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K.H.TRAGL
P.PILS
E.DEUTSCH
P.ANGELBERGER
E.HRUBY

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K.H. Tragl *
P. Pils *
E. Deutsch *
P. Angelberger
E. Hruby

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* First Medical Department, University School of Medicine, Vienna, Austria and Research Center Seibersdorf, Österreichische Studiengesellschaft für Atomenergie, Vienna Austria

Österreichische
Studiengesellschaft für Atomenergie
Ges.m.b.H.

Lenaugasse 10 A-1082 Wien

INSTITUT FÜR CHEMIE
Forschungszentrum Seibersdorf

Abstract

Accumulation of a radioactive label in tumor tissue is required for scintigraphic visualization of the tumor and has been achieved by application of bleomycin as carrier. This chemotherapeutic polypeptide drug has been labeled with ^{64}Cu. Thin layer chromatography of ^{64}Cu-bleomycin which was incubated for 22 hours at 37°C showed no changes of the chelate. When urine of the ^{64}Cu-bleomycin treated animals was chromatographed considerable amounts (9.3 %) of free ^{64}Cu appeared 2 hours after injection of the chelate and dissociation was found to be almost complete 4 hours after injection of labeled bleomycin.
The distribution of ^{64}Cu-bleomycin is similar to that found with ^{99m}Tc-bleomycin with long lasting accumulation in kidneys and liver. Highest concentration ratios of the activity in mesenchymal tumor (Yoshida) and blood respectively were found 6 hours after intravenous administration of ^{64}Cu-bleomycin.

Key words: ^{64}Cu-bleomycin, tissue-distribution, excretion, Yoshida tumor.

The scintigraphic imaging of tumors by accumulation of ^{99m}Tc-bleomycin was examined in previous experiments (1, 2). The interpretation of the results obtained with ^{99m}Tc-bleomycin, however were hampered by temperature dependent early dissociation of the chelate. The unstability of the ^{99m}Tc-bleomycin complex reduces the bleomycin directed distribution of the radioactive label and is favoring a distribution directed by TcO_4^- technetium alone. Of the metal ions available, ^{64}Cu was reported to form stable chelates with bleomycin (3) and was additionally attractive with regard to it's radioactivity decay.

^{64}Cu decays with a 12,8 h half - life by emission of beta particles and gamma rays whose energies and abundance are listed below:

beta β^-	0,573 MeV	(38 %)
beta β^+	0,656	(19 %)
EC		(43 %)

gamma	0,0075 MeV	(characteristic Ni X-rays)
	0,511	(from β^+ annihilation, 38 %)
	1,34	(0,5 %)

The most prominent 0,511 MeV gamma rays can be utilized for scintigraphic imaging with the Anger Camera using a pinhole collimator.

Materials and methods

1. Preparation of $^{64}Cu(NO_3)_2$

Approximately 1.0 mg of metallic Cu (99.999 %) was irradiated to saturation (24-48 hours) in the reactor at a thermal neutron flux of 7×10^{-13} n cm^2 s^{-1}. After decay to the desired activity, Cu was dissolved in 0.1 ml of concentrated HNO_3, freed of NO and of NO_2 by heating, and finally adjusted to 10.0 ml by addition of distilled water. The activity of ^{64}Cu was measured in a calibrated ionisation chamber and the radionuclidic purity determined with Ge (Li)-gamma spectroscopy. Specific activities up to 56 mCi/mg Cu were obtained.

2. 5.0 mg bleomycin (purchased from Lundbeck) were dissolved in 0,3 ml of 0.9 % NaCl and an aliquot of the $^{64}Cu(NO_3)$ solution containing the desired activity and less than 120 µg Cu was added. The pH was adjusted to 1.6 - 1.8 and the volume brought to 2.0 ml with 0.9 % NaCl. After stirring for one minute the solution was neutralized with 1 n $NaHCO_3$ (pH 5-7). The solution was finally sterilized by membrane ultrafiltration.
The radiochemical purity was determined by thin layer chromatography (Silica gel, methanol: 10 % NH_4-acetate 1:1). Two peaks were found representing the bleomycin components A_2 (Rf-0.4) and B_2 (Rf-0.7), which contain more than 98 % of the total ^{64}Cu activity. This ^{64}Cu-bleomycin chelate was absolutely stable for more than 24 hours at a temperature of 37°C. The specific activity of bleomycin for the consecutive experiments is kept at 0.25 mCi/mg.

3. Animal experiments.

The experiments were performed on Wistarrats weighing betwen 230 - 280 g. The animals were injected intravenously with ^{64}Cu-bleomycin (0.4 mCi/kg body weight), killed and exsanguinated 1, 2, 4, 6, 9 or 22 hours respectively after the administration of ^{64}Cu-bleomycin. The radioactivity of the tissue samples (blood, skin, heart, lung, liver, spleen, pancreas, kidneys, testicles and skeletal muscle) was determined in a well scintillation counter (Riedl) after they had been weighed in tared counting tubes. The counting rates were corrected for background and for decay of ^{64}Cu to the time of injection. They were finally expressed as per cent dose/g tissue of the injected ^{64}Cu.

In order to compare the excretion of ^{64}Cu-bleomycin with the excretion of ^{99m}Tc-bleomycin, another group of rats was treated i.v. with 0.25 mCi/kg body weight ^{99m}Tc-bleomycin (2).

In a second series of experiments an ascites form of Yoshida tumor (lymphosarcoma) was used to produce a solid tumor in the subcutanous tissue of the left hind quarters of female rats. The tumor was allowed to develop for 10 days before the injection of labeled bleomycin. The animals were treated as described before except that in this experiment tissue samples were removed from the Yoshida tumor and from the ovaries instead of the testicles.

Results

1. Thin layer chromatography

The radiochemical purity of ^{64}Cu-bleomycin was examined immediately after the reaction of the two components and after

incubation of the chelate for 1, 2, 4, 9 and 22 hours at a temperature of 37°C. During this time no dissociation of ^{64}Cu from bleomycin could be found and the chromatographic pattern was unchanged except for the overall reduction of radioactivity according to the decay of the radioisotope

2. Excretion of radioactivity in the urine

Urine of ^{64}Cu-bleomycin treated rats was obtained by puncture of the bladder. Measurement in a well scintillation counter revealed excretion of high activity in the first hour after injection of the chelate (Tab. 1) with a decrease in the following hour. If the excretion of ^{99m}Tc-bleomycin was examined in the same manner it was found to double the ^{64}Cu-bleomycin values in the first hour but to decrease to lower levels in the following hours. Since the radioactivity encountered in the urine was calculated per gram of urine it exceeded the injection dose in the ^{99m}Tc-bleomycin treated animals.

Thin layer chromatography of the urine indicated a rather stable ^{64}Cu-bleomycin complex for the first two hours of the animal experiment. Two hours after the injection of ^{64}Cu-bleomycin the fraction of free, unbound ^{64}Cu was 9.3 % of the total activity in the urine. Four hours after the administration of the chelate, however, the ^{64}Cu-bleomycin complex in the urine was almost completely dissociated. At this time free ^{64}Cu exceeded 70 % of the total activity in the urine. Examination of ^{99m}Tc-bleomycin in a similar manner revealed accelerated dissociation: the fraction of free ^{99m}Tc in the urine exceeded 20 % already one hour after the injection of ^{99m}Tc-bleomycin and was greater than 70 % two hours after administration of the chelate.

3. Distribution of ^{64}Cu in animal tissue after i.v. injection of ^{64}Cu-bleomycin.

The results of the radioactivity distribution studies in rat tissue are summarized in Table 2. They indicate rapid reception of radioactivity with consecutive slow delivery in skin, lung, and muscle white the radioactivity in liver, spleen, pancreas, testicles, ovaries, and in the implanted tumor is kept at a constant level during the observation period (22 hours). The very high radioactivity in kidney tissue is an expression of the excretory function rather than an exceptional capability to accumulate radioactivity in this tissue. This suggestion is confirmed by the high radioactivity found in the urine of the rats. Table 3 shows the concentration ratio of radioactivity in samples of the mesenchymal tumor and in blood or in different tissue samples respectively. Four hours after the injection of ^{64}Cu-bleomycin the tumor/blood ratio was found 2.4 and reaches a maximum of 3.2 six hours after administration of the chelate. At this time concentration ratio's exceeding 2.0 were found in spleen (2.16), in skin (2.6), in heart muscle (3.9), in lung (2.45), in pancreas (2.1), and in sceletal muscle (11.5). Only for liver tissue and for kidneys the tumor/tissue concentration ratio at no time reached 1.0.

Discussion

Evalution of bleomycin as a carrier of radioactive labels for scintigraphic imaging of malignant tumors has show this drug to be a helpful tool in visualization of epidermoid carcinomas and malignant lymphomas (1, 2, 4). The labels tagged to bleomycin have been ^{57}Co, ^{111}In, ^{67}Ga, and most recently ^{99m}Tc (1, 4, 5).

In fact, with ^{99m}Tc as a radioactive marker bleomycin fulfills most of the requirements expected in tumor visualization i.e. short half life, sufficient contrast of the tumor or high tumor/neighbouring tissue concentration ratios respectively. The ^{99m}Tc-bleomycin chelate complex, however, has only a moderate thermal stability: after incubation of ^{99m}Tc-bleomycin for one hour at 37°C free pertechnetate will be encountered at concentrations up to 24 % of the total radioactivity.

Since Cu^{++} was reported to form very stable chelates with bleomycin (3, 5) this metal was chosen as a radioactive label of bleomycin and its distribution, excretion and its affinity to a mesenchymal tumor were examined in an animal experiment.

In the experiments presented above, ^{64}Cu-bleomycin was shown to be superior to ^{99m}Tc-bleomycin with regard to the stability of the chelate. This was found by incubation of ^{64}Cu-bleomycin at 37°C and subsequent thin layer chromatography of the chelate. When the stability of the bleomycin chelate in the biological system was examined, thin layer chromatography of the urine of rats previously treated with either ^{99m}Tc-bleomycin or ^{64}Cu-bleomycin revealed the ^{64}Cu-bleomycin chelate to be the more stable compound. However, in the biological system the degradation of ^{64}Cu-bleomycin was accelerated and 4 hours after the injection of ^{64}Cu-bleomycin to the rats free (unbound) ^{64}Cu exceeded 70 % of the total radioactivity of the urine. With ^{99m}Tc- bleomycin this percentage of unbound label in the urine had already been found 2 hours after administration of the chelate (2).

With regard to the application of ^{64}Cu-bleomycin in scintigraphic tumor visualization its concentration ratios tumor/blood and tumor/neighbouring tissue respectively were found to equal or even surpass

the results obtained with ^{99m}Tc-bleomycin (2).

The disadvantage of any labeled bleomycin is the fact that strong accumulation of bleomycin is restricted to very specific and histologically defined tumors which confines positive imaging to a limited number of tumors. In addition, the accumulation of bleomycin in liver and kidneys gives a very high background activity and makes tumor visualization in this area almost impossible.

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Table 1

Urinary excretion of ^{99m}Tc and ^{64}Cu following i.v. administration of ^{99m}Tc - or ^{64}Cu - bleomycin resp.

Urinary excretion:

Time (hours) post injection	1	2	4	6	9	24
^{99m}Tc-(bleo)	117.3	22.8	2.1	1.1	1.1	0.14
^{64}Cu-(bleo)	53.5	13.6	3.1	2.3	0.76	0.038

(% dose / g urine)

Tabelle 2

Tissue distribution of ^{64}Cu after i.v. injection of ^{64}Cu-bleomycin (0,4 mCi/kg body weight)

Time (hours) post injection

		1	2	4	6	9	22
1	Blood	0.148 ± 0.034	0.088 ± 0.062	0.064 ± 0.033	0.057 ± 0.003	0.065 ± 0.011	0.084 ± 0.058
1	Skin	0.138 ± 0.031	0.083 ± 0.014	0.069 ± 0.022	0.070 ± 0.025	0.075 ± 0.017	0.062 ± 0.028
1	Heart	0.062 ± 0.008	0.040 ± 0.010	0.040 ± 0.010	0.047 ± 0.014	0.057 ± 0.011	0.063 ± 0.017
1	Lung	0.142 ± 0.025	0.085 ± 0.019	0.072 ± 0.024	0.075 ± 0.024	0.065 ± 0.006	0.074 ± 0.017
1	Liver	0.229 ± 0.037	0.278 ± 0.044	0.294 ± 0.058	0.243 ± 0.031	0.291 ± 0.015	0.239 ± 0.029
1	Spleen	0.064 ± 0.010	0.053 ± 0.024	0.061 ± 0.023	0.085 ± 0.043	0.083 ± 0.016	0.125 ± 0.053
1	Kidney	1.286 ± 0.050	1.244 ± 0.184	1.448 ± 0.283	1.458 ± 0.376	1.336 ± 0.209	1.179 ± 0.472
1	Pancreas	0.076 ± 0.017	0.069 ± 0.010	0.078 ± 0.013	0.087 ± 0.029	0.076 ± 0.020	0.053 ± 0.010
1	Muscle	0.058 ± 0.011	0.023 ± 0.005	0.016 ± 0.006	0.016 ± 0.004	0.020 ± 0.009	0.018 ± 0.006
2	Testicle	0.118 ± 0.034	0.105 ± 0.010	0.108 ± 0.015	0.117 ± 0.007	0.115 ± 0.003	0.089 ± 0.018
3	Ovary	0.145	0.122	0.098	0.140	0.180	0.124
3	Tumor	0.163	0.088	0.153	0.184	0.172	0.171

% dose/g tissue
(1) = 5 rats (2) = 4 rats (3) = 1 rat

Tabelle 3

^{64}Cu-bleomycin:

Tumor: tissue ratio for ^{64}Cu in rats bearing a subcutaneous implanted Yoshida tumor.

Time (hours) post injection	1	2	4	6	9	24
Blood	1.09	1.00	3.39	3.22	2.64	2.04
Skin	1.18	1.06	2.21	2.62	2.29	2.77
Heart	2.62	2.20	3.82	3.91	3.01	2.73
Lung	1.14	1.03	2.12	2.45	2.64	2.32
Liver	0.71	0.31	0.52	0.75	0.59	0.71
Spleen	2.54	1.66	2.50	2.16	2.07	1.37
Kidney	0.12	0.07	0.10	0.12	0.12	0.14
Pancreas	2.14	1.27	1.96	2.11	2.26	3.24
Muscle	2.81	3.82	9.56	11.51	8.60	9.55
Testicle	1.38	0.83	1.41	1.57	1.49	1.93
Ovary	1.12	0.72	1.56	1.31	0.95	1.38

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