals, I-131 and Tc-99m in health care has been practiced in Tanzania now for more than two decades, in the field of diagnosis and treatment of cancer. Depending on appropriately and early diagnosis, this application has provided the most reliable and effective method of cancer control in the country. In the course of using radiopharmaceuticals, the two types of wastes are generated in the form of liquid and solid. The management of radioactive wastes in Tanzania is regulated according to the national waste management regulations which are based on IAEA basic safety standard Series 115 [1, 2]. However in practice the radioactive waste regulations are not fully complied. As such there has been uncontrolled release of liquid wastes to the public sewage system. Furthermore in principle, incineration of solid waste should be carried out only if it is ensured that materials to be incinerated have no high moisture content to ensure complete combustion [3]. During such operations the licensee should notify the regulatory authority to verify if the practice is carried in proper way and as required by regulations such that the total activities released to local dump are within the clearance levels approved by regulatory authority. In most cases licensee does not follow this. Table I presents the total amount of radioactivity discharged to the public sewage for ten years starting from 1994 to 2004 at Ocean Road Cancer Institute (ORCI). The radio wastes are from excreta of patients who were injected radiopharmaceuticals for diagnostic and therapy. According to the table 1, the maximum discharge to the sewage were 80,450MBq in 2002 for Tc-99m and 7,890MBq in 2000 for I-131 and the minimum were 7,622MBq in 1994 for Tc-99m and 1,749MBq in 1998 for I-131.The calculations used to derive the data for table 1 are also given in this paper. Approximately 80% of the iodine given to a patient comes out in the urine during the first 24 h [4]. In the view of the table 1, the violation of regulatory compliances by releasing liquid to public sewage is noted. Furthermore, two methods are recommended to the users for liquid and solid wastes management as required by national waste management regulations. Some noted weakness on the regulatory enforcement and recommendation for improvement is discussed.

TABLE I: TOTAL AMOUNT OF RADIOACTIVITY DISCHARGED TO THE PUBLIC SEWAGE FROM 1994 TO 2004 AT ORCI

Year	Total Discharge to Drain (MBq)	
	Tc-99m	I-131
Jun-Dec 1994	7,622	
Jan- Dec 1995	14,737	
Mar- Dec 1996	31,306	
Jan-Dec 1997	51,720	3,528
Jan-Oct 1998	70,520	1,749
Mar-Dec 1999	69,800	3,858
Jan-Dec 2000	71,430	7,890
Jan- Dec 2001	78,900	4,201
Jan- Dec 2002	80,450	1,890
Jan- Dec 2003	79,873	6,487
Jan-Oct 2004	76,545	3,600

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^{188}Re(V) complex of cyclam, a stable rhenium-188 core potential for development of therapeutic radiopharmaceutical

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A method of labelling ^{188}Re-cyclam (1, 4, 8, 11-Tetraazacyclotetradecane) in aqueous solution with high labelling yield and high stability has been investigated. The macrocyclic ligand of cyclam bound to rhenium and formed a stable complex of dioxorhenium (v) cation (fig 1), which suitable for nuclear medicine applications.

The labelling was performed in the aqueous solution of 15 mg potassium gluconate and 1 mg stannous chloride at pH above 5. After mixing with 5 –10 mCi of ^{188}ReO-4 and 1 mg cyclam, the reaction mixture was incubated at 80 °C for 60 minutes. The Labelling efficiency and in vitro stability were determined by ITLC-SG in 0.9% NaCl system and by paper electrophoresis in 0.005 M phosphate buffer pH 7.4 at 250 V for 45 min. The results showed high labelling efficiency up to 93% (Fig 2) and high in vitro stability over 24 h, less than 2% of free $^{188}ReO_4^-$ loss from metal-ligand complex.

In addition to higher labeling yield and its in vitro stability, the labeling could be performed in aqueous media which make Re(V)-cyclam complex promising as a new Re radiopharmaceuticals for therapeutic uses.

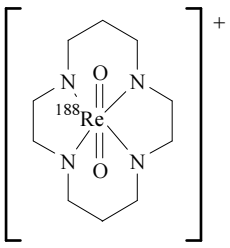

FIG. 1. Proposed structure of ^{188}Re(v) complex of cyclam.

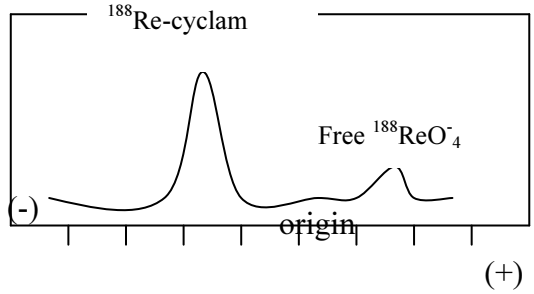

FIG. 2 P.E. chromatogram of $_{188}$Re-cyclam.

TABLE I. IN VITRO STABILITY OF ^{188}RE(V) COMPLEX OF CYCLAM

Incubation time	1 h	2 h	3 h	4 h	5 h	6 h	24 h
% free ^{188}ReO-4 loss	0.27% ± 2.50	0.46% ± 1.62	0.55% ±1.35	1.28% ±1.33	1.45% ±1.62	1.49% ±4.05	1.47% ±1.69

At present our research group have synthesized cyclam derivative for rhenium labelling to develop radiopharmaceutical for hepatic tumour therapy.

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Role of radionuclide therapy as adjuvant to palliative external beam radiotherapy for painful multiple skeletal metastasis

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The aim of this study was to evaluate the palliative efficacy of localized external radiotherapy (RT) with sistemic radionuclide (RN) therapy in patients with multiple painful osseos metastases of different primary origins.

Thirty-three patients with a refferal to the Ankara University School of Medicine Department of Radiation Oncology with painful multiple skeletal metastases from different primarily origins (breast 8, prostate 6, lung 10, GIS 3, others 6) were eligible for this trial. Local external radiotherapy was delivered to the most symptomatic region initially in all patients, and then they received either Re-186-HEDA= Rhenium 186 hidroxyethylydene disphosphonate or SM-153 EDTMP= Samarium-153 ethylene daimine tetramethylene phosphate. The mean age was 58.8 (min 35- max 82). The performance status was assessed acording to ECOG scale. At the start of treatment, end of the radiotherapy and after the 4 weeks sistemic radionuclide therapy analgesic intake and pain status were recorded by RTOG scoring system. EORTC QLQ C30 (vesion 3.0 Turkish) questionnaire was performed to evaluate the quality of life at the start of treatment, end of the radiotherapy and after the 4 week sistemic radionuclide therapy.

	ECOG	Pain Score	Analgesic Score
Pre RT vs Post RT	0.003	0.000	0.002
Post RT vs Post RN	0.157	0.067	0.020
Pre RT vs Post RN	0.008	0.000	0.001

Overall median survival was17 months, and progression free survival was 10 months. Patients with breast and prostate tumours had a higher overall and progression free survival than that of other primary tumours (p=0.0062, p=0.0080). An improved performance of 33.3% for post radiation therapy and 50% for post radionuclide therapy in ECOG scale were observed. Statistically significant correlations were found between the primary origins and decreased

pain and analgesic intake ($p< 0.05$). But no differences were observed on the self assesment quality of life questionnaire. Toxicity was acceptable.

Sistemic radionuclide therapy, as adjuvant to local external RT, is found to be reducing pain and analgesic intake particularly patients with multiple bone metastases of breast and prostate cancer with expectation of long survival. This combined treatment may be an alternative to total body or half body irradiation for multiple painful skeletal metastasis with acceptable toxicity and faster palliative response.

Treatment for microcarcinoma of the thyroid clinical experience

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According to WHO, small papillary cancer represents a papillary carcinoma < or = 1cm in dimension. The relationship between papillary microcarcinoma(PMC) and clinical papillary thyroid carcinoma (PTC) is not clear. Papillary microcarcinoma may be the earliest form of future large papillary carcinoma lesions.

Between 1997-2004 years, 120 patients with papillary microcarcinoma were investigated in our department(105 females,15 males;mean age 43±13). A surgical procedure ranging from bilateral subtotal(n=95) to bilateral total (n=25) was performed. In 25 patients tumour size was investigated to be bellow 0.5cm(25 patients≤0.5cm;95 patients=0.5-1.0 cm). Multifocal carcinoma were found in18 patients(%6.7), in 15 patients(%8) – lymphatic and 11 patients(%10.9)- capsular invasion were investigated.

In 112/120 patients(%93.3), the thyroid remnant was ablated by the first dose of I-131(2.7-5.5 GBq). Eight patients recieved a second radioiodine treatment,because of locoregional recurrence(5.5 GBq). All 120 patients remained free from disease(negative I-131 whole body scan,unmeasurable thyroglobulin levels) after a median follow up period of 45 months(16-84 months).

The treatment of patients with PMC should be no different from the treatment of patients with PTC, and thyroidectomy followed by radioiodine therapy is a possible option for treatment of papillary microcarcinoma.

Hurthle cell carcinoma - a clinicopathologic study of 13 cases

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Hurthle cell carcinoma of the thyroid is a variant of follicular carcinoma, which has been considered by many as a more aggressive disease than the usual well-differentiated carcinoma of the thyroid. Hurthle cell carcinomas exhibit unequivocal capsular and/or vascular invasion; they are aggressive tumours with a poor prognosis.

To investigate the clinico-pathologic characteristics, treatment, and outcome of Hurthle Cell Carcinoma.

During a 7-year period (1997 through 2004), 13 patients with Hurthle cell tumours were treated and monitored at the Ankara University. Forthysix percent were men and 54% were women(the female to male ratio - 7/6); mean age at diagnosis was 48.4 +/- 13.2 years (range: 29 to 84 years).

A separate non-Hurthle cell thyroid carcinoma was found within the thyroid gland in 4 (30%) of the patients--25% were papillary and 75% were follicular carcinomas. The tumours was measured from 1 to 6 cm in diameter. Three of the Hurthle cell carcinomas had extrathyroid invasion, five had intrathyroid invasion, and five were encapsulated (i.e., they had intracapsular invasion only). One (7%) of the patients had a history of low-dose, external radiation to the head and neck in childhood.

Treatment consisted of a total thyroidectomy in 12 patients(%92), and a subtotal thyroidectomy in 1 patient(%8). At surgery, lymph node metastases were present in 3 patients(23%) and lymph node dissection were performed in these patients. There was detected distant metastases at diagnosis only in one patient(lung metastases – 8% of all patients).

All of the patients had radioiodine(RAI) ablation therapy for residual thyroid tissue. RAI treatment was indicated in patients with gross residue, primary tumour size greater than 1cm, LN metastases, presence of extrathyroidal extension.

92% of the patients were ablated with a single dose of ^{131}I (3.7-5.5GBq). Only in one patient, because of the lung metastases, radioiodine treatment was twice recquired and was still followed. After a median follow-up period of 85 months, there was no recorded mortality due to the disease and 92% of the patients were categorized as disease free(criteria for ablation are as follows: negative I^{131}WBS and very low serum Tg levels).

We did not find higher incidences of local recurrences, distant metastases or mortality rates, compared to well differentiated thyroid carcinomas. Hurthle cell carcinomas of the thyroid and well differentiated thyroid carcinomas have similar biological behaviors. Their treatment should be similar, including total or near total thyroidectomy plus modified cervical node dissection when there is lymph node involvement. Radioactive iodine therapy and suppressive levothyroxin therapy should follow.When treated assertively, Hurthle cell carcinoma of the

thyroid, an oncocytic variant of follicular carcinoma, has a favorable outcome, similar to that of well differentiated thyroid carcinomas.

Radiopharmaceuticals in diagnostic and treatment of thyroid cancer

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The use of $^{131I-}$metaiodbenzylguanidine (^{131}I-MIBG), ^{131}In-octreotidi and ^{99m}Tc-DMSA provide the early diagnostic and treatment of the medullary thyroid cancer. The sensitivity of the method comprises 100% for ^{111}In-octreotidi and 60% for ^{131}I-MIBG is used for the treatment of the medullary thyroid cancer, pheochromocitoma and neuroblastoma to 35% in the case of medullary thyroid cancer [3]. The treatment of the patients has been made according to the protocols adopted by the European Association of Nuclear Medicine.

The whole body scintigraphy has been delivered during the treatment in 24,48,56 hours after the activity administration with the aim to evaluate the parameters of ^{131}I-MIBG acummulation and excretion in remnants and metastases.Regression analysis allows to choose the optimal time interval between the sequential administration of the radiopharmaceuticals. Hermatological parameters were monitored in the course of the treatment with the aim of controlling the bone marrow toxicity. The ultrasound, CT investigations and the determination of the level of calcitonin have been determined during the treatment.

Use of ^{131}I-MIBG was shown to improve the treatment outcome.

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DOTA-TATE alternative labellings with halogens and radiometals

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DOTA-D-phe-cys-tyr-D-trp-lys-thr-cys-thr (DOTA-TATE), a somatostatin analog with high affinity for somatostatin receptors expressed in cancer of neuroendocrine origin, may be labeled with different beta radionuclides for its use in targeted radiotherapy. This allows the obtanting of products with particular chemical, radiochemical and biological properties, which interact in specific way with cells that express somatostatin receptors. In this study the labelling of DOTA-TATE with ^{125}I, ^{131}I and ^{177}Lu and their biological properties were investigated, with the aim of potential application as a tumour seeking agents.

Radioiodine was introduced on the tyrosyl residue of the DOTA-TATE by oxidation of iodide with chloramine-T. The labeled peptide was isolated from the reaction mixture by sep-pak purification and/or by RP-HPLC. Lutetium-177 was introduced in the peptide via the DOTA ring. To 7,2µg of DOTA-TATE 1mg/mL, 2µL $^{177}LuCl_3$ (7.2 mCi) and 7.2 µL of gentisic acid in CH_3COONa 0.4M were added. The mixture was incubated 30 minutes at 100ºC. Purification by RP-HPLC was conducted on a C18 column eluted with a linear gradient from 10% to 40% B in 30 min (A: 0.1% TFA in water and B: 0.1% TFA in acetonitrile). Fractions containing the labeled peptide were pooled and most of the solvent removed by evaporation and re-suspended in 1 mL of saline. For the control of ^{131}I-DOTA-TATE, different gradients were used in order to optimize the separation. In the case of ^{177}Lu-DOTA-TATE analysis, gradient was from 5 to 95% B in 15 minutes. Stability at different time intervals was evaluated by RP-HPLC and by chromatography in ITLC-SG or Whatman 3MM using different mobile phases: 2-butanone (MEK); NaCl 0.9%; EtOH-HCl 0.01N 90:10 and butanol:acetic:H_2O (4:1:5) for radioidinated peptides. For ^{177}Lu-DOTA-TATE, the chromatographic systems were: CH_3COONH_4 10%/MeOH (30:70), BuOH-CH_3COOH-H_2O (5:2:1), TFA 0,1% in ACN- H_2O (1:1) and Sodium citrate 0.1M, pH:5 in Whatman 3MM (Rf of radiopeptide 0.9; 0.9-1.0; 0.9-1.0; 0.0 respectively). Biological behavior of radiolabelled peptides was evaluated using AR4-2J cell which express somatostatin receptors through internalization and externalization studies. Binding to AR4-2J cells at 1, 2, 3 and 4 h were studied, using 5 x 10^5 cells and 200.000 cpm of purified labeled conjugates per tube. Internalization of the radioconjugates at 3 and 4 h was done by further incubation in acidic conditions in order to remove the radiactivity bound to membrane. Biodistribution studies were carried out in CD-1 mice weighing 34-45g, injected with 0,5 to 74 KBq of ^{125}I-DOTA-TATE, or 1.85 to 5.50 MBq of ^{177}Lu-DOTA-TATE at 1, 2 or 4 and 24 hs post-injection. Results were expressed as percent injected dose per organ (%ID) and as percent injected dose per gram of tissue (%ID/g).

The results evidenced a labeling yield higher than 95% for ^{125}I as well as for ^{177}Lu, according with sep-pak elution profile as well as by chromatographic behavior of the reaction mixture using HPLC and ITLC-SG/saline system (radioiodine) or TFA 0,1% in ACN-H_2O (1:1)

(^{177}Lu). In the case of ^{125}I-DOTA-TATE the HPLC profile revealed that two radiochemical species were present having retention times of 23,5 and 25,0 min, while the unlabelled peptide eluted at 21,0 min, whilst for the other radiopeptides only one peak was found (11.2 min for ^{177}Lu-DOTA-TATE). Stability of the radioconjugate (at 4°C and -80°C) was confirmed during more than 8 days for the peptide labelled with ^{125}I, while with ^{177}Lu was more than 24 hs at 4°C. The binding of radiopeptides to viable AR4-2J cells increased along the time of incubation. Internalization experiments demonstrated that the radioconjugate penetrates into the cells reaching 73 ± 14 % (n=10), 32 ± 9 % (n=3) and 62 ± 18 (n=6) of the total bound activity in 3 - 4 h for the peptide labelled with ^{125}I, ^{131}I and ^{177}Lu respectively. The biological uptake pattern of the radioidinated peptide indicates urinary as well as gastrointestinal excretion. In vivo metabolization of the radioidinated peptide is evidenced by the thyroid uptake which reaches 10,2 ± 8,5 % of the injected activity in 24 h. In the case of labelling with ^{177}Lu the elimination is mainly by urinary excretion

In conclusion, DOTA-TATE labelled with radioiodine as well as with ^{177}Lu was obtained with very high yield even prior purification. The two radiochemical species evidenced for ^{125}I-DOTA-TATE by RP-HPLC could be interpreted as mono and diiodinated species. Their retention times are slightly higher than the intact unlabelled peptide, allowing the preparation of a labelled molecule with high specific activity. Binding experiments using AR42J cells indicated that the radioconjugates are able to recognize and bind to somatostatin receptors present in viable tumour cells. Biodistribution in normal mice shows a different pattern when labelling with radioiodide and radiolantanide. These findings opens the possibility of the use of cocktails of DOTA-TATE labelled with different radionuclides in order to minimize the radiation dose to organs not compromised with the tumour.

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Intracoronary radionuclide therapy with liquid ^{188}Re-filled balloons: Radiopharmaceutical and dosimetric studies

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Percutaneous transluminal coronary angioplasty (PCTA) associated with radioactive liquid-filled balloons has demostrated useful to inhibit the growth of neointimal tissue and as consequence offers a potential method to decrease restenosis rate. Near 40 to 60% of the patients suffered from restenosis, being necessary to reiterate angioplasty.

The spectrum of late effects from the variety of sources types, dosimetric aspects and delivery systems has not became clear. An attractive radionucleid for this purpose is ^{188}Re, that is eluated from a ^{188}W/^{188}Re generator. The generator can be used during 6 months, depending on the initial activity. The specific activity can be increased by a concentration system up to a maximum of 20 GBq/mL.

The present study had the purpose of optimizing the relation risk/benefit during a procedure of brachytherapy with ^{188}Re associated to angioplasty. The possibility of balloon rupture exists, with the passage of the radioactive solution towards the patient bloodstream. In order to increase the security during the treatment the evaluation of different agents such as ^{188}Re-DTPA, ^{188}Re-Citrate and ^{188}Re-EC vs $^{188}ReO_4^-$ (as the standard agent) was performed. Labelling procedures were optimized considering pH, temperature, reaction time and carrier adition. Dosimetric studies using Mirdose 3, after iv injection to Wistar rats of the three radiopharmaceuticals were performed. Biodistrubion at 1 and 3 hs post-administration and dose estimation showed no difference between all the radiopharmaceuticals, presenting low uptake in thyroid and stomach, and faster renal elimination than $^{188}ReO_4^-$, with smaller residence times in all the organs. As the preparation of ^{188}Re-Citrate and ^{188}Re-EC required less strong conditions than ^{188}Re-DTPA, one of these radiopharmaceuticals could be selected to improve the security of the procedure.

A second point of this study was the evaluation of a number of safety requirements in order to estimate radiation dose delivered to operating personnel. Ambiental film dosimeters, with and without β-emitters shielding, were placed in the Radiopharmacy Laboratory; exposition dose above the shielding wall and at different points within this laboratory were also determined, radiation doses in the Catheterization Laboratory were measured while each patient procedure was being performed; dose rates for all the personnel positions were registered. If each brachytherapy procedure demands 20 min each year the Nuclear Medicine physician could participate in 52 doses administrations, 108 times for the Cardiologist and 600 procedures for the radioprotection official. Elutions ^{188}W/^{188}Re generator demands 60 min. Radiopharmacist could participate in 50 procedures, with manual concentration devices. In our case radioprotection measures were carried out by the Radiopharmacist. All radiation exposition rates measured in this study were significantly lower than those reported for brachytherapy performed with ^{192}Ir.

Finally absorbed doses estimated by Monte Carlo method comparing the procedures carried out with ^{188}Re or ^{192}Ir, both to patient and to the staff, were performed. Exposition rates using lucite and lead shielding were estimated. Dose deposition around the balloon in the patient, considering both distance and radial distribution were also estimated. From these results it was possible to stablished that most of the dose was given in the first 3 mm for ^{188}Re. However the ^{192}Ìr device showed a penetration of approximately 10 cm; this has as consequence a greater non-target tissue irradiation. Dose distribution around the balloon showed that after ^{188}Re irradiation, the dose outside the balloon falled quickly to zero, which may enable to diminish the border effect, while in the case of ^{192}Ìr the previous effect could be accentuated as the gamma penetration is much greater.

Intravascular brachyterapy is a skilled, high precission procedure requiring the expertise of a multidisciplinary team. Radiation dose estimated to the operating staff and patient demostrated that it is a safe method, if unnecesary exposition is limited and radioprotection measures are implemented and registered. Alternatives radiopharmaceuticals were evaluated, both considering labelling procedures as dosimetric criteria.

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Labelling of new formulation of tin-sucralfate freeze-dried kit with technetium-99m and its biological evaluation

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Sucralfate, the aluminum hydroxide complex sulfated sucrose, promotes the healing of peptic and duodenal ulcers [1,2] in the acid medium of stomach, sucralfate becomes viscous and binds to denuded mucosa forming a protective coating. Many authors reported the use of Technetium-99m sucralfate in the clinical imaging of gastric and duodenal alcers [3] and inflammatory bowel disease [4] We therefore investigated the preparation of a new formulation of tin-sucralfate (F.D.K.) directly labeled with $^{99m}TcO_4$ and it's in vivo biological evaluation in mice. The lyophilized form containing 100 mg sucralfate and 11.3mg dihydrated stannous chloride. The optimal PH values of the labeled preparation were found to be from 4.0 to 10.0. The range of sucralfate amount studied (50-500 mg) not affacted the radiochemical purity of the labeld complex. The radiochemical purity and the stability of the labeled preparation that assessed by filteration were more than 95% .Technetium-99m sucralfate was radiochemical stable upto a specific activity of 1000 mCi per gram which was more stable than earlier published value (4) and without any radiolytic decomposition (Table I). The biological behaviour of ^{99m}Tc- eluate was evaluated in two groups of ulcrated fasted and non-fasted mice. The data of organ distribution of ^{99m}Tc-sucralfate in ulcerated mice (Table II) showed that more than 99% of the administered dose was accumulated in the stomach (87.92%) and intestine (11.43%). The radioanalytical results together with the in vivo biological behaviour of the labeled preparation demostrate it's stability, efficacy and usefulness in medical applications for the detection of gastrointestinal ulcers.

TABLE I. DATA OF THE STABILITY OF ^{99m}Tc - SUCRALFATE VERSUS TOTAL RADIOACTIVITY

Ref.	Radioactivity /mCi	Activity mCi/ Sucralfate mg	Incubation time /hr					
			1 h		3 h		24 h	
			*A	*B	*A	*B	*A	*B
1	10.0	1.0	98.6	1.4	97.4	2.6	97.5	2.5
2	8.0	0.8	99.6	0.4	99.1	0.9	98.3	1.7
3	5.0	0.5	98.5	1.5	98.2	1.8	97.2	2.8
4	3.0	0.3	97.1	2.9	97.0	3.0	96.6	3.4
5	1.0	0.1	99.5	0.5	99.1	0.9	98.6	1.4
6	0.5	0.05	97.3	2.7	97.1	2.9	96.7	3.3

*A=Percentage of labeled complex (^{99m}Tc - sucralfate).
*B= Percentage of free pertechnetate ($Na^{99m}TcO_4$).

TABLE II. REPRESENTS THE BIODISTRIBUTION DATA OF THE LABELED COMPLEX (^{99m}Tc-SUCRALFATE) 90 MIN. POST ORAL ADMINISTRATION IN THE FASTING AND ULCERATED MICE

Organ	% dose/organ			Mean ± s.d.
	Mouse No.1	Mouse No.2	Mouse No.3	
Blood	0.15	0.05	0.15	0.12 ± 0.058
Liver	0.03	0.02	0.04	0.07 ± 0.060
Lungs	0.04	0.02	0.04	0.03 ± 0.012
Spleen	0.03	0.02	0.03	0.03 ± 0.005
Stomach	85.22	90.64	87.80	87.89 ± 2.711
Intestine	14.21	9.07	11.00	11.43 ± 2.596
Kidneys	0.05	0.02	0.04	0.04 ± 0.015
Carcass	0.27	0.16	0.90	0.44 ± 0.399

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Residual activity and adsorption studies on commercial radiopharmaceutical kits and syringes

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In this study, which investigates the residuel activity and adsorption of various commercial radiopharmaceutical kits (MDP, MAG-3, DMSA, DTPA, MAA, MIBI, HMPAO), the effect of time and incubation period in syringes were determined.

The residuel radioactivity and adsorptions of various commercial radiopharmacuetical kits in two different types of intact syringes (K, L) and the needles- plungers and the body of these syringes were measured at two different times (0 and 20 min.).

The radiopharmaceuticals prepared were withdrawn into two different types of syringes first and then immediately evacuated. The syringe, plunger and body, needle were measured in the dose calibrator. These syringes were then rinsed with the water and the radioactivity adsorbed on the syringe, plunger- body- needle were examined.

Different results were obtained from each radiopharmaceuticals. The residuel activity and adsorption didn't change by time for some radiopharmaceuticals (A,B) whereas time played an important role the others (C,D, E, F). Adsorption was observed mostly the in needles and plungers of syringes with elastomeric tips. The values of residuel activity decreased significantly by washing and it turned into adsorbed radioactivity.

When eight radiopharmaceuticals were examined the adsorbed radioactive dose increased together with residuel activity doses as the incubation period in the syringes got longer.

The increase in the incubation time of the radiopharmaceuticals after before and withdrawal application to the patient causes increased adsorption of activity and residuel activity in the syringe, plunger body and needle. It is concluded that the use of completely plastic syringes than plungers with elostomeric tips is more advisable in Radiopharmacy. Because these elastomeric tips have more adsorption tendency for the substances.

Localization of ^{131}I-labelled monoclonal antibody ERIC1 in a subcutaneous xenograft model of neuroblastoma in SCID mice

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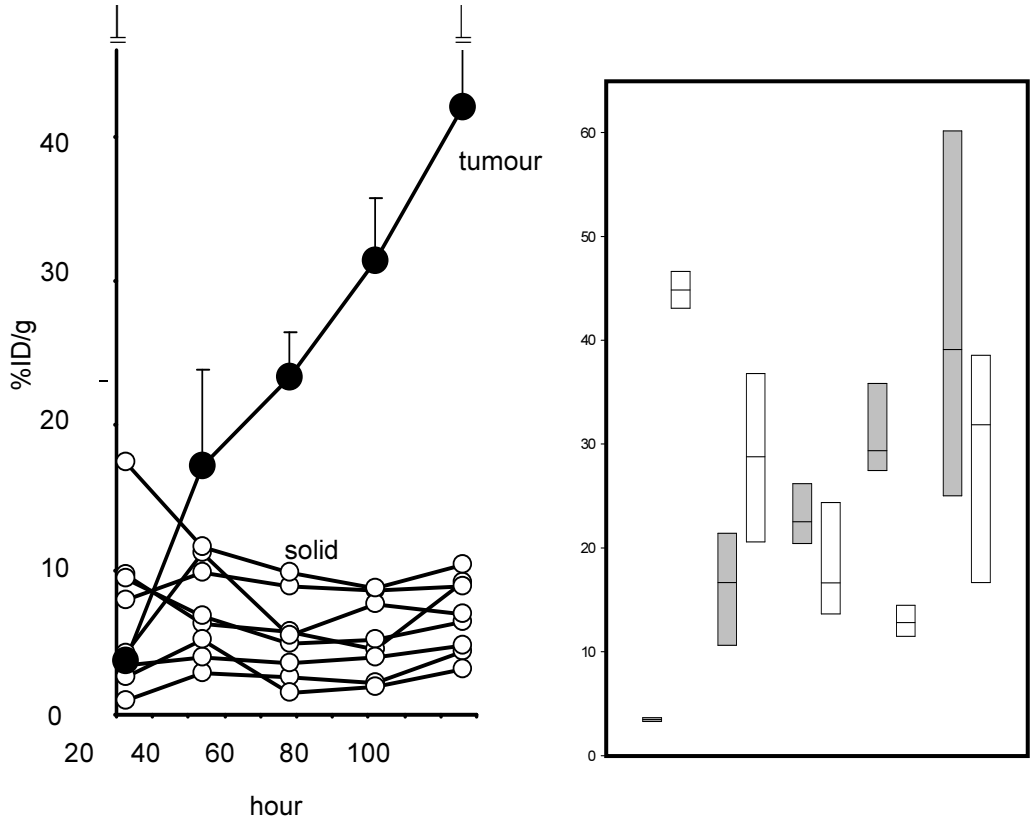
Our purpose was to evaluate a novel strategy of immunolocalization of human neuroblastoma by targeting the neural cell adhesion molecule (NCAM), which is over-expressed on neuroblastoma. Neuroblastoma is the most frequent solid extracranial childhood tumour and also the most common neoplasm in the first year of life. The most frequent metastatic sites are bone, bone marrow and liver. The neural cell adhesion molecule (NCAM) is constitutively expressed on nearly 100% of all neuroblastomas. NCAM has been successfully used as a target for immunotoxines and bi-specific antibodies in multiple myeloma, small cell lung cancer, glioma or neuroblastoma, in vitro and in vivo.

NCAM expression was quantified on established neuroblastoma cells by real time PCR and NCAM expression on the cell surface shown by flow cytometry. A SCID mouse model using IMR5-75 neuroblastoma cells to induce subcutaneous tumour growth was established. ^{131}I was used to label monoclonal NCAM specific ERIC1 antibodies generating the ^{131}I-ERIC1 antibody, which shows a high affinity to NCAM also after labelling (K_D= 9 x 10^{-8}). Groups of five to six mice received intravenous injections of 3-9 MBq of ^{131}I-ERIC1 on day 0 into the tail vein. At time points 2.5 h, 24 h, 48 h, 72 h and 96 h post injection mice were sacrified by cervical dislocation. Blood, liver, spleen, kidneys, muscle, femur, thyroid, intestines, lung, tumour and urine were removed and weighed. Organ-specific radioactivity was measured in a well-counter as well as that of the tail and remnant cadavers. Data were calculated as dose per gram of tissue (%ID/g) and tumour to non-tumour ratios (T/NT ratio). For blocking experiments mice were pre-injected with 140µg of unlabelled ERIC1 into the tail vein 24 h prior ^{131}I-ERIC1 application. Mice from these blocking experiments were measured after 72 h.

Measurement of organ-specific radioactivity showed low organ-specific uptake (5.33 %ID/g after 72 h), which continuously decreased over the 96 hour investigation period, demonstrating clearance of radioactivity.

In contrast, tumours accumulated radioactivity continuously up to a peak of 42.07 %ID/g at the 96 hour time point (31.07 %ID/g at 72 h). This specific uptake could be blocked by unlabelled ERIC1 antibodies.



Biodistribution of ^{131}I-ERIC1 in IMR5-75 tumour bearing SCID mice; mice were killed 2.5, 24, 48, 72 or 96 h after injection of ^{131}I labelled ERIC1 antibodies and organs specific radioactivity were measured in a scintillation counter:

A) closed circles = tumour; open circles = solid organs (kidney, liver, thyroid gland, muscle, femur, intestines, spleen, lung);

B) bBox plot to showing median and range of tumour specific radioactivity and radioactivity in the peripheral blood; for exact numerical values see Ggrey boxes = tumour; white boxes = peripheral blood

These results indicate that ^{131}I-labelled ERIC1 has the ability to probe NCAM-expressing tumour cells in vivo with high efficiency and is a promising reagent for the diagnosis and treatment of NCAM-positive human tumours, especially for neuroblastoma.

^{188}Re-labelled anti-CD20 monoclonal antibody: Labelling and quality control studies

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Immunotherapy with human chimeric antibody rituximab (Rituxan, IDEC pharmaceuticals) has been a major advance in treatments of patients with CD20-positive B-cell non Hodgkin´s lymphoma (NHL). Radioimmunotherapy (RIT) uses the targeting features of monoclonal antibody to deliver radiation from an attached readionuclide and it is an appeling concept that has received widespread attention. Here, we report our experience using rhenium-188 (^{188}Re)-radiolabeled chimeric anti-CD20 antibody (rituximab). A stable antibody-labeling technique had been developed for ^{188}Re. The ^{188}Re-direct labeling of anti-CD20 monoclonal antibody, the methods for quality control: paper chromatography, instant thin layer chromatography-silica gel (ITLC-SG) and HPLC technique, the immunoreactivity and biological recognition of the target antigen assessment of the radiolabeled molecule, in vitro stability and the assessment of in vivo stability through biodistribution studies in normal WISTAR rats are described.

For the direct radiolabeling, the reduction of monoclonal antibody (mAb) was performed with 2-mercaptoethanol (2-ME), based on Schwarz´s method [1] at a molar ratio 2000:1 (2-ME:mAb). By means of this method some of the disulfide bonds of the antibody are reduced to sulfhydryl groups (we obtained 4–5 groups) and these groups provide sites for the formation of very strong bond between the reduced rhenium and the antibody. The methodology used in this work has been tested in a phase I radioimmunotherapy clinical trial [2] using the humanized mAb hR3 for loco-regional treatment of brain tumours.

The labeling efficiency (> 95 %) of this method showed that the final product needs no further purification for clinical purposes (low level formation of colloidal species).

In vitro stability studies of the labeled anti-CD20 were performed at room temperature at 4 h, 24 h and 48 h in cysteine, human serum and saline. In the presence of normal human serum, during the first 4h, transchelation of about 15% of the ^{188}Re to serum proteins occurs, also ^{188}Re radiolabeled was trancomplexed to cysteine after 1h incubation at 37ºC.

Biodistribution studies (in vivo stability) were executed at 1, 4, 24 and 48 h after injection of the radiolabeled antibody in Wistar rats. Among all organs there was a slight level of activity in kidneys (10.41%), lungs (14.72%) and blood at 4 h. These values were reduced at 24 h. The remaining organs (heart, liver, stomach, spleen, etc.) had very low accumulation of the radiotracer. These results showed that there was no accumulation in any organ.

The reduction step with 2-ME to generate thiol groups may affect antibody immunoreactivity. Preliminary experiments (Table I) showed that good binding percentages (70-90) could be obtained with this antibody using fresh concentrate of leukocytes (1.5×10^6).

TABLE I. CELL BINDING PERCENTAGES FOR ANTI-CD20 USING 1.5×10^6 CELLS/ASSAY

No.	Antibody	Total cpm	Cpm in pellet	Percentage %	Binding percentage
1	^{188}Re-CD20	16941	13697	80.8	68.9
	^{188}Re-CEA	14030	1670	11.9	
2	^{188}Re-CD20	2366595	2296612	97.0	92.5
	^{188}Re-CEA	3078743	139395	4.5	

The immunoreactivity of the reduced labeled antibody was determined to ensure that the radiolabeling procedure did not influence the binding activities to receptor. The value determined for specific binding was very high (70–90%), showing good possibilities for in vivo therapeutic applications.

The direct labeling of antibodies with ^{188}Re is progressing with a larger number of researches using the reduction method for this purpose and such methods appear to be a popular tool in freezed-dried kit formulation for clinical trials.

The use of ^{188}Re-direct labeling anti-CD20 as a potencial RIT agent may offer many benefits and advantages: i) the direct radiolabeling approach will allows very high yields of rhenium-labeled antibody to be obtained without the need for postlabeling prurification; ii) a kit could be developed tha would merely require the mixing of perrhenate with the other reagents in a single vial; and iii) the whole process could be made simple and fast enough to be performed with a minimum of manipulation in the interest of safety and convenience [3].

In conclusion, we have proven a methodology that allows the obtaining of preparations of ^{188}Re labeled mAb anti-CD20, in this case it is another possibility in the development of mAb based radiopharmaceutical for therapy of NHL using a monoclonal antibody with having proven effectiveness.

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A novel finding: Anti-androgen flutamide kills androgen-independent PC-3 cells: A radiolabelled methyl-choline incorporation into tumour cells

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[Methyl-^{11}C]-choline was introduced to image many types of cancers especially the prostate cancer [1]. Al-Saeedi et al. reported that the incorporation of [Methyl-^{3}H]-choline into breast tumour (MCF-7) cells correlated strongly with proliferation as determined by [Methyl-^{14}C]-thymidine uptake [2]. Also, Al-Saeedi, et al. showed that the chemotherapy using MCF-7 cells treated with 5-Fluorouracil (5-FU) induced modulation in [Methyl-^{3}H]-choline incorporation and certain mechanisms for this modulation were reported. In this study, the androgen-dependent prostate tumour (LNCaP) cells were treated with the well known pure anti-androgen drug, flutamide, for three days. The cells were then incubated with [Methyl-^{3}H]-choline for 10 mint to detect the effect of flutamide on both cell proliferation and choline incorporation. At the same time, a preliminary work was established using androgen-independent PC-3 cells treated with flutamide as controls in this study. PC-3 cells were treated with a range of doses of flutamide inhibiting growth by 20[Methyl-^{3}H]-Choline Incorporation into MCF-7 Cells: Correlation with Proliferation: choline kinase and phospholipase D assay**.**

[Methyl-^{3}H]-Choline Incorporation into MCF-7 Cells: Correlation with Proliferation: choline kinase and phospholipase D assay**.**

- 70%. Treated and control cells were incubated with [Methyl-^{3}H]-choline for 10 min, then in non-radioactive medium to simulate the rapid blood clearance of [Methyl-^{11}C]-choline tracer in control and treated PC-3 cells, and then extracted with organic and aqueous solvents to determine its effect on the intracellular distribution of this tracer.

Interesting results showed that flutamide killed the androgen-independent prostate cancer cells, PC-3 and mechanisms responsible for flutamide-induced modulation on [Methyl-^{3}H]-choline incorporation were reported. The PC-3 cells' proliferation was inhibited by flutamide. In addition, treatment of PC-3 cells with flutamide for 3 days resulted in a build up of cells in S phase and [Methyl-^{3}H]-choline incorporation per a cell was found to be decreased in treated compared to untreated cells. flutamide inhibits PC-3 cells' proliferation by certain mechanism (unknown) other than the well-known androgen receptor (AR) mechanism, which accordingly induced modulation in [Methyl-^{3}H]-choline incorporation into the PC-3 cells.

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[Methyl-3H]-choline incorporation into MCF-7 cells: Correlation with proliferation: Choline kinase and phospholipase D assay

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[Methyl-^{11}C]-choline is a new positron emission tomography (PET) imaging agent, which was introduced to image prostate cancer but the identity of metabolites producing the ^{11}C signal in tumour cells during [Methyl-^{11}C]-choline-PET imaging in prostate cancer patients still is unknown. Al-Saeedi et al. [1] demonstrated that choline incorporation is related to cellular proliferation. Choline kinase (ChoK) enzyme is the first step to phosphorylate choline to form phosphocholine (PCho) and subsequently phosphatidylcholine (PtdCho), the most constituent of cellular membrane. Yamashita and Hosaka (1997) reported that ChoK mRNA and protein levels are highest in exponential phase and decline in the stationary phase of growth. In this study, whether ChoK level is related to cellular proliferation or not was investigated. In addition, the activity of phospholipase D (PLD) was determined in both rapidly proliferating and confluent cells. There is some evidence that indicates that PLD activity is involved in enhancing cell proliferation and transformation. The hydrolysis of PtdCho by PLD leads to the formation of choline**.**

The activity of ChoK was determined after homogenizing MCF-7 cells pellet in ice-cold 25 mM Tris/HCl buffer (1.5 ml), pH 7.4, using a Dounce homogenizer. The homogenate was centrifuged at 500 g for 5 minutes to remove cell nuclei and debris, and protein content was determined for each sample. ChoK assay mixture contained 40 mM Tris/HCl with unlabelled carrier of 0.25 mM choline, pH 7.4, [Methyl-^{3}H]-choline (37 kBq; 2.22x10^{6} d.p.m./sample), 2 mM ATP, 10 mM $MgCl_2$ was added to 96 μg of homogenate protein in a total volume of 0.1 ml. After incubation for 20 minutes at 37°C, the reaction was stopped by the addition of an ice-cold mixture of methanol and chloroform in a total volume of 1.5 ml. Then lipid-metabolite extraction was carried out. The total radioactivity in each aqueous phase was determined. Then [Methyl-^{3}H]-PCho separation from [Methyl-^{3}H]-choline was carried out using ion exchange chromatography on Dowex H^+ columns. In another experiments, measurement of PLD activity in MCF-7 cells grown to different proliferative fractions was carried out by radiolabelling of endogenous substrates with 37 kBq/ml medium/flask [9,10-^{3}H]-myristic acid followed by the addition of a primary alcohol [1-Butanol (1-BtOH)] to the medium of the cells and detection of metabolically stable phosphatidylalcohol produced by the PLD-catalyzed transphosphatidylation reaction using the transphosphatidylation assay.

From ChoK assay, the measured elute of [Methyl-^{3}H]-PCho in cells was standardised to cell protein values and expressed as pmol mg^{-1} protein^{-1} min^{-1}. Mean (±SD) of [Methyl-^{3}H]-PCho was 484.04±20.23 pmol mg^{-1} protein^{-1} min^{-1} (n=6) for exponentially growing cells, and 70.35±9.83 pmol mg^{-1} protein^{-1} min^{-1} for confluent cells (n=6). [Methyl-^{3}H]-PCho content was significantly higher in the exponentially growing cells compared with the confluent cells using student *t*-test (P<0.001). Also, the results from exponentially growing cells and confluent cells showed that 98.59±0.28% and 97.39±0.22% respectively of [9,10-^{3}H]-myristic acid was incorporated into PtdCho. The transphosphatidylation [^{3}H]-PtdBut reaction (measure

of PLD activity) results were normalized to their corresponding protein contents and expressed as the mean (n=6) [d.p.m./μg protein ± SD (mean S phase ± SD)]. [^{3}H]-PtdBut accumulation was significantly higher (P<0.001) in the exponentially growing cells [196.39±2.21 d.p.m./μg protein (39.69±4.00%)] compared to the confluent cells [99.10±1.35 d.p.m./μg protein (9.33±0.82%)]. The activity of ChoK as well as PLD were significantly higher in the exponentially growing cell populations, resulting in increased phosphocholine (PCho) production, approximately 60% of the aqueous fraction of the cell, in the exponentially growing cells compared to the confluent cells. Moreover, TLC results indicate that betaine was about 20% of the aqueous fraction of the cell.

This study indicates that the major water-soluble choline metabolite was PCho as a consequence of increased ChoK and PLD activity.

Key words: Radiopharmaceuticals; Choline; Phosphocholine; PLD; PET; Tumour.

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Production of uncommon radionuclides for nuclear medicine at the TESLA Accelerator Installation

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The Channel for production of radionuclides (H4) of the TESLA Accelerator Installation has been designed for routine production of common radionuclides as ^{123}I and ^{18}F to be used for the preparation of radiopharmaceuticals for clinical diagnostic procedures in nuclear medical centers in the region, and experimental production of novel radionuclides for research purposes. The diversity of beams that will be delivered by the VINCY Cyclotron makes this installation perfectly suited for the production of a variety of radionuclides, what is ultimately required for the development of new radiotracers for imaging and therapy.

Due to its high resolution three-dimensional molecular imaging capabilities, Positron Emission Tomography (PET) became the technique of choice for nuclear medical diagnostics. PET has evolved around the use of short-lived radionuclides of natural organic elements ^{11}C, ^{13}N, ^{15}O, and as a hydrogen analogue ^{18}F. The very short half-life of these radionuclides limits their applicability to the close vicinity of an accelerator, where they are produced. Even though a number of longer-lived positron emitting radionuclides are known, which nuclear properties satisfies the requirements for PET imaging (see Table I), they are not yet commonly used in nuclear medicine. The diversity of physical, chemical and biochemical properties of these radionuclides justifies further exploration into this area of inorganic PET in order to take advantage of the opportunities offered by such radionuclides in PET research. In addition to the practical benefits of medium half-life radiotracers, certain applications particularly in oncology require tracer kinetics to be followed for periods exceeding the limits of conventional short-lived PET radionuclides.

The medium half-life PET radiotracers could find application in microdosimetry of therapeutic procedures based on radionuclides. Many of the therapeutic radionuclides are not convenient for SPECT or PET imaging; therefore their in-vivo biodistribution in patients, and consequently the patient specific microdosimetry, cannot be traced during the treatment procedure. The simultaneous administration of the same radiopharmaceutical labeled with a therapeutical radionuclide and its positron-emitting analogue would enable the determination of the biodistribution of the therapeutic radionuclide in every patient, and the calculation of the dose delivered to the tumour and critical organs.

Based upon available radiobiologic and experimental evidence it is clear that α-particle emitters have great potential for use in endoradiotherapy. The high linear energy transfer (LET) and short range of α-particles allow for very high potency and specificity. High potency is achieved because one to three tracks through the cell nucleus are sufficient to sterilize the hit cell. Specificity arises due to the relatively short, 30-90 μm range of α-particles in tissue.

The spectrum of malignant diseases that may be treated with α-emitters includes most common cancers when single cells or small clusters of cells are the potential target. Several clinical applications are envisaged in specific clinical situations, such as leukemia, in the relapsed patient, especially after a second remission. Local intracavitary administration holds

promise, for example, treating metastases in the abdominal cavity following surgical resection of ovarian cancer.

TABLE I. DECAY PROPERTIES OF SOME MEDIUM HALF-LIFE POSITRON EMITTING RADIONUCLIDES THAT CAN BE PRODUCED AT THE TESLA ACCELERATOR INSTALLATION

Radio-nuclide	$t_{1/2}$ (h)	β^+ decay (%)	E_{β^+} (MeV)	**Radio-nuclide**	$t_{1/2}$ (h)	β^+ decay (%)	E_{β^+} (MeV)
^{52}Fe	8.2	57	0.804	^{76}Br	16	57	3.98
^{57}Ni	36	40	0.85	^{82m}Rb	6.5	26	0.80
^{61}Cu	3.3	62	1.21	^{83}Sr	33	24	1.23
^{64}Cu	12.7	19	0.575	^{86}Y	15	34	3.15
^{66}Ga	9.5	57	4.15	^{89}Zr	78	22	0.90
^{68}Ga	1.1	89	1.90	^{94}Tc	4.9	11	0.816
^{73}Se	7.1	65	1.32	^{120}I	1.4	46	4.59
^{75}Br	1.6	76	1.74	^{124}I	100	22	2.14

Many opportunities exist for developing a wide range of suitable carriers, not only antibodies, but other agents such as peptides or small molecular metabolites, to bind the α-emitting radionuclides to the targeted cells. Antibodies can deliver agents intracellularly if they bind to internalizing cell bound recognition sites as antigens. This will increase the probability that an α-emitter will kill the targeted cell. Access of the α-emitter to the tumour is limited by diffusion kinetics and the best access is likely to be via blood vessels.

There are only few α-emitting radionuclides that can fulfill the requirements for this specific nuclear medical application. ^{211}At turned out to be the most promising candidate, because of its proposed halogen behavior. However, the in vivo stability of astatine labeled compounds is in most cases insufficient. New approaches in conjugation of chelating ligands with biospecific macromolecules (like monoclonal antibodies) or biospecific peptides allow today the stable labeling of these tracers with metallic radio-nuclides, especially when the ionic charge is $\geq$+3. ^{149}Tb ($t_{1/2}$=4.118 h, E_α=3967 keV, range=28 μm in tissue) is such a radionuclide, recently proposed as the most promising alpha emitter for endoradiotherapy. This radionuclide is partially decaying by positron emission, thus it might be used also for patient specific dosimetric studies during the therapeutic procedure.

There are in principle two main routes for ^{149}Tb production at the TESLA Accelerator Installation: light particle induced (p or ^{3,4}He) reactions on ^{152}Gd and heavy ion induced nuclear reactions such as ^{141}Pr(^{12}C,4n)^{149}Tb or ^{142}Nd(^{12}C,5n)^{149}Dy$\rightarrow$^{149}Tb. The yields of the most promising reactions that can be used for the production of this radionuclide are sufficient for clinical studies.

Obviously, the TESLA Accelerator Installation offers unique possibilities for the production of a number of uncommon radionuclides and for the development of new radiopharmaceuticals that might change the profile of future nuclear medicine.

Facilities for national production of cold kits in Venezuela

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In 1995 the Faculty of Pharmacy at the Universidad Central de Venezueal accepted the responsibility to develop radiopharmacy as a professional practice subject to corresponding sanitary regulations. The programme took into account the differents aspects that conform to the professional practice, and the Radiopharmaceutical Research and Development Center (CEIDRAF) was created under the auspices of the the Pharmaceutical Research Institute in the same faculty, that would be responsible for planning the programme. The most convenient strategy to consider each of the regarded aspects was carefully designed. Some of these aspects were identified to be: human resource capacitation, cold kits for technetiun radiopharmaceuticals production, and research and development in the radiopharmacy area. The work is focused specifically in the design of a laboratory for the national production of cold kits. It would be the first and perhaps the only one to produce this class of pharmaceuticals in Venezuela.

Based on the character of the radiopharmaceuticals as parenteral drugs, facilities that conforms the cGMP design criteria for production of this class of medicaments has to be carefully considered: production laboratories provided with the necessary clean areas and well equipped to adequately accomplis the production and quality control activities.

In our case, the design and building of the facilities was supported by nationals institutions, specifically, by the Universidad Central de Venezuela, and the equipment was provided mainly through the regional (ARCAL) and technical cooperation projects of the IAEA. As a result of the support, at this time we have the facilities that allows us to offer to the nuclear medicine services in our country, most of the first and second generations of cold kits for technetium radiopharmaceuticals with the pharmaceutcal quality necessary for clinical use. Our production will assure supply and accesibility of the mentioned products. In addition, we have the capacity to produce these goods in order to cover our national demand.

In this poster we are showing some aspects of our facilities, such as: the layout of the clean and controlled areas, layout of equipment, design of support systems, and proposals for personnel, materials and final products.

We include some pictures of the quality control laboratory as well. Additionally, the development has been complemented with a Quality Control and Assurance Programme documented in the Quality Manual elaborated according to the Venezuelan COVENIN-ISO 9001:2000 Guidelines and the Validation Master Plans elaborated according to the FDA Baseline Guide, WHO (GMP: 32th report), BPR (IAEA/ARCAL XL) and USP 27.

Finally we would like to remark that this development could be taken taken as a demonstration of the impact on developing countries of adequate management of the IAEA support in all the aspects related with peaceful application of nuclear energy.

Radiosynthesis and bio-evaluation of ^{123}I-labelled 2-methyl-4-nitroimidazole derivatives as potential infection imaging agents

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The experimental use of [^{131}I]Ornidazole **1**, a radioiodine-labelled 2-methyl-5-nitroimidazole derivative, as a potential inflammation- and infection-seeking agent, has recently been reported [1, 2]. One of the claimed advantages of [^{131}I]ornidazole was its fast accumulation at the target site (within 10 min), in contrast to ^{67}Ga and radiolabelled antibodies and leukocytes which require periods of up to 24 h. In the present study two new ^{123}I-labelled 2-methyl-4-nitroimidazole derivatives **2** and **3** were radiosynthesised and their infection-seeking properties in rabbits were investigated, using ^{67}Ga and [^{123}I]Ornidazole as controls.

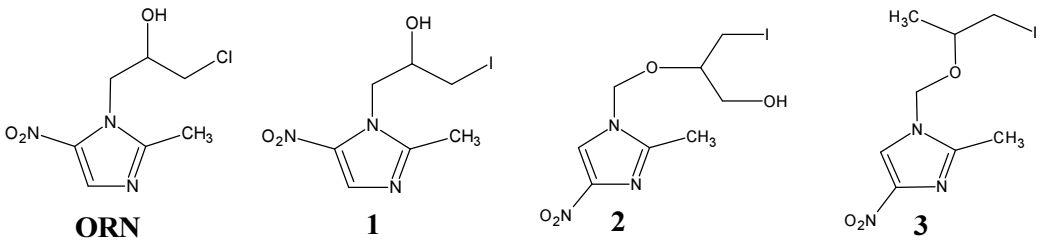

FIG. 1. Chemical structures of iodinated nitroimidazole derivatives.

[^{123}I]Ornidazole **1** was prepared from ornidazole (**ORN**) via a halogen exchange reaction. Compounds **2** and **3** were prepared via an iodide-for-tosylate substitution reaction. Their labelling precursors were prepared via *N*-3-alkylation of 2-methyl-5-nitroimidazole with pre-synthesized chloromethyl ethers containing an ester group. Ester hydrolysis and tosylation of the resulting alcohols gave the desired precursors. Labelling with n.c.a. Na[^{123}I] was conducted in acetone at elevated temperatures. The labelled compounds were purified by means of column chromatography on silica gel. Infection was induced in the right thigh of the rabbits by injection with *E. coli* and allowed to develop over 24 h. The rabbit was immobilized on the camera and the tracer was injected intravenously into the ear. Static images were collected over various intervals. Uptake was measured as a percentage of the count ratio between ROI's on the right versus the left thigh.

Radiochemical purities of the radiolabelled nitroimidazole tracers varied between 90 and 95%. All the tracers showed increased uptake at the area of induced infection after 6 h and 24 h, but the uptake was lower than in the case of ^{67}Ga. Tracer **3** showed a slightly superior uptake after 6 h than the others at the same time. Images obtained with tracer **2** at shorter time intervals (30–60 min.) did not show significantly higher infection uptake than at longer intervals (3–6 h). ^{123}I activity was also detected in the heart, liver, kidneys, bladder and thyroid.

TABLE I. COUNT RATIO AFTER VARIOUS TIME INTERVALS IN RIGHT (R) VERSUS LEFT (L) THIGH MUSCLE OF RABBITS INDUCED WITH E. COLI IN THE RIGHT THIGH MUSCLE, AND INJECTED WITH RADIOIODINATED TRACERS **1**, **2**, **3** AND ^{67}GA

Tracer	R/L Count Ratio (%)				
	30 min	1 h	3 h	6 h	24 h
[^{123}I]**1** with infection	-	-	-	126	150
- without infection	-	-	-	96	95
^{67}Ga with infection	-	-	-	147	248
[^{123}I]**2** with infection	126	112	98	103	126
- without infection	93	98	94	94	94
^{67}Ga with infection	-	96	118	135	189
[^{123}I]**3** with infection	-	-	-	156	144
- without infection	-	-	-	94	94
^{67}Ga with infection	-	-	-	185	271

* Data not acquired.

The tabulated data suggest that radiolabelled nitroimidazoles might not be suitable as infection imaging tracers. It might, however, be worthwhile to investigate their suitability as radiosensitizers of hypoxic cells.

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Feasibility study of the utilization of medical isotope production reactor (MIPR) for ^{99}Mo production

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Compared with the conventional target irradiation approach, production of ^{99}Mo from the fuel solution of a MIPR has the advantages of simplified fuel handling and processing, much lower cost and so on[1].

A production process of ^{99}Mo from the fuel solution of a MIPR was investigated using stable elements and natural U, and acidic granular alumina (Al_2O_3) of 0.136 mm to 0.093 mm as the adsorbent. In column separation, the sample solution was passed through the pre-saturated Al_2O_3 column. Then the column was successively washed with HNO_3 of the same concentration as the sample solution, H_2O and 0.01mol/L NH_4OH solution of a volume 5 times of the Al_2O_3 bed respectively. Finally, Mo was eluted with 1mol/L NH_4OH of a volume 5 times of the Al_2O_3 bed. The concentration of Mo in the effluent and eluate was determined by AAS to calculate the adsorption efficiency, desorption efficiency and recovery yield of Mo.

Change of HNO_3 concentration between 0.01mol/L and 0.5mol/L had no observable effect on the separation of Mo. With increase of temperature of sample solution from 25 to 90 ^oC, the adsorption efficiency of Mo had no considerable change, but the desorption efficiency decreased almost linearly.

Under the conditions of a ratio of the high to diameter of Al_2O_3 bed(H/D) between 1 and 6, a flow rate(V) between 0.05ml/ml/min and 2ml/ml/min, the adsorption efficiency of Mo was not affected, but the desorption efficiency was greatly affected. When the H/D was less than 3, the recovery of Mo decreased with increase of V. When the H/D was larger than 3, at a V between 0.5ml/ml/min and 2ml/ml/min, the recovery of Mo was ~95%.

Under the conditions of Mo concentration in a range of 4.2 mg/mL to 43.8mg/mL, H/D between 3 and 6, ratio of sample solution volume to that of Al_2O_3 bed between 6.6 and 50.6, and flow rate from 0.2ml/ml/min to 1.0ml/ml/min, the mean overall recovery yield of Mo was 89.1%(n=3) by two-steps' separation.

The results of separation of Mo from U, Cs, Sr and I show that the radionuclidic purity of ^{99}Mo product can meet the demand of the European Pharmacopoeia EP(table 1).

On a 1:1 mock-up of circulation loop for fuel solution transfer and radionuclide extraction, using a column loaded with 1L Al_2O_3 as extraction column and another column loaded with 60mL Al_2O_3 as purification column, trails of separation of Mo from 100L $UO_2(NO_3)_2$ solution in a medium of 0.1mol/L HNO_3 and containing 5000g U, Sr, Cs, Ce, Zr, Ru, Te, I were conducted. The overall recovery yield of Mo was 67.7%±3.2%(n=3), while the contents of U, Sr, Cs, Ce, Zr, Ru, Te, and I in separated Mo meet the specification of EP.

TABLE I. CONTENTS OF U, CS, SR AND I IN SEPARATED MO AND THEIR COMPARISON WITH THE SPECIFICATIONS OF EP

Element	U	Sr	Cs	I
F_1/%[1]	3.2×10^{-5}	5×10^{-4}	$<8\times10^{-5}$	5×10^{-3}
F_2/%[2]	9.7×10^{-10}	2.5×10^{-7}	$<6.4\times10^{-9}$	2.5×10^{-5}
S/%[3]	$\Sigma\alpha\leq1\times10^{-9}$	$^{90}Sr\leq6\times10^{-8}$ $^{80}Sr\leq6\times10^{-7}$	$\Sigma\beta\gamma\leq1\times10^{-4}$	$^{131}I\leq5\times10^{-5}$

Notes:
1) F_1 is the experimental data of ratio of element content in the elate by one-column separation to that added.
2) F_2 is the calculated data of ratio of element content in the elate by two-column separation to that added.
3) S is the ratio of radioactivity of the radionuclide to that of ^{99}Mo in ^{99}Mo product in the specification of EP.

In conclusion, by a two step separation using Al_2O_3 as the absorbent, ^{99}Mo being adsorbed in 0.1mol/L HNO_3 solution and eluted with 1 mol/L NH_4OH solution, a overall ^{99}Mo recovery yield of more than 65% can be obtained, while the content of α emitters, ^{89,90}Sr, ^{137}Cs and ^{131}I etc. in ^{99}Mo product can meet the specification of EP. Therefore, MIPR can be utilized for the production of medical ^{99}Mo.

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The CCHEN's cyclotron radioisotope programme and its impact after two years of operation

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The Chilean Nuclear Energy Commission (CCHEN) through its brand new Cyclotron Laboratory has introduced cyclotron-produced short-lived radionuclides and its radiopharmaceuticals for the first time in Chile. Neutron deficient radionuclides are now routinely available upon request for PET diagnosis procedures

The Cyclotron Laboratory has been conceived upon an IBA's Cyclone 18/9 accelerator. Accelerated beam particles are protons (18 MeV) and deuterons (9 MeV) with intensities up to 60 and 40 μA, respectively. The machine is housed in its Cyclotron Vault, 18 ft below ground level. An external transport beam line connects to an Experimental Area for further atomic or nuclear physic's charged particles beam, non radionuclidic production, applications purposes.

In addition to the provided external beam line, there are seven internal targets fully dedicated to production. Two targets (large and small oxygen-18 enriched-water capacity) are currently dedicated routinely to Fluoride-18 production. The produced activities are transferred remotely to the Hot Cells Laboratory to label selected molecules. A second small volume target will be acquired and installed in the near future to warrant expected radionuclide availability.

Products from short lived positron emitters such as ^{18}F-fluordeoxyglucose and ^{18}F-sodium fluoride are used at present for radiopharmaceutical applications such as medical evaluation of a patient's health and, also, to carry out clinical research.

Furthermore, radioisotope and radiopharmaceutical products under development include Galium-67 in the form of ^{67}Ga-Citrate and ^{67}Ga-chloride. Development of ^{11}C organic derivatives and, later on, ^{62}Cu from a ^{62}Zn generator is expected to start sometime in the future.

The present work will present how the introduction of cyclotron's technology and guaranteed availability of short lived positron emitters radioisotopes can have a positive impact on nuclear medicine practice. As of today, with more than one thousand PET diagnoses already performed using ^{18}FDG, nuclear medicine and, primarily, oncologist physicians are assembling a data base with the most significant results and improvements for precise oncology stage determination.

It is, indeed, quite evident for patients to have an ambulatory non-invasive option for prognosis, while, at the same time, improving their quality of life.