Skeletal Localization of Samarium-153 Chelates: Potential Therapeutic Bone Agents

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A series of stable complexes of ^{153}Sm has been produced using multidentate acetate and phosphonate ligands. Biodistribution studies in unanesthetized rats showed varying degrees of bone and soft-tissue uptake for these complexes. Of the complexes studied, [^{153}Sm] ethylenediaminetetramethylenephosphonate (EDTMP) showed the best combination of high bone uptake, low nonosseous uptake, and rapid blood clearance which warranted its further investigation in rabbits. Rabbit studies confirmed the [^{153}Sm]EDTMP results obtained in rats. Blood clearance in rabbits was found to be more rapid than [^{99m}Tc] methylene diphosphonate (MDP). Scintigraphic images were virtually indistinguishable from [^{99m}Tc]MDP images. Lesion/normal bone ratios were determined from digitized images obtained using a drill hole model and found to be ~17:1. Based on these excellent biodistribution characteristics, [^{153}Sm] EDTMP could be therapeutically useful in treating metastatic bone cancer.

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Half of the people with breast, lung, and prostate cancer will develop bone metastases sometime during the course of their disease (*1*). Bone pain is a frequent complication and its relief often creates a therapeutic dilemma for the attending physician. Although various methods of analgesia and external beam radiation are the initial approach to therapy, systemic treatment with radionuclides such as phosphorus-32 and more recently strontium-89 has been used with inconsistent results, the most adverse being bone marrow suppression (*2,3*). Rhenium-186 (^{186}Re) complexed to diphosphonate ligands has also been suggested for use as a potential therapeutic agent (*4*). Complexation of ^{186}Re with diphosphonate ligands in aqueous solution produces multiple chelate entities which have less than desirable stability characteristics. We concluded that multidentate aminophosphonate ligands (MAPLs) should form more stable chelates with fewer structural forms than diphosphonate complexes.

Samarium-153 was considered a promising beta-emitting radionuclide for complexation with MAPLs because of its desirable physical characteristics and ready availability. Samarium-152 is an inexpensive lanthanide with a high thermal neutron capture cross-section (204b). Samarium-153 has 810 (20%), 710 (50%), and 640 (30%) keV beta particle emissions (B^-(avg) = 0.29 MeV), a 103-keV gamma-ray emission (28%), 55 keV-conversion electrons (41%), and a half-life of 46.8 hr (*5*). This short half-life allows for the delivery of fractionated dose regimes while the 103-keV gamma ray is ideal for following the in vivo deposition of the chelate via conventional gamma-ray scintigraphy.

Recently we demonstrated that stable ^{153}Sm chelates could be readily produced using phosphonate ligands (*6*). The assessment of their biologic characteristics has now been used to determine which of the chelates deserves further investigation as a potential therapeutic agent.

MATERIALS AND METHODS

Ligands

The following ligands were used for chelation of ^{153}Sm: nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), nitrilotrimethylenephosphonic acid (NTMP), ethylenediaminetetramethylenephosphonic acid (EDTMP), diethylenetriaminepentamethylenephosphonic acid (DTPMP), hydroxyethylethylenediaminetriacetic acid (HEDTA), hydroxyethylethylenediaminetrimethylenephosphonic acid (HEDTMP), bis-(aminomethyl) - dicyclopentadienyltetramethylenephos-

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phonic acid (DCPDTP), bis-(aminomethyl)-norbornyltetramethylenephosphonic acid (NBTP), methylene diphosphonic acid (MDP) hydroxymethane diphosphonic acid (HDP), N, N-dimethylaminomethylene diphosphonic acid (DMAD), 2,3-dicarboxypropane-1-hydroxy-1,1-diphosphonic acid (HEDP).

Preparation of Complexes

Sm_2O_3 was obtained as 99.06% enriched $^{152}Sm_2O_3$. The ^{153}Sm was prepared by neutron irradiation in the University of Missouri Research Reactor using a thermal flux of 8.5×10^{13} n/cm^2 sec and a resonance flux of 1.7×10^{12} n/cm^2 sec. A weighed amount of Sm_2O_3 was flame sealed into a quartz vial under vacuum and welded into an aluminum can. Following irradiation the sample was opened, dissolved in 1–4 N HCl and brought to a stock concentration of approximately $1.2 \times 10^{-3}M$ with deionized water. When necessary, a carrier solution of the same molarity was added, to attain the desired specific activity. The Dow Chemical Company provided all the ligands except the methylenediphosphonate (MDP) used for technetium-99m (^{99m}Tc) complexes, which was purchased commercially as a kit.† Stannous pyrophosphate (PYP) was also purchased commercially.‡ The structures of the multidentate aminophosphonate ligands are shown in Figure 1.

To form each samarium-153 (^{153}Sm) complex 20–85 mg/ml of ligand were used. The amount of ligand needed to achieve quantitative complex formation (*6*) was first dissolved in deionized water followed by the addition of concentrated base. The ^{153}Sm stock and carrier solutions were added so that the final samarium concentration was $\sim 3 \times 10^{-4}M$ with a specific activity of 1 to 10 mCi/ml (37–370 MBq/ml). The pH was adjusted to >10 and the solution heated to 60°C for ~30 min to facilitate complexation. After heating, the pH was adjusted to 7.0 with 4–5*M* HCl.

Complex yields were determined by separating the complexed metal and uncomplexed ^{153}Sm species on a 0.5-ml C25 Sephadex cationic exchange column. The ionic samarium (Sm^{+3}) and insoluble $Sm(OH)_3$ were retained on the column while the anionic complex was eluted with two 9-ml volumes of isotonic saline.

The elutions and columns were counted using the 103-keV gamma photon of ^{153}Sm and the complex yield (% complex) was obtained from the formula:

$$\% \text{ Complex} = 100 \times \left(1 - \frac{\text{column counts}}{\text{total counts}}\right).$$

Samples were counted in a NaI(Tl) well counter and corrected for background. All of the complex yields were >98% except [^{153}Sm]NBTP (95.8%), [^{153}Sm]MDP (97%) and [^{153}Sm]HDP (93.2%). Their specific activities ranged from 1–10 mCi/ml (37-370 MBq/ml).

Rats

Two-hour biodistribution study in rats. Biodistribution of the agents was performed in 160–220g male Sprague Dawley rats. Fifty to one hundred microliters of the ligand complex were injected into the tail veins of unanesthetized rats and each rat was housed individually in a cage for 2 hr. At 2 hr postinjection the animals were killed. One-milliliter samples of blood were taken by cardiac puncture and weighed. The whole animals were then weighed and dissected. The excised tissues were washed with normal saline, blotted, and weighed prior to counting. Cage droppings and kill papers were collected and counted with the bladder to quantify the urine activity. One femur was excised and dissected free of the soft tissues before weighing and counting.

All tissues remaining after the dissection were counted and marked as carcass. The samples were counted using an inverted NaI(Tl) thyroid detector/scaler.

Long-term biodistribution of [^{153}Sm]EDTMP in rats. Samarium-153 EDTMP was prepared as previously described using a ligand concentration of $8 \times 10^{-2}M$ and a samarium concentration of $3 \times 10^{-4}M$, the complex yield was >99%. Fifty microliters of the complex were injected into the tail veins of male Sprague-Dawley rats. Each rat was housed in a metabolic cage equipped to collect urine. They were killed in groups of five by cervical dislocation at 15 min, 30 min, 1 hr, 2 hr, 5 hr, 24 hr, 48 hr, and 72 hr postinjection. A 1-ml sample of blood was drawn from the heart and weighed immediately after killing. The whole animal was then weighed and subsequently dissected with special care being taken to separate the blood and urine on the kill papers, the tissue washings and the urine collected from the cages. All tissues were washed with isotonic saline, blotted, weighed, and counted. Animals maintained for 24 hr or more were provided with ad lib standard rat chow and water.

Counting was performed as previously described for all tissues except blood and muscle in the longer time period groups which were counted in a NaI(Tl) well detector and compared with diluted standards.

Calculations. Calculated values of the % dose and % dose/

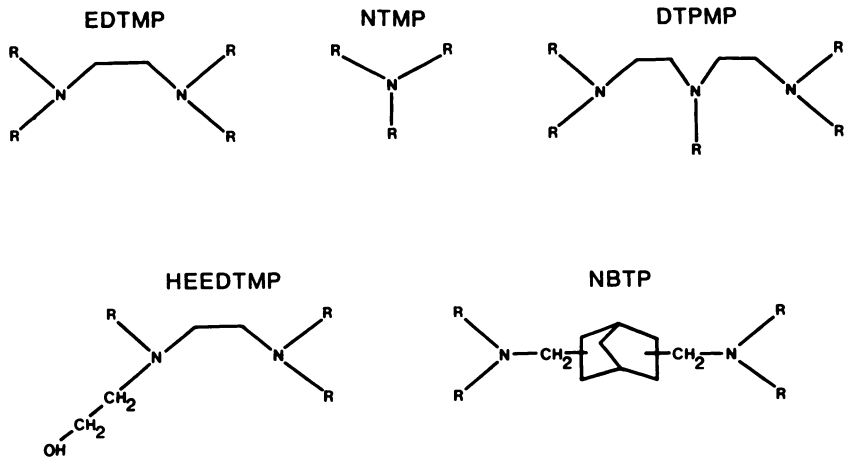

FIGURE 1
Structures of the aminomethylene phosphonate ligands used in this study. R = $CH_2PO_3H_2$

g of the tissues were derived from the total organ counts minus the injection site counts minus the background counts. Values for the samples counted in the well counter were determined by using the average value of triplicate injection standards and employing a correction for the percent recovered. Bone/blood and bone/muscle ratios were determined from the percent dose/g values of those organs.

Rabbits

Three-hour biodistribution study in rabbits. The biodistribution of [^{153}Sm]EDTMP was determined in 2.35 ± 0.14 kg male New Zealand white rabbits. EDTMP was complexed to the ^{153}Sm by the method described earlier yielding a complex with a specific activity ranging from 1–10 mCi/ml (37–370 MBq/ml). A separate group of animals (2.62 ± 0.14 kg) was injected with [^{99m}Tc]MDP prepared from a commercial kit[§] with a specific activity of ~10 mCi/ml (370 MBq/ml). A cannula inserted through a marginal ear vein and flushed with heparinized isotonic saline was injected with 50–100 μl of the [^{99m}Tc]MDP. The cannula was then removed and counted. The measured activity was compared to the standards.

All of the animals were placed in individual cages, equipped for urine collection, for 3 hr. At 3 hr a 2-ml blood sample was removed by cardiac puncture and the animals were killed.

Urine in the intact bladder was removed en block for counting along with the voided urine. The whole liver, kidneys, and a sample of muscle were taken, washed with isotonic saline, blotted, and weighed. Sections of the liver removed from each lobe were weighed. A sample of bone marrow was carefully dissected from the femoral shaft and weighed as was the stripped femur from the contralateral limb.

Except for the bone marrow, all samples were counted with an inverted NaI(Tl) detector and values for the % dose and % dose/g were calculated from the injected counts. The bone marrow samples were counted in a well counter and the results were compared with a diluted standard and used to calculate the % dose and the % dose/g.

Blood clearance. Blood clearance was determined in five New Zealand white rabbits. One hundred to two hundred microliters of either [^{153}Sm]EDTMP, [^{99m}Tc]MDP or [^{99m}Tc] PYP were injected into the rabbits ear vein and 1-ml samples of blood were intermittently withdrawn from a cannula inserted in the vein of the opposite ear for the next 3 hr. The blood samples were counted in a well counter and the % administered doses were calculated.

Lesion/normal uptake. Bone uptake of [^{153}Sm]EDTMP was compared with [^{99m}Tc]MDP using a modification of Subramanian's drill hole model technique (*7*). Under pentobarbital anesthesia a drill hole was made in the proximal tibia of five male New Zealand white rabbits (2.7–3.2 kg) and the lesions were allowed to heal for 9–11 days. The [^{99m}Tc]MDP was injected into the ear vein and 2 hr and 40 min later the animals were anesthetized (using Ketalar and Rhompin). At 3 hr postinjection the drilled tibia was imaged using an Anger camera equipped with a pinhole collimator. The image was digitized and stored in the interfaced PDP 11/40 computer for analysis. The procedure was repeated the following day using 200–250 μl of [^{153}Sm]EDTMP (0.5–1 mCi/ml) (18.5–37 MBq/ml) with the spectrometer window adjusted for the 103-keV gamma photon of ^{153}Sm. The percentage of ^{99m}Tc activity remaining in the ^{153}Sm window was <0.6%. The two digitized images were then analyzed for their relative uptake of radioactivity in the lesion versus the nonlesion areas of the tibia. Lesion/normal (L/N) ratios for each agent were then calculated and compared in the same lesion.

RESULTS

Preparation of Complexes

With the concentration of ligand used in these studies, complex yields of the ^{153}Sm chelates were >98%, with only three exceptions, and all were easily achieved at alkaline pH (>10). Before the chelates were injected into the animals their pH was lowered to ~7 with no detectable complex dissociation. Table 1 includes the uptake (% ID) of the various ^{153}Sm chelates in the organs of unanesthetized rats 2 hr after i.v. injection. Corresponding values of the % ID/g body wt. are presented in Table 2. Each of the multidentate chelates of ^{153}Sm localize differently than unchelated ionic ^{153}Sm.

Diphosphonate ligands that form ^{99m}Tc complexes and exhibit excellent skeletal imaging properties were also complexed to ^{153}Sm and studied in rats. At pH 10 ^{153}Sm formed complexes with five diphosphonates (HEDP, MDP, HDP, DPD, and DMAD) in yields >95%. Each complex remained stable when the chelate solutions were reduced to pH 7 except [^{153}Sm]HDP which demonstrated only 93% complex yield at the lower pH.

Rats

Two-hour biodistribution study in rats. Samarium-153 HEDP and [^{153}Sm]DMAD were the two diphosphonate chelates that demonstrated significant bone localization (22.1 ± 1.4% and 32.0 ± 4.2%, respectively), however, both showed high blood and muscle activity 2 hr postinjection (Table 3). The ^{153}Sm complexed to the other three diphosphonates showed >75% liver uptake and <2.5% skeletal uptake. Ionic ^{153}Sm also showed high liver uptake (71.6 ± 5.4%) with 10.5 ± 1.9% localized in bone.

Four of the multidentate phosphonate ligands (NTMP, EDTMP, HEEDTMP, and NBTP) chelated ^{153}Sm and demonstrated high skeletal uptake with low blood concentration at 2 hr postinjection in rats. Samarium-153 DTPMP had 29.9 ± 9.9% of the ID in bone with the rest appearaing in the urine (i.e., 73.7 ± 8.2%) at 2 hr postinjection. The skeletal uptake of [^{153}Sm]NTMP, -EDTMP and -NBTP were all >55% (Table 1). The % ID of [^{153}Sm]NBTP and -NTMP were 5.46 ± 0.80%[¶] and 4.10 ± 0.7%, respectively, in the liver compared with 0.277 ± 0.066% ID for the [^{153}Sm] EDTMP.

Localization of [^{153}Sm]EDTMP in nonosseous tissues was also low. The bone/blood and bone/muscle ratios of [^{153}Sm]EDTMP were 1,843 ± 1274 and 1,459 ± 505, respectively. Both of these uptake ratios of [^{153}Sm] EDTMP were higher than those observed with [^{99m}Tc] MDP (Table 2).

TABLE 1
Uptake (% ID) of ^{153}Sm Multidentate Chelates in Rats* 2 hr Following i.v. Injection

	% Injected dose/organ†											
Complex	$^{153}Sm^{+3}$‡	[^{153}Sm]NBTP	[^{153}Sm]DCPDTP	[^{153}Sm]NTA*	[^{153}Sm]EDTA	[^{153}Sm]HEDTA*	[^{153}Sm]DTPA	[^{153}Sm]NTMP	[^{153}Sm]EDTMP	[^{153}Sm]DTPMP	[^{153}Sm]HEEDTMP	[^{99m}TC]MDP
Organ												
Blood	1.38	0.363	0.055	0.641	0.383	0.076	0.191	0.901	0.032	0.158	0.035	0.590
	0.382	0.047	0.040	0.266	0.034	0.024	0.051	0.133	0.016	0.117	0.006	0.245
Heart	0.116	0.046	0.032	0.117	0.049	0.020	0.019	0.052	0.010	0.012	0.008	0.015
	0.029	0.030	0.007	0.058	0.016	0.006	0.026	0.016	0.009	0.009	0.010	0.006
Lung	8.84	0.303	1.53	0.265	0.131	0.041	0.039	0.177	0.021	0.021	0.022	0.045
	3.50	0.101	0.475	0.112	0.041	0.008	0.025	0.021	0.007	0.021	0.020	0.006
Liver	71.62	5.50	82.1	8.60	4.35	1.163	0.292	3.92	0.252	0.270	0.451	1.28
	5.39	0.896	1.31	1.81	1.38	0.253	0.121	0.673	0.038	0.127	0.072	0.557
Spleen	2.570	0.053	3.02	0.097	0.049	0.011	0.028	0.047	0.006	0.013	0.001	0.029
	1.165	0.018	0.36	0.039	0.030	0.007	0.030	0.012	0.004	0.010	0.001	0.010
Stomach	0.196	0.441	0.141	0.530	0.202	0.101	0.025	0.628	0.241	0.081	0.055	0.049
	0.096	0.180	0.059	0.093	0.058	0.035	0.024	0.279	0.158	0.087	0.019	0.017
Large intestine	0.241	0.266	0.071	0.458	0.297	0.126	0.189	0.316	0.095	0.209	0.032	0.119
	0.043	0.041	0.020	0.159	0.045	0.031	0.070	0.063	0.024	0.226	0.017	0.030
Small intestine	0.590	0.487	0.248	1.17	0.466	0.778	0.542	0.972	0.752	0.340	0.165	0.393
	0.154	0.095	0.301	0.775	0.090	0.515	0.356	0.614	0.391	0.332	0.026	0.253
Urine	2.266	37.20	4.14	32.50	51.72	53.618	95.204	38.34	49.11	73.66	0.366	47.77
	0.472	3.435	4.36	5.053	5.398	2.555	1.392	0.981	3.921	8.202	0.064	2.230
Kidney	0.808	1.00	0.335	1.22	0.754	0.550	0.564	1.07	0.254	0.445	49.966	0.575
	0.198	0.279	0.086	0.442	0.159	0.081	0.058	0.108	0.035	0.062	4.260	0.048
Muscle	1.389	3.12	0.587	2.18	1.17	0.531	1.11	2.25	0.233	0.906	0.229	1.22
	0.482	2.20	0.156	0.951	0.192	0.039	0.592	0.366	0.073	0.701	0.047	0.788
Femur	0.420	2.30	0.324	2.33	1.73	1.903	0.007	2.37	2.31	1.20	2.286	2.17
	0.077	0.159	0.033	0.198	0.243	0.128	0.003	0.065	0.162	0.395	0.296	0.095
Carcass	12.20	54.69	7.65	49.92	38.16	39.401	3.06	49.70	44.22	22.15	45.305	44.95
	1.88	10.63	0.794	2.816	5.267	1.987	1.661	1.887	3.291	5.594	4.928	1.955
Skeleton§	10.50	57.51	8.10	56.84	43.31	47.578	0.185	59.29	57.71	29.92	57.156	54.14
	1.93	3.986	0.83	4.544	6.086	3.209	0.086	1.624	4.042	9.875	7.387	2.375

* Sprague-Dawley rats, body weight = 160–220 g.
† Mean and standard deviation ($\bar{X}$/s.d.) of the % injected dose (% dose) are given; N = 5 for all values except N = 4.*
‡ ^{153}Sm injected Sm-chloride at pH 4.
§ Based on estimates assuming: blood volume = 6.5% of body wt. and skeletal dose = %ID femur × 25.

TABLE 2
Uptake/g (%ID/g) of [^{153}Sm]Multidendate Chelates in Rats* at 2 hr Following i.v. Injection

	% Injected dose/g†											
Complex	$^{153}Sm^{+3}$‡	[^{153}Sm]NBTP	[^{153}Sm]DCPDTP	[^{153}Sm]NTA*	[^{153}Sm]EDTA	[^{153}Sm]HEDTA*	[^{153}Sm]DTPA	[^{153}Sm]NTMP	[^{153}Sm]EDTMP	[^{153}Sm]DTPMP	[^{153}Sm]HEEDTMP	[^{99m}Tc]MDP
Organ												
Blood	0.116	0.029	0.004	0.054	0.035	0.006	0.016	0.081	0.002	0.014	0.003	0.047
	0.029	0.004	0.003	0.022	0.005	0.002	0.004	0.008	0.002	0.011	0.001	0.017
Heart	0.127	0.059	0.041	0.160	0.082	0.029	0.028	0.075	0.013	0.015	0.011	0.020
	0.031	0.032	0.011	0.082	0.033	0.012	0.038	0.018	0.011	0.010	0.015	0.008
Lung	7.21	0.195	0.851	0.207	0.106	0.034	0.033	0.146	0.015	0.015	0.019	0.036
	2.56	0.083	0.267	0.088	0.041	0.008	0.022	0.023	0.005	0.015	0.017	0.006
Liver	9.18	0.639	8.58	1.05	0.654	0.133	0.041	0.542	0.027	0.035	0.055	0.145
	2.06	0.066	0.589	0.297	0.207	0.046	0.019	0.068	0.005	0.022	0.009	0.064
Spleen	3.67	0.076	4.24	0.126	0.085	0.019	0.087	0.073	0.009	0.018	0.002	0.037
	1.35	0.026	1.07	0.056	0.042	0.012	0.096	0.021	0.005	0.016	0.002	0.009
Stomach	0.049	0.098	0.038	0.169	0.055	0.024	0.006	0.125	0.041	0.032	0.011	0.009
	0.008	0.024	0.020	0.086	0.025	0.009	0.004	0.059	0.029	0.045	0.004	0.003
Large intestine	0.026	0.024	0.008	0.043	0.030	0.015	0.021	0.033	0.009	0.023	0.004	0.012
	0.007	0.003	0.002	0.017	0.007	0.004	0.010	0.007	0.003	0.026	0.002	0.003
Small intestine	0.059	0.046	0.028	0.112	0.042	0.084	0.057	0.085	0.070	0.036	0.020	0.036
	0.017	0.010	0.037	0.091	0.009	0.055	0.041	0.061	0.037	0.035	0.002	0.021
Kidney	0.502	0.576	0.200	0.715	0.509	0.332	0.356	0.701	0.147	0.271	0.245	0.345
	0.097	0.156	0.055	0.243	0.136	0.045	0.072	0.099	0.022	0.026	0.045	0.021
Muscle	0.018	0.038	0.007	0.028	0.016	0.007	0.011	0.031	0.003	0.012	0.003	0.015
	0.006	0.028	0.002	0.013	0.003	0.001	0.009	0.004	0.001	0.009	0.001	0.009
Femur	0.626	3.32	0.534	3.82	2.94	3.263	0.011	3.98	3.72	1.93	3.950	3.36
	0.180	0.240	0.067	0.378	0.366	0.343	0.006	0.351	0.259	0.776	0.561	0.210
Bone/blood	5.6	118.0	163.0	86.1	84.1	556.2	0.8	49.9	1833.0	224.0	1308.01	78.0
	1.7	19.9	70.4	50.0	11.5	212.0	0.6	7.2	1274.0	153.0	159.9	23.0
Bone/muscle	38.5	117.0	79.6	171.5	184.1	487.6	1.0	133.0	1459.0	221.0	1314.6	277.0
	15.7	53.4	16.0	106.2	26.5	28.8	0.5	22.6	505.0	126.0	188.0	101.0

*Sprague-Dawley rats; body weight = 160–220g
† Mean and standard deviation (X/SD) of % injected dose/g are given; N = 5 except for N = 4.*
‡ Sm^{+3} injected as Sm chloride at pH 4.

TABLE 3
Uptake (% ID) of [^{153}Sm]Diphosphonates in Rats* at 2 hr Postinjection

	% Injected dose/organ						
Complex	[^{153}Sm] HDP	[^{153}Sm] DPD	[^{153}Sm] DMAD	[^{153}Sm] MDP	[^{153}Sm] HEDP	$^{153}Sm^{+3}$	[^{99m}Tc]MDP
Organ							
Blood†	0.083	0.254	3.007	0.226	13.12	1.38	0.590
	0.008	0.061	0.865	0.186	2.61	0.382	0.245
Heart	0.020	0.477	0.166	0.073	0.265	0.116	0.015
	0.008	0.294	0.036	0.018	0.058	0.029	0.006
Lung	0.747	1.271	0.597	2.594	0.799	8.84	0.045
	0.225	0.713	0.083	1.109	0.483	3.50	0.006
Liver	88.967	76.071	13.326	84.52	3.507	71.62	1.28
	1.720	7.449	1.248	3.83	0.860	5.39	0.557
Spleen	6.842	5.314	0.098	8.803	0.103	2.570	0.029
	1.195	0.505	0.022	2.799	0.018	1.165	0.010
Stomach	0.083	0.159	2.880	0.178	1.425	0.196	0.049
	0.044	0.021	1.258	0.044	0.322	0.096	0.017
Large intestine	0.044	0.264	0.538	0.076	0.647	0.241	0.119
	0.014	0.193	0.070	0.076	0.092	0.043	0.030
Small intestine	0.096	0.491	1.180	0.151	1.12	0.590	0.393
	0.025	0.256	0.288	0.070	0.241	0.154	0.253
Urine	0.272	3.463	28.651	0.197	41.27	2.266	47.77
	0.204	1.320	1.902	0.145	4.61	0.472	2.230
Kidney	0.128	3.400	1.439	0.446	0.994	0.808	0.575
	0.081	2.681	0.260	0.193	0.438	0.198	0.048
Muscle	0.458	4.605	10.279	1.878	10.54	1.389	1.22
	0.132	1.710	2.615	0.972	2.12	0.482	0.788
Femur	0.084	0.084	1.280	0.066	0.819	0.420	2.17
	0.022	0.019	0.172	0.017	0.054	0.077	0.095
Carcass	2.619	8.873	48.311	2.845	47.00	12.20	44.95
	0.925	1.599	2.988	0.614	4.00	1.88	1.955
Skeleton†	2.106	2.089	31.989	1.645	20.50	10.50	54.14
	0.539	0.483	4.286	0.430	1.35	1.93	2.375

* Sprague-Dawley rats, 160–220 g.
† Activity in: total blood assuming blood volume = 6.5% of body wt. total skeleton estimated by multiplying %ID in femur by 25.

The biodistribution of the ^{153}Sm complexes of the multidentate acetate ligands (NTA, EDTA, HEDTA, and DTPA) showed that [^{153}Sm]DTPA nearly completely cleared from the blood with no significant bone uptake and 95.2 ± 1.4% excreted into the urine 2 hr after injection. In contrast a high percentage of [^{153}Sm] EDTA, HEDTA and NTA localized in bone (Tables 1 and 2). The liver uptake of [^{153}Sm]HEDTA and NTA were 4.49 ± 1.43% and 9.98 ± 1.90% ID, respectively, and even though these chelates showed excellent skeletal localization, their mean bone/blood and bone/muscle ratios were much lower than [^{153}Sm]EDTMP.

Long-term biodistribution in rats. Retention of the [^{153}Sm]EDTMP in bone was measured in rats over a 3-day period. The results are shown in Table 4 and indicate that the % ID present in the skeleton (corrected for ^{153}Sm decay) did not change significantly in the first 72 hr. Samarium-153 blood activity however, was lower at 24, 48, and 72 hr but kidney and liver uptake did not change.

Rabbits

Because [^{153}Sm]EDTMP showed good selective skeletal localization and low blood levels the rat studies were extended to evaluation in rabbits. These results are summarized in Table 5 and show that [^{153}Sm] EDTMP also demonstrates high skeletal uptake and low ^{153}Sm activity in blood and muscle 3 hr postinjection in rabbits. The ^{153}Sm activity remaining in the blood of unanesthetized rabbits following i.v. injection of [^{153}Sm]EDTMP was plotted as a function of time (Fig. 2). The results demonstrate that [^{153}Sm]EDTMP clears the blood more rapidly than [^{99m}Tc]MDP or [^{99m}Tc]PYP and are consistent with the relative 3-hr values.

Scintigraphic images of the [^{153}Sm]EDTMP skeletal uptake in the rabbits were taken at 3 hr postinjection. These images (Fig. 3A) demonstrate a pattern of localization that is consistent with the quantitative organ distribution studies and reveal high selective skeletal uptake. No significant nonosseous tissue accumulation

TABLE 4
Biodistribution of [^{153}Sm]EDTMP in Rats* as a Function of Time After Injection

Time/organ	% Injected dose/organ†							
	15 min	30 min	1 hr	2 hr	5 hr	24 hr	48 hr	72 hr
Blood‡	5.852	2.304	0.532	0.032	0.008	0.007	0.006	0.006
	0.553	0.476	0.395	0.016	0.019	0.002	0.001	0.001
Heart	0.147	0.069	0.017	0.010	0.006	0.006	0.008	0.007
	0.025	0.012	0.006	0.009	0.003	0.005	0.008	0.008
Lung	0.342	0.150	0.051	0.021	0.016	0.007	0.012	0.015
	0.039	0.038	0.016	0.007	0.005	0.002	0.011	0.008
Liver	0.959	0.526	0.322	0.252	0.370	0.349	0.458	0.492
	0.137	0.108	0.073	0.038	0.080	0.021	0.079	0.115
Spleen	0.055	0.025	0.011	0.006	0.009	0.007	0.009	0.007
	0.004	0.005	0.003	0.004	0.007	0.006	0.006	0.004
Stomach	0.390	0.999	0.166	0.241	0.059	0.042	0.069	0.053
	0.175	0.648	0.136	0.158	0.033	0.024	0.081	0.042
Large intestine	0.617	0.259	0.089	0.095	0.27	0.055	0.081	0.058
	0.084	0.044	0.023	0.024	0.17	0.013	0.071	0.022
Small intestine	0.795	0.507	0.342	0.752	0.330	0.045	0.061	0.055
	0.069	0.125	0.166	0.391	0.257	0.009	0.040	0.041
Kidneys	1.745	0.805	0.466	0.254	0.364	0.250	0.286	0.216
	0.334	0.069	0.126	0.035	0.055	0.087	0.028	0.036
Muscle	8.851	4.574	1.662	0.223	0.064	0.125	0.120	0.097
	0.848	2.134	1.630	0.073	0.043	0.038	0.020	0.024
Femur	1.902	2.118	2.302	2.308	2.341	2.082	2.381	2.276
	0.148	0.095	0.109	0.162	0.173	0.116	0.151	0.160
Carcass	61.763	49.604	46.558	44.221	46.460	39.965	43.982	41.708
	2.669	3.848	2.094	3.291	1.834	2.103	1.130	1.403
Urine	28.375	42.150	46.868	49.108	46.075	54.680	49.796	52.541
	3.095	4.548	2.730	3.921	2.851	2.418	1.531	1.423
Skeleton§	47.557	52.955	57.549	57.710	58.535	52.041	60.231	56.911
	3.696	2.363	2.733	4.042	4.105	2.895	1.165	4.004

* Sprague Dawley rats; body wt. = 190–210 g, N = 5.
† Mean and s.d. ($\bar{X}$/s.d.) of % ID/organ.
‡ Assumes blood volume = 6.5% of body wt.
§ Based on % dose femur × 25.

is observed. For qualitative comparison a skeletal image of a 2.3-kg rabbit injected with [^{99m}Tc]MDP is presented in Fig. 3B.

Lesion/Normal Uptake

The uptake of radiolabeled chelates in lesions vary consistently when compared to normal bone (*7*). To estimate the selectivity of [^{153}Sm]EDTMP to localize in bone lesions, the lesion/normal bone ratio of [^{153}Sm] EDTMP was compared to [^{99m}Tc]MDP in the same lesion. The ratios presented in Table 6 show that the lesion/normal bone uptake ratio was not significantly different from [^{99m}Tc]MDP, confirming its selectivity.

DISCUSSION

Samarium-153 and other rare earth radionuclides have been proposed as potential diagnostic skeletal imaging agents (*8–10*). To our knowledge, no [^{153}Sm] chelate has been considered for the treatment of bone lesions. Although advantageous in its bone uptake (Table 1) [^{153}Sm]HEDTA showed some liver localization which diminishes somewhat its attractiveness as a therapeutic radiopharmaceutical. O'Mara et al. (*9*), reported that when less stable ^{153}Sm complexes are injected they demonstrate increased uptake by reticuloendothelial organs and attributed this observation to "in vivo" radiocolloid formation. Our results using molecularly small diphosphonate ligands (i.e., MDP, HDP, and DPD) to complex ^{153}Sm are consistent with their observations since >70% of their injected dose appears in the liver. On the other hand, [^{153}Sm]HEDP and [^{153}Sm]DMAD which are molecularly larger diphosphonate ligands, localize less in the liver than the other diphosphonate complexes, but still at unacceptably high liver and blood levels (Table 3). This implies that small ^{153}Sm diphosphonate ligands are inappropriate to use as therapeutic bone agents.

Samarium-153 NTA and [^{153}Sm]EDTA show skeletal uptake similar to [^{153}Sm]HEDTA (Table 1), with larger amounts depositing in the liver. In contrast, [^{153}Sm] DTPA shows minimal liver as well as bone uptake and is rapidly excreted into the urine. The differential bone uptake of [^{153}Sm]EDTA and [^{153}Sm]DTPA may result

TABLE 5
Biodistribution of [^{153}Sm]EDTMP and [^{99m}Tc]MDP in Rabbits at 3 hr. % Dose (s.d.) n = 5

Organ	[^{153}Sm]EDTMP	[^{99m}Tc]MDP
Blood	0.122	0.873
	(0.100)	(0.128)
Liver	0.955	0.661
	(0.425)	(0.093)
Kidney	0.712	0.866
	(0.197)	(0.222)
Urine	34.3	38.9
	(4.37)	(9.59)
Muscle	0.357	0.873
	(0.158)	(0.140)
Femur	3.30	2.53
	(0.25)	(0.33)
Marrow	0.148	0.174
	(0.037)	(n = 2)
Skeleton	66.3	50.8
	(4.98)	(7.42)
Bone/blood	867	52.9
	(759)	(7.5)
Bone/muscle	1194	345
	(433)	(71)
Bone/marrow	134	92
	(36)	(n = 2)

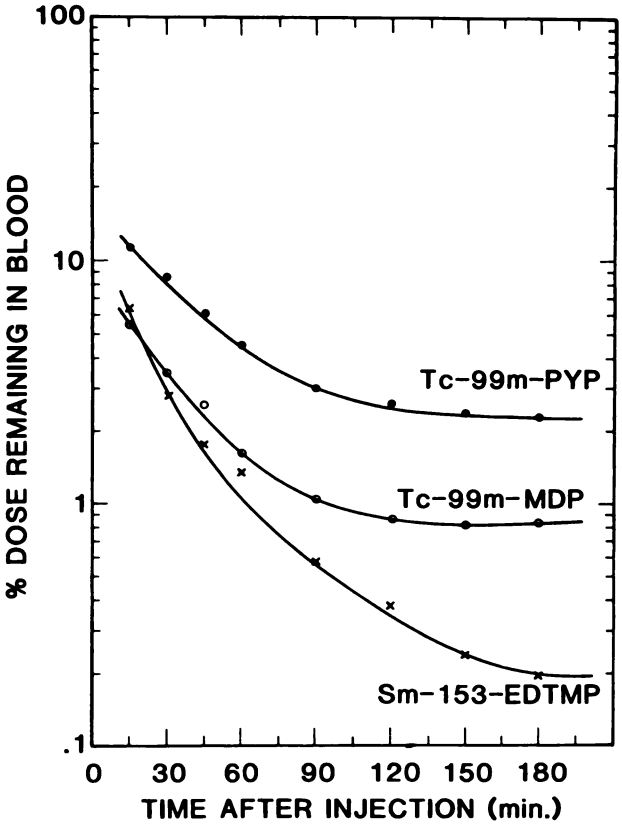

FIGURE 2
Blood clearance of [^{153}Sm]EDTMP, [^{99m}Tc]MDP, and [^{99m}Tc]PYP in rabbits.

from the greater stability (*11*) of the DTPA chelate or the greater number of DTPA chelating groups on the molecule that may block or reduce the ability of the samarium to attach to the binding sites on bone. This result lends further emphasis to the importance of chelate structure on skeletal uptake and the rate of clearance from blood.

Samarium-153 NTMP and [^{153}Sm]NBTP behave like [^{153}Sm]NTA and [^{153}Sm]EDTA exhibiting good bone uptake but some liver accumulation (Table 1 and 2). Samarium-153 NBTP was studied because excellent skeletal images have been obtained with [^{99m}Tc]NBTP. Samarium-153 DTPMP resembles [^{153}Sm]DTPA in the efficient manner it clears from blood and other soft tissues (including liver) but, differs significantly by its moderate skeletal uptake (29.9 ± 9.9%). In contrast, [^{153}Sm]EDTMP shows high skeletal uptake and rapid blood and nonosseous tissue clearance (Tables 1 and 2). The amount of blood ^{153}Sm activity 2 hr after the injection of [^{153}Sm]EDTMP is lower than any of the other chelates examined. Samarium-153 EDTMP gives better bone/blood and bone/muscle ratios than commercial [^{99m}Tc]MDP preparations and once deposited in bone it shows no significant clearance over the next 3 days.

The rabbit studies confirmed the [^{153}Sm]EDTMP results (Table 5) obtained in the rats. Uptake throughout the skeleton is excellent while the nonosseous localization at 3 hr postinjection is very low (Fig. 3A). Samarium-153 EDTMP clears the blood more rapidly than [^{99m}Tc]MDP (Fig. 2). Its low muscle and bone marrow activity at 3 hr postinjection relates to its extremely efficient blood clearance (Table 4). Marrow deposition (0.15 ± 0.04% ID) compares to the level reported by Subramanian et al. (*7*) for [^{99m}Tc]MDP. The skeletal images in Figure 3 show that the skeletal uptake of [^{153}Sm]EDTMP is as selective as [^{99m}Tc]MDP and is increased at sites of bone growth (e.g., joints). This suggests that it should concentrate in bone lesions like the ^{99m}Tc-diphosphonates.

The selective uptake of [^{153}Sm]EDTMP in lesion was documented using a modified drill hole technique (Table 6). Relative lesion/normal bone (L/NB) uptake ratios were calculated from the digitized images and compared with the [^{99m}Tc]MDP images. The L/NB ratio was ~5, but this low value was not representative of the actual L/NB ratio since the digitized images used for analysis were obtained from a two-dimensional image of the skeleton and did not take into account the activity from the tissues surrounding the lesion. By using the same lesion and the same approximate geometry a valid comparison can be made between [^{99m}Tc]MDP and [^{153}Sm]EDTMP. Subramanian et al. (*7*), reported that the uptake of [^{99m}Tc]MDP in callous tissue of the drill hole lesion is 17–18 times that of normal bone. From our results we estimate that [^{153}Sm] EDTMP has a L/NB ratio of ~17/1. The high lesion concentration of [^{153}Sm]EDTMP, and its low blood and

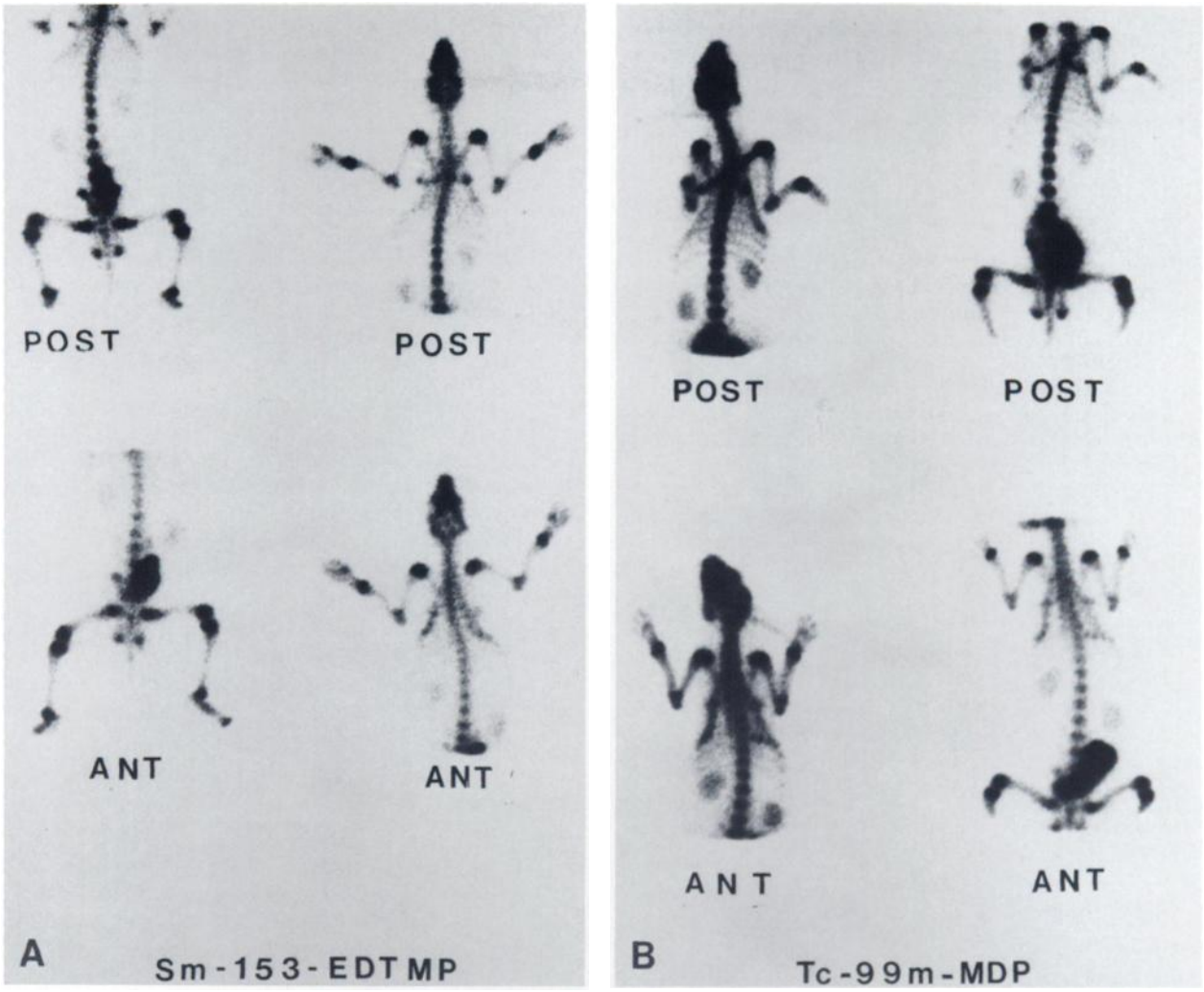

FIGURE 3
Anterior and posterior skeletal images obtained with A: [^{153}Sm]EDTMP and B: [^{99m}Tc]MDP. All images were obtained 3 hr after injection of the agent.

bone marrow activity suggest that this complex should be therapeutically effective in treating metastatic bone disease.

In summary, skeletal uptake of phosphonate and acetate ligands complexed to ^{153}Sm were studied in rats. Both ligand types chelated the radionuclide and produced agents with high skeletal uptake, but many showed significant liver deposition and undesirable high blood activity. Samarium-153 EDTMP was the chelate demonstrating highest skeletal uptake and lowest blood and nonosseous tissue activity. Due to these attributes we conclude that [^{153}Sm]EDTMP could be therapeutically useful in treating metastatic bone cancer. The gamma photon emission from ^{153}Sm gives the added advantages of allowing one to monitor [^{153}Sm]EDTMP skeletal uptake by scintillation imaging and quantitating its uptake in specific lesions.

TABLE 6
Lesion/Normal Bone Ratios from a Drill Hole Model

Animal no.	L/N ratio		[^{153}Sm]EDTMP / [^{99m}Tc]MDP
	[^{99m}Tc]MDP	[^{153}Sm]EDTMP	
1	3.73	3.33	0.89
2	4.12	4.72	1.15
3	5.09	4.64	0.91
			0.98 ± .14

NOTES

* Oak Ridge National Laboratory, Oak Ridge, TN.

† Du Pont Company, No. Billerica, MA and Amersham International, Buckinghamshire, UK.

‡ Mallinckrodt Incorporated, St. Louis, MO.

¶ The complex yield of 95.8% may account for the percent activity appearing in the liver if all of the uncomplexed ^{153}Sm was in the form found with the free Sm injectate.

§ Amersham International, Buckinghamshire, UK.

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REFERENCES

1. A cancer journal for clinicians, American Cancer Society. 1984; 34: Number 1.
2. Winston MA. Radioisotope therapy in bone and joint disease. *Semin Nucl Med* 1979; 9:114–120.
3. Firusian N, Mellin P, Schmidt CG. Results of Sr-89 therapy in patients with carcinoma of the prostate and incurable pain from bone metastases: a preliminary report. *J Urol* 1976; 116:764–768.
4. Weininger J, Ketring AR, Deutsch E, et al. Re-186 HEDP: a potential therapeutic bone agent [Abstract]. *J Nucl Med* 1983; 24:P125.
5. Table of isotopes seventh edition. Lederer CM, Shirley VS, eds. New York: John Wiley and Sons, 1978: 858.
6. Goeckeler WF. The preparation and characterization of several acetate and phosphonate complexes of Sm-153 for use as radiotherapeutic bone agents. Ph.D. Thesis, University of Missouri-Columbia, MO, 1984.
7. Subramanian G, McAfee JG, Thomas FD, et al. New diphosphonate compounds for skeletal imaging, comparison with methylene diphosphonate. *Radiology* 1983; 149:823–829.
8. Rosoff B, Siegel E, William GL, et al. Distribution and excretion of radioactive rare-earth compounds in mice. *IJARI* 1963; 14:129–133.
9. O'Mara RE, McAfee JG, Subramanian G. Rare earth nuclides as potential agents for skeletal imaging. *J Nucl Med* 1969; 10:49–51.
10. Subramanian G, McAfee JG, Blair RJ, et al. Dy-157-HEDTA for skeletal imaging. *J Nucl Med* 1971; 12:558–561.
11. Sillen G, Martell A, eds. Stability constants of metal ion complexes, supp 1. London: Burlington House, 1971.