[Chem. Pharm. Bull. 32(10)4049—4053(1984)]

Radioactive Metal Complexes of Ethylenediamine-*N*, *N*-diacetic Acid. Biodistribution of Radioactivity in Mice Bearing Tumors

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(Received January 28, 1984)

Complexes of ethylenediamine-*N*, *N*-diacetic acid (EDDA) with radioactive metal ions and ^{3}H-labeled EDDA were prepared and the biodistribution of the radioactivity in mice bearing Ehrlich tumor was studied. Radioactive complexes studied were those of ^{51}Cr, ^{57}Co, ^{59}Fe, ^{64}Cu, and ^{67}Ga. μ-Oxo ^{57}Co EDDA was prepared by treatment of ^{57}Co EDDA with hydrogen peroxide.

The tumor/blood ratios of radioactivity indicated that ^{57}Co EDDA and μ-oxo ^{57}Co EDDA were concentrated in the tumor tissues, whereas other complexes and ^{3}H-EDDA were not. The tumor tissues were clearly visualized in scintigrams after the administration of the ^{57}Co complexes to mice.

Keywords——ethylenediamine-*N*, *N*-diacetic acid; Ehrlich tumor; tumor scintigraphy; radiopharmaceutical; metal chelate; ^{57}Co; ^{51}Cr; ^{59}Fe; ^{64}Cu; ^{67}Ga

Our recent studies[1] have shown that ^{99m}Tc radioactivity was concentrated in tumor tissues in experimental animals a few hours after the administration of the complex of ethylenediamine-*N*, *N*-diacetic acid (EDDA) with ^{99m}Tc. The tumor tissues were clearly visualized in scintigrams. In animals that did not bear tumors, ^{99m}Tc activity was not accumulated in any specific organ and was excreted through the kidneys. For further studies on tumor scintigraphic agents, knowledge on the mechanism of localization of the EDDA complex of ^{99m}Tc (^{99m}Tc EDDA) in tumors is required.

To shed light on this problem, we prepared several radioactive metal complexes of EDDA as well as ^{3}H-labeled EDDA and studied their biological behavior in mice bearing Ehrlich tumor. The present paper deals with the results of the study.

Experimental

Materials——EDDA was prepared by the reported method.[2,3] ^{3}H-Labeled EDDA (^{3}H-EDDA) was synthesized by using ^{3}H-labeled iodoacetic acid (specific activity, 20 mCi/mmol). 3-Oxopiperazine-1-acetic acid (cyclic EDDA) and ^{3}H-labeled cyclic EDDA were prepared from EDDA and ^{3}H-EDDA, respectively, by treatment with acid. Other chemicals were of analytical grade and were obtained from commercial sources. Radioactive materials used for biodistribution studies and for the preparation of radioactive metal complexes were $^{51}CrCl_3$ (specific activity, 150 mCi/mg Cr), $^{57}CoCl_2$ (carrier-free), $^{59}FeCl_3$ (3—20 mCi/mg Fe), $^{64}CuCl_2$ (1 mCi/mg Cu), and $^{67}GaCl_3$ (carrier-free). Sources of the radioactive materials were Amersham International Ltd. (^{51}Cr, ^{57}Co, ^{59}Fe), Tokai F-25 Japan Atomic Energy Research Institute (^{64}Cu), and Dai-ichi RI Laboratories (^{67}Ga).

Preparation of Radioactive Metal Complexes——Solutions of radioactive metal complexes used for biological studies were prepared by mixing aqueous solutions of radioactive metal ions (0.1—0.3 ml, 50—100 μCi) with EDDA in saline (0.2—0.5 mmol in 1.0 ml; pH, 7.0). Formation of radioactive metal complexes was confirmed by means of thin layer chromatography.[1] Naturally, the amount of EDDA was in a large excess over that of metal ions. To

prepare μ-oxo complex, ^{57}Co EDDA solution was oxidized by addition of 50 μl of 3.3% H_2O_2.

Biodistribution——ICR mice (male; body weight, 30±2 g) were implanted with Ehrlich ascites tumor cells at the right foreleg, then left for a period of 2—3 weeks for tumor growth (to a size of 1.0—1.5 cm in diameter). The solutions of the radioactive metal ions or their complexes were injected intravenously in the tail vein of the tumor-bearing mice. The animals were killed at selected times. Blood, organs, and tumor tissues were removed and weighed, and their radioactivity was measured with an autogamma scintillation spectrophotometer. The per cent of the injected dose/ml blood or /g organ and the tumor/blood (g/ml) and tumor/muscle (g/g) ratios of radioactivity were calculated.

Biodistributions of ^{3}H-labeled compounds were studied similarly. Tissues were solubilized with 1.5 ml of a tissue solubilizer (NCS) in liquid scintillation vials by heating overnight at 50 °C in a water bath. Blood plasma (0.1 ml) was mixed with 1.5 ml of NCS. The solutions were mixed with 10 ml of aquasol-2 and their radioactivity was measured with a liquid scintillation counter.

For gamma-emitting isotopes, sequential scintigrams were also made by using a scintillation camera with a pinhole collimator.

Results

The results of the biodistribution study are summarized in Tables I and II. ^{57}Co EDDA was concentrated in the tumor tissues. The administration of ^{57}Co EDDA solution treated with hydrogen peroxide (*i.e.*, μ-oxo complex of dimeric Co EDDA (μ-oxo Co EDDA)[3]) further increased the tumor/blood and tumor/muscle ratios. This complex was also concentrated in abscesses.

The results of biodistribution of inorganic forms of ^{57}Co are included in Table I.

Table I. Distribution of ^{57}Co Radioactivity in Mice[a]

Compound	$^{57}CoCl_2$	$^{57}CoCl_3$[b]	^{57}Co EDDA	μ-Oxo ^{57}Co EDDA	μ-Oxo ^{57}Co EDDA[c]
Organ					
Blood	2.05±0.20	3.11±0.56	0.094±0.013	0.086±0.014	0.085±0.018
Muscle	0.45±0.01	0.44±0.01	0.062±0.006	0.045±0.005	0.044±0.003
Tumor	1.79±0.55	2.05±0.66	0.835±0.051	1.190±0.045	3.311±0.815[c]
Liver	9.85±0.99	9.13±0.98	0.413±0.031	0.574±0.081	0.792±0.273
Spleen	1.11±0.10	1.16±0.01	0.103±0.004	0.098±0.006	0.130±0.014
Stomach	1.34±0.36	1.12±0.30	0.233±0.071	0.166±0.049	0.197±0.063
Intestine	1.84±0.35	1.75±0.46	0.225±0.038	0.188±0.035	0.263±0.105
Kidney	6.97±1.21	5.87±0.90	1.616±0.315	1.369±0.213	1.133±0.341

a) Values are % dose/ml blood and % dose/g organ 3 h after administration. Mean±S.D. of 4 animals.
b) $^{57}CoCl_2$ was treated with H_2O_2.
c) Distribution in mice bearing abscesses. See ref. 1.

Table II. Distribution of Radioactivity in Mice[a]

Compound	^{3}H-EDDA	^{3}H-Cyclic EDDA	^{51}Cr EDDA	^{59}Fe EDDA	^{59}Fe EDDA[b]	^{64}Cu EDDA	^{67}Ga EDDA
Organ							
Blood	1.11±0.21	1.41±0.25	0.21±0.05	6.53±0.45	6.11±0.14	0.90±0.12	1.98±0.21
Muscle	0.37±0.08	0.85±0.10	0.07±0.02	0.81±0.15	0.98±0.06	0.10±0.01	0.31±0.07
Tumor	0.79±0.11	0.85±0.12	0.29±0.05	0.77±0.09	1.51±0.15	0.96±0.10	0.67±0.11
Liver	2.66±0.55	2.27±0.53	1.21±0.16	4.89±0.71	3.34±0.17	11.45±1.23	1.10±0.21
Spleen	0.57±0.10	0.75±0.08	0.21±0.02	34.66±3.85	36.93±3.45	0.81±0.12	0.68±0.10
Stomach	0.33±0.11	0.90±0.13	0.09±0.03	1.30±0.24	0.94±0.04	0.46±0.03	0.47±0.11
Intestine	1.10±0.25	0.55±0.10	0.11±0.04	1.63±0.35	1.80±0.54	1.15±0.24	0.27±0.08
Kidney	13.64±1.55	8.68±1.65	2.13±0.15	2.26±0.27	2.16±0.16	13.72±1.31	1.52±0.35

a) Values are % dose/ml blood and % dose/g organ 3 h after administration. Mean±S.D. of 3 animals.
b) ^{59}Fe EDDA was treated with H_2O_2.

Significant amounts of radioactivity were present in blood and in organs. These results indicate very slow clearance of radioactivity. The tumor/blood and tumor/muscle ratios were small. The tumor tissue did not significantly accumulate Co and its radioactivity should reflect that in blood.

In contrast to Co complexes, no significant radioactivity was concentrated in the tumor by the administration of ^{3}H-EDDA, ^{3}H-cyclic EDDA, or EDDA complexes of ^{51}Cr, ^{59}Fe, ^{64}Cu and ^{67}Ga. High uptake of radioactivity by the liver was noted with ^{64}Cu EDDA. Accumulations of ^{3}H-EDDA, ^{3}H-cyclic EDDA, and ^{51}Cr EDDA in the kidneys indicated urinary excretion. The blood clearances of radioactivity were very slow with ^{59}Fe and ^{67}Ga EDDA. Considerable radioactivity was concentrated in the spleen with ^{59}Fe EDDA. No significant changes in ^{59}Fe biodistribution were observed after the treatment of ^{59}Fe EDDA with hydrogen peroxide.

Sequential scintigrams after administration of μ-oxo ^{57}Co EDDA to a mouse bearing Ehrlich tumor are shown in Fig. 1. The image of the tumor became recognizable at 1.5 h and was clearly visualized 2—7 h after the administration. The image was observable for more than 24 h. The quality of the scintigrams was as satisfactory as could be obtained with ^{99m}Tc

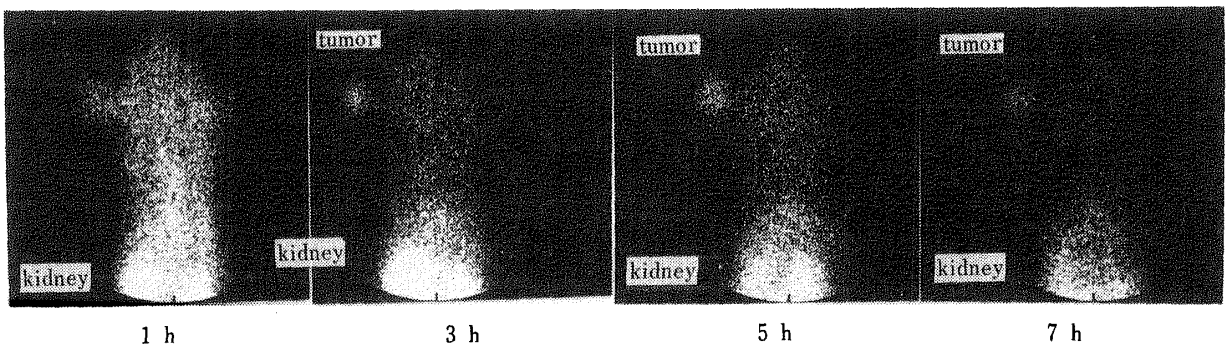

Fig. 1. Sequential Scintigrams of a Mouse Bearing Ehrlich Tumor with μ-Oxo ^{57}Co EDDA (Anterior Projection)

Times after the administration are indicated below the scintigrams.

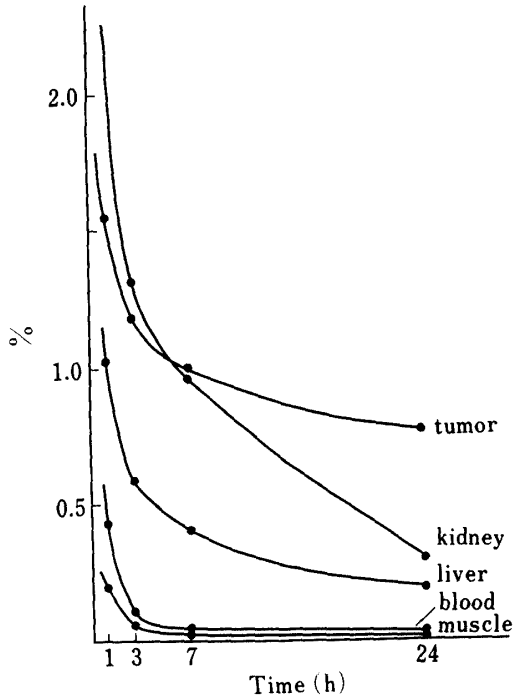

Fig. 2. Time–Course of Biodistribution of μ-Oxo ^{57}Co EDDA

TABLE III. Effect of Carrier Co on Tumor/Blood Ratio of μ-Oxo EDDA

Co/EDDA[a]	Carrier-free	1/4	1/2	1	2
% dose/g tumor	1.190	1.173	1.237	1.911	5.105
% dose/ml blood	0.086	0.184	0.217	1.495	5.565
Tumor/blood	13.837	6.375	5.700	1.278	0.917

a) Ratio of the amounts of Co and EDDA (mol/mol). EDDA, 35.2 mg/ml.

EDDA.[1]

Figure 2 shows the time–course of biodistribution of μ-oxo ^{57}Co EDDA. Clearances from blood and muscle were very rapid. Various amounts of $CoCl_2$ were added as a carrier in the preparation of μ-oxo ^{57}Co EDDA. The tumor/blood ratio of the radioactivity was highest with carrier-free ^{57}Co and decreased with increase in the amount of the carrier (Table III).

Discussion

The results of the biodistribution study of the ^{3}H-labeled chelating agents show that EDDA and cyclic EDDA do not have an affinity for tumor tissues. This finding is not unexpected, since chelating agents structurally related to EDDA do not concentrate in any organ or tissue and are excreted from the kidneys.[4]

From the results of the biodistribution of the radioactive complexes, we can conclude that there are two kinds of EDDA complexes, tumor-localizing and nonlocalizing ones. Among the EDDA complexes studied, those of ^{57}Co and ^{99m}Tc concentrated in tumor tissues. The reason for these significant differences between the complexes is not fully understood at present.

There are two possible explanations for the phenomena. One is based on the rate of ligand exchange of the complexes. The preparations used in the study contained a tracer amount of radioactive metal ion, which was in the form of EDDA complex, and an excess amount of EDDA. Radioactive EDDA complex and EDDA should be diluted in blood, where considerable concentrations of other chelating molecules and metal ions are present. The EDDA complex may undergo ligand exchange *in vivo*. It is well known that trivalent Co is inert to ligand exchange reactions.[5] Our previous studies[1] showed that ^{99m}Tc EDDA was also inert to ligand exchange and was stable *in vivo*. The ligand exchange reactions are expected to be fast with other metal ions. If such ligand exchange reactions are taken into account, it seems likely that radioactive ions of Cr, Cu, Fe and Ga may not be in the form of EDDA complexes when they reach the tumor tissues. This might explain the differences between the EDDA complexes.

The other explanation is based on the chemical forms of the complexes. The tumor/blood ratio of ^{57}Co radioactivity was enhanced by treatment of the Co complex with H_2O_2. The treatment converts the Co chelate of EDDA to dimeric μ-oxo Co(III) complex.[3] The structure of ^{99m}Tc EDDA complex is not fully understood but may be dimeric or polymeric.[6] On the other hand, there is little doubt that EDDA complexes of the other metal ions are in the forms of normal metal chelates. Thus, the differences in the biodistribution might reflect the chemical structures of the complexes.

The addition of carrier Co to μ-oxo ^{57}Co EDDA decreased the tumor/blood ratio. This result may be interpreted in terms of saturation of the hypothetical receptor site in the tumor tissues.

Much work remains to be done before the mechanism of the accumulation of the EDDA

complexes can be understood. Since no stable isotope of Tc exists, chemical and biological studies of this element meet with many difficulties. The present finding that Co EDDA complex accumulated in tumors should provide a new approach. At present, a radioactive Co that is safely applicable for human scintigraphy is not available from commercial sources, but several stable and radioactive Co isotopes are readily available, and can be used in chemical and animal studies. Investigations along this line are in progress and the results will be reported in due course.

Acknowledgements Financial support from the Ministry of Education, Science and Culture and from the Ministry of Welfare is gratefully acknowledged. Thanks are due to Mr. Masato Ohya, Miss Mutsumi Matsuoka and Miss Junko Miura for technical assistance.

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