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Evaluation of ^{99m}Tc-Labeled Amino Acids as Radiopharmaceuticals. III.[1] ^{99m}Tc Complexes of Ligands related to Ethylenediamine-*N*,*N*-diacetic Acid for Tumor Visualization

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Seventeen ^{99m}Tc-labeled ligands were evaluated as tumor scanning agents by preparing sequential scintigrams of mice bearing intramuscularly transplanted Ehrlich tumor. The ligands were labeled by the $SnCl_2$ method and were administered intravenously to the mice. The ligands studied were ethylenediamine-*N*,*N*-diacetic acid (*u*-EDDA) and structurally related compounds. ^{99m}Tc complexes of *u*-EDDA, ethylenediamine-*N*,*N'*-diacetic acid, *N*-hydroxyethyliminodiacetic acid, and propylene-1,3-diamine-*N*,*N*-diacetic acid achieved clear visualization of Ehrlich tumors. The image of the tumor was less clearly visualized with the complexes of iminodiacetic acid, *N*-methyliminodiacetic acid, nitrilotriacetic acid, ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetic acid, *N*-hydroxyethylethylenediamine-*N*,*N'*,*N'*-triacetic acid, hydrazine-*N*,*N*-diacetic acid, and 3-oxopiperazine-1-acetic acid.

Keywords——radiopharmaceuticals; tumor scintigraphy; ^{99m}Tc; Ehrlich tumor; ethylenediamine-*N*,*N*-diacetic acid; ethylenediamine-*N*,*N'*-diacetic acid; *N*-hydroxyethyliminodiacetic acid; propylene-1,3-diamine-*N*,*N*-diacetic acid; complexane

In the previous paper of this series,[1] we reported that tumor tissues were clearly visualized in the scintigrams of mice bearing intramuscularly transplanted Ehrlich tumor a few hours after the administration of ethylenediamine-*N*,*N*-diacetic acid (*u*-EDDA) labeled with ^{99m}Tc. ^{99m}Tc labeled *u*-EDDA (^{99m}Tc *u*-EDDA) has been proved effective for scintigraphic visualization of various malignant tumors in experimental animals.[2] These results prompted us to investigate ^{99m}Tc complexes of various other ligands structurally related to *u*-EDDA.

Studies of this kind should reveal the structural requirements in *u*-EDDA analogs for ^{99m}Tc accumulation in the tumor and provide a sound basis for the molecular design of new tumor scintigraphic agents.

The present paper describes the results of scintigraphic studies on mice bearing Ehrlich tumor. The ligands studied are listed in Table I.

Table I. Ligands used in the Study

No.	Name	Abbreviation	Structure
1	Ethylenediamine-*N*,*N*-diacetic acid	*u*-EDDA	$H_2N(CH_2)_2N(CH_2COOH)_2$
2	Ethylenediamine-*N*,*N'*-diacetic acid	*sym*-EDDA	$HOOCCH_2NH(CH_2)_2NHCH_2COOH$
3	Iminodiacetic acid	IDA	$HN(CH_2COOH)_2$
4	*N*-Methyliminodiacetic acid	MeIDA	$CH_3N(CH_2COOH)_2$
5	Nitrilotriacetic acid	NTA	$N(CH_2COOH)_3$
6	Hydrazine-*N*,*N*-diacetic acid	HzDA	$H_2NN(CH_2COOH)_2$
7	Ethylenediamine-*N*,*N*,*N'*,*N'*-tetraacetic acid	EDTA	$(HOOCCH_2)_2N(CH_2)_2N(CH_2COOH)_2$

No.	Name	Abbreviation	Structure
8	*N*-Hydroxyethylethylenediamine-*N*,*N*′,*N*′-triacetic acid	HEDTA	$(HOCH_2CH_2)(HOOCCH_2)N(CH_2)_2N(CH_2COOH)_2$
9	*N*-Hydroxyethyliminodiacetic acid	HIDA	$HOCH_2CH_2N(CH_2COOH)_2$
10	Propylene-1,3-diamine-*N*, *N*-diacetic acid	PDDA	$H_2N(CH_2)_3N(CH_2COOH)_2$
11	Ethylenediamine-*N*, *N*-dipropionic acid	*u*-EDDP	$H_2N(CH_2)_2N(CH_2CH_2COOH)_2$
12	Ethylenediamine-*N*, *N*′-dipropionic acid	*sym*-EDDP	$HOOCCH_2CH_2NH(CH_2)_2NHCH_2CH_2COOH$
13	Taurine-*N*,*N*-diacetic acid	taurine-DA	$HO_3SCH_2CH_2N(CH_2COOH)_2$
14	Triethylenetetraamine	trien	$H_2N(CH_2)_2NH(CH_2)_2NH(CH_2)_2NH_2$
15	*N*-Acetylethylenediamine-*N*′, *N*′-diacetic acid	Ac-EDDA	$CH_3CONH(CH_2)_2N(CH_2COOH)_2$
16	*N*-Carbobenzoxyethylenediamine-*N*′,*N*′-diacetic acid	Z-EDDA	$C_6H_5CH_2OCONH(CH_2)_2N(CH_2COOH)_2$
17	3-Oxopiperazine-1-acetic acid	*cyclic*-EDDA	$HN(CH_2)_2N(CH_2COOH)CH_2CO$

Experimental

Materials——^{99m}Tc pertechnetate was obtained from a generator (Dainatech, Dainabot Radioisotope Lab. Inc.). *sym*-EDDA, IDA, MeIDA, NTA, EDTA, HEDTA, *sym*-EDDP, and trien were obtained from commercial sources. *u*-EDDA,[3] HzDA,[4] HIDA,[5] taurine-DA,[6] Ac-EDDA,[3] and *cyclic*-EDDA[3] were synthesized according to the methods described in the cited references.

u-EDDP was prepared by the reaction of *N*-acetylethylenediamine with 3-chloropropionic acid and subsequent hydrolysis of the product. Z-EDDA was prepared by carbobenzoxylation of *u*-EDDA with carbobenzoxy chloride.

PDDA was synthesized by the following procedure: Propane-1,3-diamine (70% aqueous solution) was mixed with about 1/3 equimolar amount of ethyl acetate and allowed to stand for 3 d at room temperature. The reaction mixture was distilled under reduced pressure and the fraction of bp 140—150°C (5 mmHg) was collected. It was identified as N^1-acetylpropane-1,3-diamine from its nuclear magnetic resonance (NMR) spectrum. An aqueous mixture of N^1-acetylpropane-1,3-diamine and 2 equimolar amounts of sodium chloroacetate was heated with stirring in a water bath at 80°C for 10 h, during which time the pH of the mixture was maintained at 9—11 by adding aqueous NaOH. The mixture was heated for an additional 5 h, during which time no appreciable change in pH occurred. After the addition of NaOH (about 4 mol per mol of *N*-acetylpropane-1,3-diamine), the solution was refluxed gently for 7 h, concentrated under reduced pressure, acidified with conc. HCl to pH 4.0, and filtered. The filtrate was treated with EtOH. The white precipitate that formed was separated and recrystallized from EtOH–water. mp 238°C. *Anal.* Calcd for $C_7H_{14}N_2O_4$–H_2O: C, 40.38; H, 7.75; N, 13.45. Found: C, 40.31; H, 7.75; N, 13.45.

^{99m}Tc Labeling——To 1.0 ml of aqueous solution (pH 7.0) containing 20 mg of a ligand, 0.15 ml of a 2.0 mg/ml solution of $SnCl_2$ was added. The pH of the mixture was adjusted to pH 7.0 with 0.1 M NaOH. The solution was passed through a 0.2 μm Millipore filter into a sealed vial. A saline solution of 10 mCi of ^{99m}Tc-pertechnetate was added to the vial. The mixture was shaken gently and allowed to stand for 10 min at room temperature. The labeling efficiency was evaluated chromatographically using a 0.5 mm silica gel plate (Merck F_{254}; 100×8 mm) developed with the following solvent systems: (a) ethanol/water (7: 3) (b) 1-butanol/acetic acid/water (4: 1: 1). The radiochromatogram was made with a TLC scanner (Aloka 101).

Scintigraphic Study——ICR mice (male; 30±2 g, body weight) were intramuscularly implanted with Ehrlich ascites tumor cells at the right foreleg.

The mice were left for a period of 3 weeks for the growth of the tumor. A saline solution of ^{99m}Tc-labeled ligand (0.1 ml, 0.5—1.0 mCi) was injected through the tail vein in the mice showing tumor growth. Sequential scintigrams were made at predetermined intervals using a scintillation camera (Toshiba GCA 202) with a pinhole collimator.

Results and Discussion

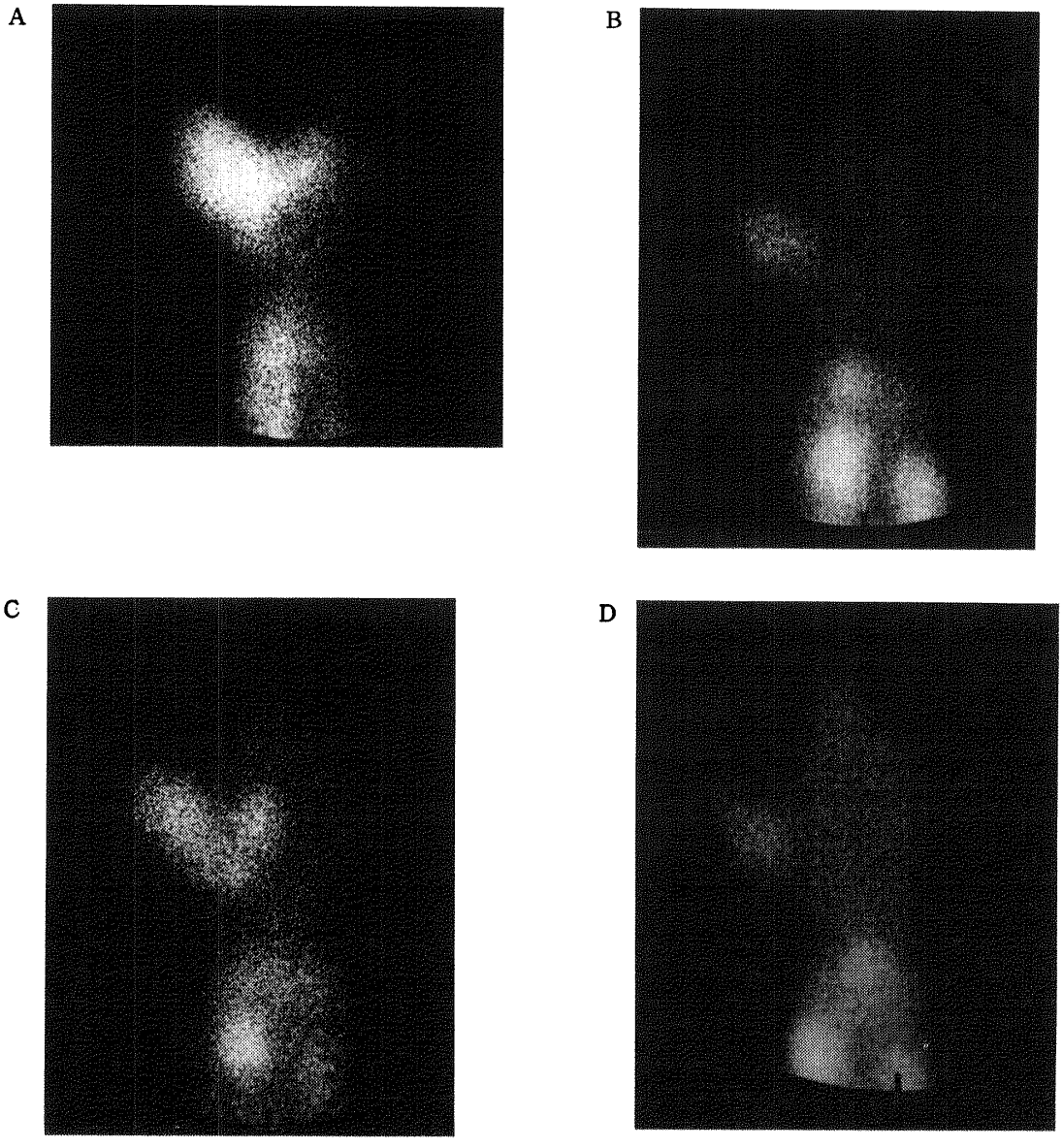
All ligands listed in Table I were labeled with ^{99m}Tc in yields consistently greater than 99% with the exception of *u*-EDDP and *sym*-EDDP. Both of them formed a colloidal precipitate

in the procedure described in the experimental section and hence were not used in the scintigraphic study.

Scintigrams of ^{99m}Tc complexes of the ligands are shown in Fig. 1. As communicated previously,[1] the tumor was very clearly visualized 2—5 h after the administration of ^{99m}Tc u-EDDA. The tumor was also clearly visualized by ^{99m}Tc complexes of *sym*-EDDA and HIDA. These two ^{99m}Tc-labeled compounds might thus be useful as new radiopharmaceuticals for tumor scintigraphy. It was noted in the sequential scintigrams that hepatic uptake was somewhat higher with ^{99m}Tc *sym*-EDDA and the radioactivity in blood was higher with ^{99m}Tc HIDA than with ^{99m}Tc *u*-EDDA. These results suggest that ^{99m}Tc *u*-EDDA is superior to the other two ^{99m}Tc complexes as a tumor-visualizing agent.

In the scintigram of ^{99m}Tc PDDA, the tumor was visualized fairly well. The blood clearance of the radioactivity was much slower than in the case of ^{99m}Tc *u*-EDDA. This resulted in greater background activity and a slightly obscured image of the tumor.

IDA, MeIDA, NTA, EDTA, and HEDTA formed quite stable complexes with ^{99m}Tc. These complexes did not accumulate in any specific organ and were excreted slowly by the kidney. In the scintigrams taken several hours after administration of these complexes, the tumor was slightly visualized and high radioactivity was found in the kidneys. Nearly the



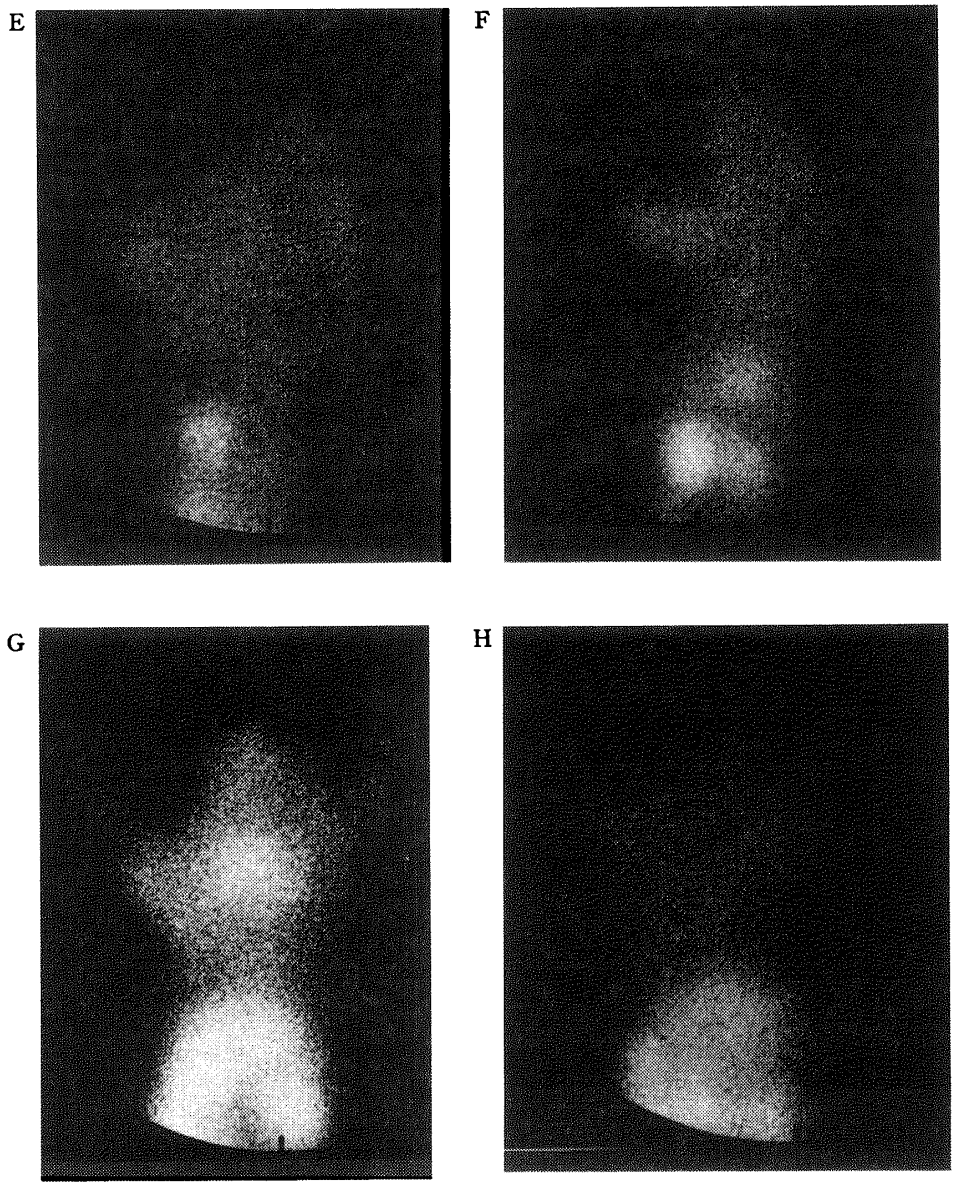


Fig. 1. Scintigrams obtained with ^{99m}Tc-Labeled Ligands administered to Mice bearing intramuscularly Transplanted Ehrlich Tumor at the Right Foreleg (Anterior Projection)

Ligands and times after administration are: A, *u*-EDDA, 3.0 h; B, *sym*-EDDA, 3.0 h; C, HIDA, 3.0 h; D, PDDA, 3.0 h; E, EDTA, 3.5 h; F, HEDTA, 1.5 h; G, trien, 3.0 h; H, Ac-EDDA, 3.0 h.

same scintigrams were obtained with ^{99m}Tc HzDA, although blood clearance of the radioactivity was much slower with this compound.

In the scintigrams with ^{99m}Tc taurine-DA and ^{99m}Tc trien, the thyroid gland and stomach were visualized and the tumor was not. This suggests that these complexes were not very stable *in vivo* and ^{99m}Tc-pertechnetate was liberated.

Under acidic conditions, *u*-EDDA is readily dehydrated intramolecularly to form *cyclic*-EDDA. The tumor was slightly visualized by ^{99m}Tc *cyclic*-EDDA. Contamination of *u*-EDDA by *cyclic*-EDDA may cause the obscurity and poor reproducibility in the scintigrams of ^{99m}Tc *u*-EDDA.

In the scintigrams of ^{99m}Tc complexes of Ac–EDDA and *z*-EDDA, the tumor was not visualized. The acylation of the amino group of *u*-EDDA resulted in loss of the affinity to the tumor of ^{99m}Tc complexes. ^{99m}Tc Ac–EDDA was excreted mainly by the kidney, as were most of the ^{99m}Tc complexes in the present study. On the other hand, considerable hepato-

biliary excretion was noted with ^{99m}Tc Z-EDDA. ^{99m}Tc-labeled *N'*-*p*-toluenesulfonyl-ethylenediamine-*N*,*N*-diacetic acid has been proved to be an excellent hepatobiliary tracer.[7] Increase of lipophilicity of the ligand causes an increased hepatobiliary excretion and hence is not favorable for tumor scintigraphy.

Among the ^{99m}Tc complexes of seventeen ligands studied, those of *u*-EDDA, *sym*-EDDA, HIDA, and PDDA achieved clear visualization of the intramuscularly transplanted Ehrlich tumor. These complexes did not accumulate in any specific organ and were excreted by the kidney. These properties may be attributed to the hydrophilic nature of the ligands and to appropriate rates of blood clearance. ^{99m}Tc complexes of well-known complexanes such as IDA, NTA, and EDTA did not accumulate in the tumor.

A hydrophilic functional group seems to be indispensable for the present purpose, in addition to the chelating groups.

References and Notes

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