Antibodies and isotopes, a chemical approach to tumour targeting

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Introduction

In the production of antibodies for tumour scanning or therapy relatively little emphasis has been placed on the choice of isotope. Only indium-111 has been routinely reported as a replacement for iodine isotopes in antibody scintigraphy. The introduction of indium-111 as a protein label was almost entirely for physical reasons, its gamma ray energy and lack of beta decay makes this isotope both a more effective imaging agent and reduces the dose to the patient.[1]
Biochemically, iodine and indium behave quite differently as antibody labels. These differences are clearly seen in-vivo after the label is lost from the protein. Indium-111, a trivalent metal ion, is prone to numerous charge interactions with biological molecules resulting in pronounced liver uptake in some cases. Iodine, as the negatively charged iodide is rapidly taken into the thyroid, however this may be simply blocked with inactive iodine treatment. For tumour location the affinity of tri-valent indium for biological structures may also be seen in the persistence of activity at tumour sites, a distinct advantage as compared to iodinated antibodies.[2]
It is known that only a small fraction of a labelled antibody actually binds to a target tumour. The rest, perhaps 99% of the injected dose, will eventually be broken down and relocated to positions primarily determined by the individual chemistry of the isotope used. Therefore knowledge of an isotopes chemistry ss well aa its physical characteristics may be import ant in improving the ratio of tumour to background activity for both scanning and therapeutic studies.
In this study, scandium-47 and yttrium-90 have been used as representatives of potential cytotoxic labels. Both isotopes have a high yield of energetic beta particles and half-lives of the same order as indium-111. In addition they are both members of Group III and so may be used as a base for chemical comparisons in the future with radiotoxic isotopes from other chemical groups.

Methods

Isotopes

The isotopes were produced by parent-daughter decay from commercially obtained parent isotopes. Strontium-90 and calcium-47 were both obtained from Amersham International. Approximately 0.4MBq aliquots of these elements were bound to 2g of Dowex 50W-X8 cation exchange resin in 0.1M HCl in 1×5cm columns. Both resins were flushed with 0.1M sodium citrate pH 4.2 followed by

10 ml of 0.25M sodium acetate for the strontium-90 column. Both columns were left for the build up of daughter activity, which was eluted by either citrate (Sc-47) or acetate (Y-90) buffer. Routinely this technique gave a product containing less than 0.5% of the parent isotope.

Antibody

A sheep polyclonal anti-CEA antibody was used. Prior to use this was coupled with DTPA using the cyclic anhydride method.[3] The protein used here had, on average, 2 moles DTPA/mole protein. Protein labelling was achieved by mixing the 100µg of protein with up to 200µl of activity in the elution buffer. Labelling efficiencies varied between 41-55% (Sc-47) and 68-83% (Y-90) In one case, 100µg of DTPA modified protein was labelled with Iodine-125 by the Chloramine-T method and 2.5 µg of this injected together with the yttrium-90 preparation. (Figs 1 and 2)

Animal Experiments

Adult female Balb/c mice were used weighing between 21-24g. All antibody injections were via i/p administration. This has been shown to give maximum transfer to the blood compartment within one hour. Injections of free DTPA as the sodium salt were also via the i/p route.
Animals were sacrificed in triplicate at various time periods after injection and samples of blood and tissue collected.

Results and Discussion

Blood clearance curves for I-125, Y-90 and Sc-47 labelled antibodies are shown in Figures 1 and 3. Liver uptake for the three isotopes is shown in Figures 2 and 4.
The clearance from the blood for all these isotopes is very similar. The lower value for the 2 hour Sc-47 blood activity probably represents a poor i/p injection, since one animal had depressed activity in all its organs, reflected in the increased standard error of this point. The uniform clearance rates suggest that all three of these labels are similarly stable in the blood of mice. However in contrast to I-125, both metallic isotopes are deposited into the liver, the liver activity increasing as activity is lost from the blood. It is probable that the breakdown of antibodies by the liver is responsible for both the blood clearance and the subsequent relocation of the free metallic ions into associstion with liver structures.
More Sc-47 activity is deposited into the liver than is seen with Y-90, this may reflect the higher stability constant Sc-47 exhibits for binding to a range of chelator molecules. Thus when released from the DTPA bond within the liver, it binds with greater affinity to its eventual liver location.[4] Though indium has a higher stability constant for DTPA binding than either of these Group III elements, it does not accumulate to the same extent within the liver. [5] This indicates that binding within the liver is not entirely determined by the ability of these metals to form stable chelate systems. Other factors, determined

by the chemistry of each element, must be of importance in the retention of these elements in the liver.

The substantial liver uptake of scandium and yttrium, and consequent high dose, precludes the use of these isotopes as cytotoxic agents unless the liver uptake can be supressed. Immunologically, antibodies of different classes or their fragments might be used, though it may only relocate, not eradicate, the dosimetric problem of non-tumour associated activity [6]. Apart from alternative isotopes with different chemical behaviour, methods aimed at removing liver activity may be employed.

The strategy employed here, of using DTPA to mobilise metallic ions, has been used succesfully in both animals and man to remove radioactive heavy metals.[7] In this study, yttrium-90 activity within the liver was reduced with repetitive i/p injections of 30 μM/Kg bodyweight of DTPA in acetate buffer. (Figure 5) Increasing the DTPA concentration gave poorer results and dissolving the DTPA in saline (not shown) rather than sodium acetate gave no change in liver activity.

At least part of the reason for the failure of DTPA to remove more activity may lie in the very short residence time of DTPA in the body, 70% is removed from the blood within 15 min after injection and rapidly excreted.[8] Consequently it is unlikely that the DTPA will remain in the liver, at the site of isotope deposition, for long enough to allow ligand exchange. For this particular application DTPA encapsulated within liposomes may be appropriate. Liposomes are very effective liver localising agents, once within the target cell they may slowly release entrapped reagents. Using liposomes containing DTPA has enhanced the clearance of plutonium from the liver of mice, in comparison to administration of free DTPA.[9]

In the context of antibody directed radioactive metals, chelation treatment would offer a system for lowering the non-tumour dose, as liposomes are not taken into the majority of tumours, thus enhancing the tumour to background ratio. To achieve this, however, will require further work on the chemical nature, and kinetics, of the binding of metallic ions within the liver.

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DTPA: Diethylene triamine penta acetic acid
CEA: Carcino embryonic antigen

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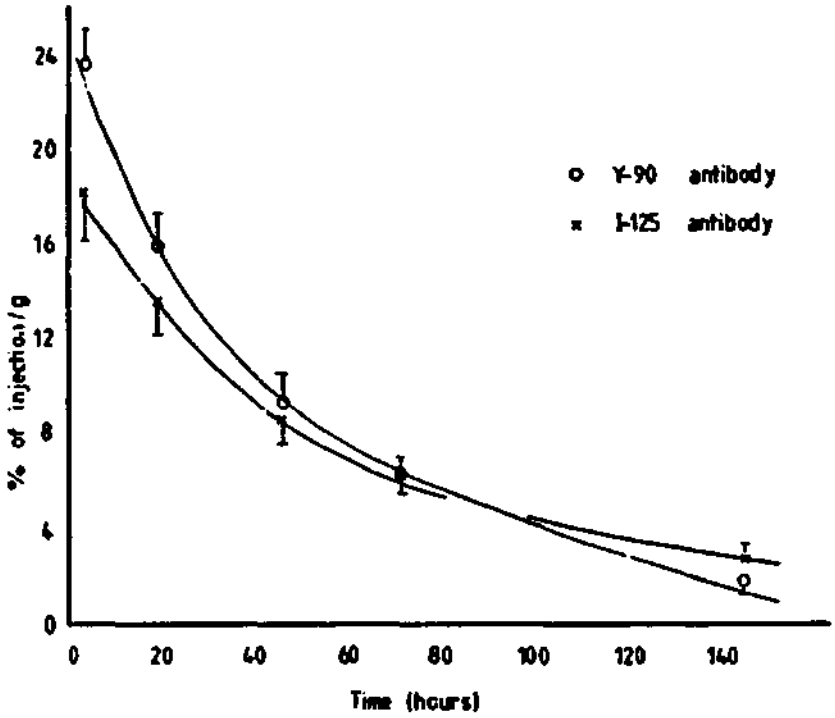

Figure 1: Blood clearance of anti-CEA antibody labelled with either I-125 or Y-90 injected into the same animal (n=3)

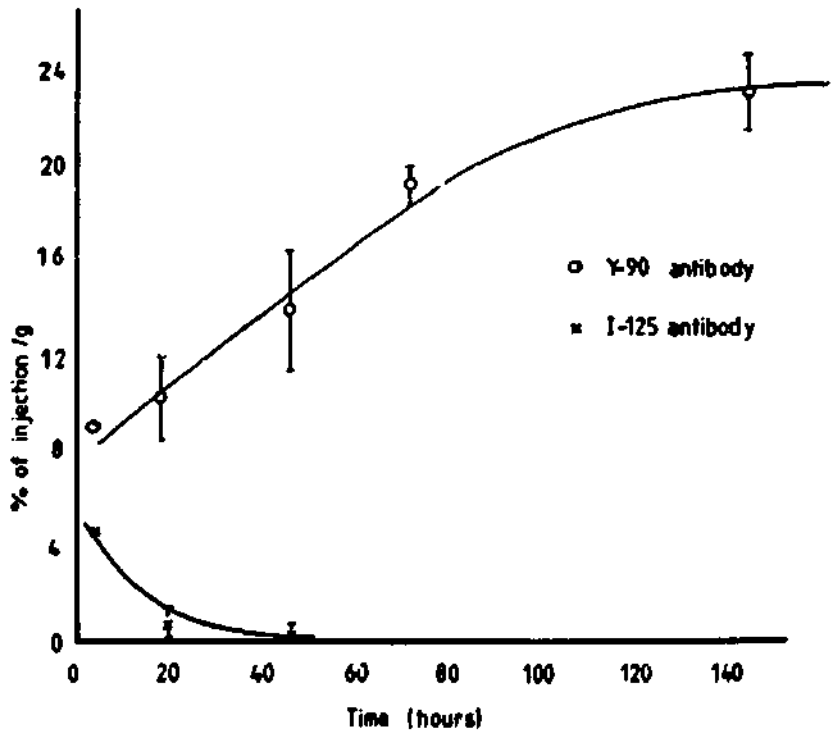

Figure 2: Uptake of radioactivity by liver after injection of both I-125 and Y-90 separately labelled antibodies into the same animal (n=3)

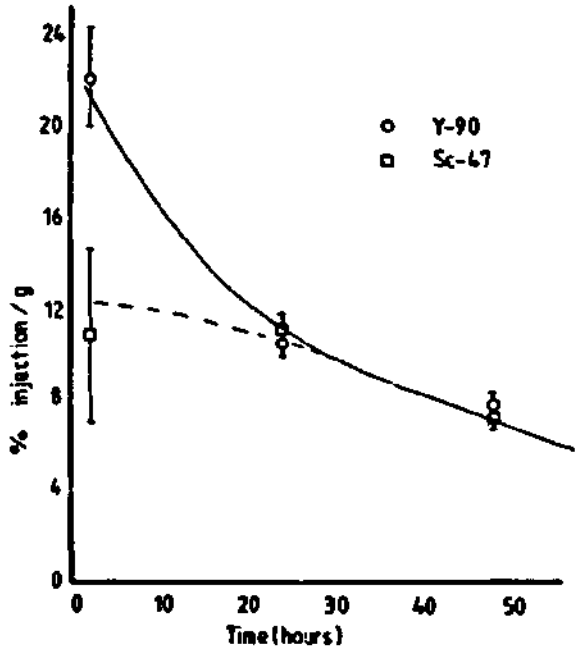

Figure 3: Blood clearance of anti-CEA antibody labelled with either Y-90 or Sc-47 injected into separate animals (n=3/isotope)

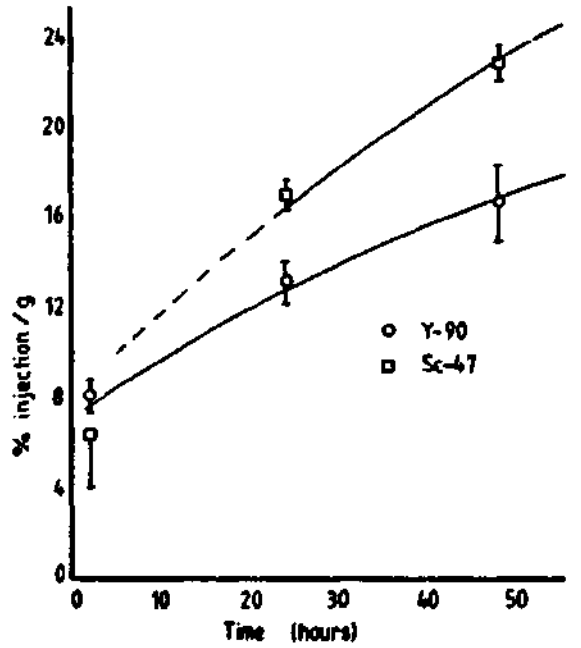

Figure 4: Uptake of radioactivity by liver after injection into separate animals of antibody labelled with either Sc-47 or Y-90 (n=3/isotope)

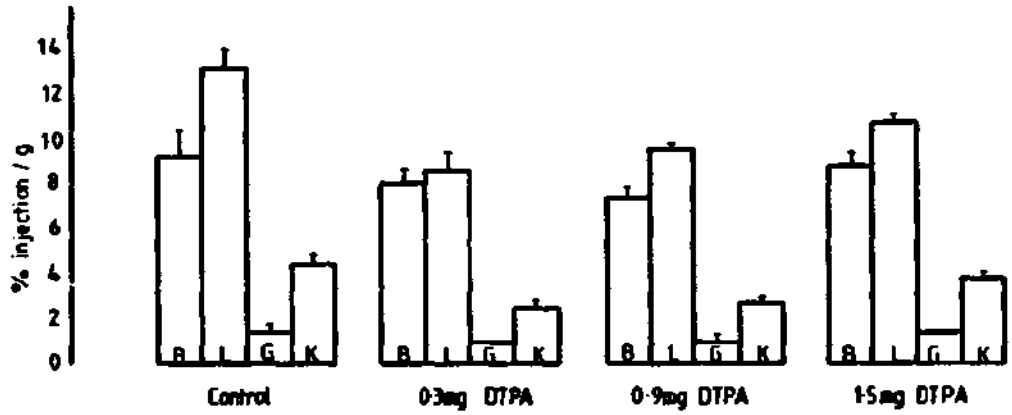

Figure 5: Clearance of activity from B-blood, L-liver G-gut and K-kidney in mice after an initial i/p injection of Y-90 labelled antibody, followed by five separate i/p injections through 48 hours, of 0.1ml of DTPA. Tissue analysis carried out at T+48 hours after antibody injection. (n=3)