Notes

Tissue Distribution of Technetium-99m and Carbon-14 Labeled *N*-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic Acid

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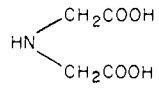
The synthesis, radiochemical labeling, and tissue distribution characteristics of *N*-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid are described. The radiopharmaceutical prepared by labeling with ^{99m}Tc was rapidly eliminated through the hepato-biliary system of mice. Parent ^{14}C compound was eliminated primarily through the kidney. The ^{99m}Tc ion appears to have a greater influence than the organic carrier molecule on the distribution of the radiopharmaceutical.

Numerous radiopharmaceutical agents composed of radioactive metal ions bound to chelating compounds have been developed as organ-imaging agents in nuclear medicine. Generally, these agents consist of a combination of γ-emitting radionuclidic cations with existing organic compounds which have established complexing properties. For example, the tetracyclines,[1] DTPA,[2] and other compounds[3] have been chelated or complexed with reduced technetium-99m to form radiopharmaceuticals. Sundberg et al.[4] have coupled an EDTA derivative to proteins for the purpose of binding radioactive metals to a macromolecule-bound chelating group.

Incorporation of a chelating moiety into a relatively low-molecular-weight drug analogue may create an agent capable of binding with technetium-99m while retaining the biological actions and tissue distribution characteristics of the parent drug. Iminodiacetic acid (1) is a metal



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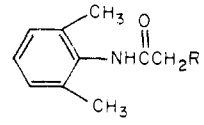

2, R = $N(CH_2CH_3)_2$
3, R = $N(CH_2COOH)_2$
4, R = Cl
5, R = NH_2

complexing moiety which strongly binds transition metals[5] and can be readily incorporated into organic molecules. We wish to report the synthesis and biological distribution of an iminodiacetic acid containing radiopharmaceutical agent which is structurally related to the antiarrhythmic drug lidocaine (2).[6]

Results and Discussion

Substitution of the diethylamino group of 2 with iminodiacetic acid produced the drug–chelate analogue 3 which is capable of complexing with ^{99m}Tc and other organ-imaging radionuclides. Carrier compound 3 was synthesized by displacement of the halogen atom of the chloroacetyl compound 4 with the disodium salt of iminodiacetic acid (1). A complex between reduced technetium, prepared by stannous reduction of TcO_4^-, and 3 was formed under acidic conditions. Paper chromatography with physiological saline and paper electrophoresis of the Tc-3 chelate showed the absence of radioactive bands associated with pertechnetate or technetium–tin colloid. While the exact radiochemical structure of Tc-3 remains to be determined, the paper electrophoretic data, which shows a single sharp peak with a migration distance intermediate between that of colloid and pertechnetate, suggests that Tc-3 exists in one discrete radiochemical form.

The tissue distribution of Tc-3 in mice was determined at various time intervals following intravenous administration. Table I shows the percent injected dose of radioactivity in blood, liver, intestine, and kidney. The technetium was rapidly extracted from the blood by the liver and was ultimately eliminated through the hepatobiliary system. After 60 min, a large portion of the dose (76%) was accounted for in the liver, gall bladder, and intestine. Urinary excretion of label was found to be a minor pathway for the elimination of Tc-3 in mice and dogs.[7]

The dissimilarity in tissue distribution and blood concentrations of Tc-3 compared to literature values for [^{14}C]lidocaine[8] led to studies on the influence of the metal ion on the disposition of Tc-3. A comparison of the distribution of 3 with and without the technetium label was undertaken with the aid of [^{14}C]-3. The ^{14}C-label introduced into 3 was located in the methylene groups of the iminodiacetic acid moiety and was produced by an alternate pathway to 3 (see Experimental Section, method B). Amino compound 5, prepared by the action of ammonia on 4, was treated with chloroacetic acid-^{14}C to produce [^{14}C]-3 with a specific activity of 1.12 μCi/mg. Tissue distribution determinations for [^{14}C]-3 in mice were carried out by a similar procedure using the same milligram quantity as in the Tc-3 experiments. Clearance of [^{14}C]-3 from the blood was as rapid as Tc-3 blood clearance.

In Table I is shown the percent ^{14}C dose in blood, liver, intestine, and kidney. A comparison of the major routes of elimination of Tc-3 and [^{14}C]-3 in mice is shown in Figure 1. The main elimination pathway of radioactivity after administration of [^{14}C]-3 was via the kidney, which is more in keeping with the urinary elimination of lidocaine[9] and its metabolites[10] than with the hepatobiliary fate of Tc-3. At 60 min after injection, most (74%) of the ^{14}C dose was accounted for in the urine with less than 2% found in the liver or intestines.

Evidence supporting that a Tc-3 complex was being monitored and that extensive dissociation in vivo of Tc-3 to colloidal technetium or pertechnetate had not occurred was gained by a comparison of the distribution of Tc-3 to

Table I. Percent Injected Radioactivity at Various Times in Tissues of Mice Following Intravenous Administration of Tc-3 and [^{14}C]-3[a]

	Time, min	Blood	Liver	Intestine	Kidney	Urine
Tc-3	5	3.3[b] (2.6-3.8[c])	28.2 (21.3-44.2)	38.3 (27.8-46.3)	1.8 (1.1-3.0)	
	30	1.1 (0.6-1.8)	10.2 (3.4-14.7)	60.2 (47.6-70.1)	0.6 (0.2-1.0)	
	60	0.8 (0.3-1.4)	8.0 (0.9-19.2)	68.3 (54.1-73.8)	0.8 (0.6-1.3)	
[^{14}C]-3	5	7.9[b] (6.8, 8.9[d])	5.7 (6.1, 5.2)	3.8 (4.0, 3.6)	7.5 (7.6, 7.4)	31.9 (34.6, 29.3)
	30	0.5 (0.3, 0.7)	1.3 (1.0, 1.6)	1.8 (1.5, 2.1)	1.0 (0.3, 1.8)	74.2 (61.0-82.7[c])
	60	0.2 (0.1, 0.2)	0.4 (0.4, 0.5)	1.4 (1.5, 1.3)	1.7 (3.3, 0.2)	74.0 (58.9-82.8[c])

[a] Mice were injected in the tail vein with Tc-3 (ca. 0.5 μCi) or [^{14}C]-3 (2.23 μCi). Radioactivity in lung, spleen, or heart was less than 1% of the injected dose. In the Tc experiments, less than 2% of the dose was accounted for in the stomach. [b] Represents percent dose average value. [c] Represents range of four mice. [d] Represents mouse 1 and 2, respectively.

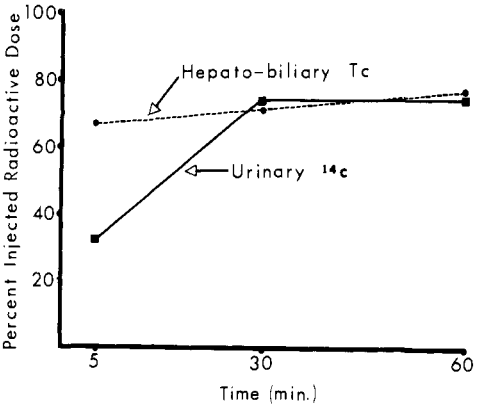

Figure 1. Major pathways of elimination of radioactivity in mice following intravenous injection of Tc-3 and [^{14}C]-3. Each value is in terms of the total injected radioactive dose and represents an average of at least two mice. The hepato-biliary clearance of Tc (•- - -•) consists of the combined counts found in liver, gall bladder, and intestines. The urinary excretion of ^{14}C (▪——▪) includes the urinary bladder and contents.

the literature values for colloidal technetium and pertechnetate distribution. The absence of significant quantities of radioactivity in the stomach and spleen indicates that the distribution of Tc-3 is different from the distribution of either pertechnetate[11] or colloidal technetium.[12] Furthermore, the short transit time of Tc-3 through the liver is inconsistent with the biological distribution of technetium–tin colloid which is known to have a biological half-life in the liver in excess of 2 days.[12] This suggests that the technetium remains in chelate form during its course through the animal. Additionally, the chromatographic and tissue distribution characteristics of the technetium eliminated in the bile 1 h after Tc-3 administration were evaluated as a measure of the in vivo stability of Tc-3. Paper chromatography of the radioactive biliary contents in saline produced a chromatogram identical with that of Tc-3 before injection. Tissue distribution studies obtained from reinjection of the radioactive biliary contents into a second set of mice showed a pattern well correlated with Tc-3 distribution.[7] The distribution pattern of Tc-3 along with the properties of the technetium excreted in the bile after Tc-3 administration strongly suggests that the Tc-3 chelate remains intact in the animal and is eliminated in an unchanged form. The possibility of metabolism of Tc-3 to Tc-labeled metabolites of 3 which undergo biliary excretion has not been studied further.

The difference in fate of Tc-3 compared to [^{14}C]-3 may be related to the formation of the radioactive metal–3 chelate. Although the effect of the technetium atom on the electronic and spatial characteristics of 3 and the possibility of dimer or trimer formation have yet to be studied, it seems reasonable that, in the chelate, donation of the nitrogen unshared electron pair to the electropositive technetium atom is likely. The resulting partially positive iminodiacetic acid nitrogen or the positive character of the reduced technetium may possess properties similar to a cationic quaternary nitrogen and may play a role in the rapid hepato-biliary excretion of Tc-3. A relatively nonspecific active transport system which is well described for the excretion of cations[13] may be responsible for the biliary elimination of a number of quaternary ammonium antiarrhythmic compounds.[14] Tc-3 may be eliminated in the bile by such a transport process while [^{14}C]-3 follows the usual urinary pathway for the excretion of polar substances.

Knowledge of the structural features which influence biological disposition is essential in the design of organ-imaging radiopharmaceuticals.[15] By radiolabeling the carrier molecule 3 and preparing the Tc-3 chelate for distribution studies, we have shown that the fate of 3 is dramatically different from the disposition of Tc-3. The strong influence of the radiochemical ion on the target organ specificity of Tc-3 should be taken into account in the design of radiopharmaceuticals containing an iminodiacetic acid moiety.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. ^{1}H NMR spectra were obtained on a Jeolco C-60HL NMR spectrometer and the chemical shifts are expressed in parts per million relative to Me_4Si or sodium 2,2-dimethyl-2-silapentane-5-sulfonate. Electron impact mass spectra were obtained with a Du Pont 21-490 mass spectrometer operating at 70 eV. A Beckman paper electrophoresis cell (Durram Type) Model R-Series D system was used for paper electrophoresis. Chromatograms were scanned with a Packard Model 385 radiochromatogram scanner. Liquid scintillation counting and γ counting of samples were performed on a Packard Tri-Carb Model 2002 spectrometer.

ω-Chloro-2,6-dimethylacetanilide (4). Following the procedure of Lofgren,[16] 2,6-dimethylaniline (4.85 g, 40.0 mmol) in glacial HOAc (30 ml) was treated with chloroacetyl chloride (4.97 g, 44.0 mmol) to yield 6.1 g (77%) of pure 4: mp 147–149° (lit.[17] mp 145–146°); ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6, CH_3), 4.18 (s, 2, CH_2Cl), and 7.10 ppm (s, 3, aromatic protons); mass spectrum *m/e* (rel intensity) 197 (55, M^+), 148 (100, $M^+ - CH_2Cl$).

ω-Amino-2,6-dimethylacetanilide Hydrochloride (5·HCl). A solution of 4 (4.4 g, 22.0 mmol) in 135 ml of absolute EtOH saturated with NH_3 was stirred at room temperature for 3 days. The mixture was evaporated to dryness and the residue was dissolved in 0.1 N HCl (50 ml) and then extracted with two 50-ml portions of $CHCl_3$. The aqueous layer was adjusted to pH 10 with 1 N NaOH and extracted with $CHCl_3$ (5 × 50 ml) to yield, after evaporation of the combined $CHCl_3$ layers, crude 5 (1.1 g, 28%, mp 78–80°). The HCl salt of 5 was prepared by bubbling HCl gas through an EtOH–Et_2O solution of 5. Recrystallization from EtOH gave an analytical sample: mp 302° dec; ^{1}H NMR (Me_2SO-d_6) δ 2.24 (s, 6, CH_3), 3.92 (s, 2, CH_2), and 7.22 ppm (s,

3, aromatic protons); mass spectrum *m/e* (rel intensity) 178 (100, M^+). Anal. ($C_{10}H_{15}N_2OCl$) C, H, N.

***N*-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic Acid (3). Method A.** A solution of 4 (2.0 g, 10.0 mmol) and disodium iminodiacetic acid (1.8 g, 10.0 mmol) in EtOH–H_2O (3:1) was held at reflux for 48 h. The mixture was cooled and then evaporated to dryness to leave a yellow solid. The residue in 25 ml of H_2O was extracted with Et_2O (3 × 25 ml). The pH of the aqueous layer was then adjusted to 3.2 with concentrated HCl and the precipitate which formed on cooling was recrystallized from H_2O to give 0.5 g (20%) of **3**: mp 215–216°; ^1H NMR (Me_2SO-d_6) δ 2.20 (s, 6, CH_3), 3.57 (s, 2, $NCOCH_2N$), 3.63 (s, 4, CH_2COO), 7.19 ppm (s, 3, aromatic protons). Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

Method B. A solution of **5** (1.1 g, 5.0 mmol) in 95% EtOH (50 ml) was added dropwise to a stirred solution of chloroacetic acid (0.95 g, 10.0 mmol) in 95% EtOH (50 ml). During the addition, the pH of the mixture was maintained above 7 with 4 N NaOH. The resulting mixture was heated to reflux for 24 h. The solvents were evaporated and the residue was recrystallized from H_2O buffered to pH 3 to give the final product (0.32 g, 25%) which gave ir and NMR spectra and melting point identical with a sample of **3** produced by method A. Anal. ($C_{14}H_{18}N_2O_5$) C, H, N.

***N*-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic Acid-*methylene*-^{14}C ([^{14}C]-3).** Following the procedure of method B, a solution of **5** (147 mg, 0.69 mmol) in 95% EtOH (15 ml) was allowed to react with chloroacetic acid-α-^{14}C (250 μCi/mg, Amersham Searle) diluted with carrier chloroacetic acid (147 mg, 1.5 mmol). The crude product was recrystallized from H_2O to give 104 mg (51%) of [^{14}C]-**3**: mp 185–190°; 1.12 μCi/mg; chemical and radiochemical purity was confirmed by TLC on silica gel with BuOH–HOAc–H_2O (4:1:1) and $CHCl_3$–EtOH (1:1).

***N*-(2,6-Dimethylphenylcarbamoylmethyl)iminodiacetic Acid ^{99m}Tc Chelate (Tc-3).** A solution of **3** (0.15 g, 0.51 mmol) in 3 ml of 0.1 N NaOH was adjusted to pH 3.5 with 1 N HCl. To this mixture was added 0.3 ml of a 1 N HCl solution containing $SnCl_2$ (0.6 mg, 3.3 μmol) while maintaining a pH of 3.5 by the dropwise addition of 1 N NaOH. After 5 min at room temperature, 5–50 μCi of $^{99m}TcO_4^-$ was added.

Radiochemical Purity. The radiochemical purity of Tc-3 was examined by ascending paper chromatography in physiological saline and by paper electrophoresis for 1.5 h at a constant voltage of 300 V in a 0.05 M phosphate buffer, pH 6.8. The radiochromatograms of Tc-3 were compared with those found for both the pertechnetate anion and for technetium–tin colloid prepared by the method of Lin and Winchell[12] and by the method used to prepare Tc-3 except that **3** was omitted from the reaction mixture. The two colloids appeared indistinguishable under both analytical systems. Paper chromatography yielded R_f values of 0.0, 0.74, and 0.95–1.0 for technetium–tin colloid, pertechnetate, and Tc-3, respectively. When subjected to paper electrophoresis, technetium–tin colloid, pertechnetate, and Tc-3 were observed to migrate 0.0, 7.3, and 3.2 cm, respectively. Less than 0.1% of Tc-3 was present as either technetium–tin colloid or as pertechnetate. Greater than 95% of the radioactivity was recovered after filtration of the Tc-3 solution through a 0.22-μ membrane filter (Millipore Corp.). Radiochemical analysis as above of the filtrate was identical with unfiltered Tc-3.

Tissue Distribution Studies. Male Swiss–Webster mice (20–25 g) were administered [^{14}C]-3 or Tc-3 intravenously in the tail vein. Each mouse received a dose of 2.0 mg (4.96×10^6 dpm) of [^{14}C]-3 in 0.25 ml of distilled H_2O adjusted to pH 5.7 with 1 N NaOH or 0.1–1.0 μCi of Tc-3 in 0.1 ml of the pH 3.5 $SnCl_2$ solution described above. Liver, lung, kidneys, spleen, heart, intestines, tail, and urine were counted at 5, 30, and 60 min after injection. In the ^{14}C experiments, mice were clamped to prevent urination, and urinary bladders plus contents were counted. Counts in the tail in all cases were found to be less than 5% of the injected dose and were subtracted from the total dose to obtain the total injected dose. Organ and blood samples in the Tc experiments were counted in test tubes. For ^{14}C samples lung, kidney, spleen, and heart (100–200 mg) were solubilized with 2 ml of NCS (Amersham Searle) and 2 drops of H_2O in liquid scintillation vials with plastic tops by heating to 50° in a water bath overnight. The resulting solutions were counted in 13 ml of a toluene cocktail [0.2 mM POPOP (Packard) and 27 mM PPO (Packard) in reagent grade toluene]. All ^{14}C values were corrected for efficiency using a toluene-^{14}C internal standard. Blood was collected at the time of sacrifice using a 200-μl capillary tube. A syringe containing NCS (2.0 ml) was used to wash the blood from the tube into a scintillation vial. After heating to 50° in a water bath overnight, 13 ml of cocktail was added and the samples were bleached with 0.6 ml of a 0.8 M solution of benzoyl peroxide in toluene. Additional NCS was sometimes necessary to afford a clear solution. Lung and spleen samples were bleached with 0.3 ml of benzoyl peroxide solution. Liver and intestine samples were homogenized in a 4:1 water to organ mixture with a Potter-Elvehjem tissue homogenizer and 0.3-ml aliquots were solubilized for counting. The urinary bladders containing urine were solubilized with NCS and aliquots were counted.

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