INDIUM-113m-LABELED POLYFUNCTIONAL PHOSPHONATES AS BONE-IMAGING AGENTS

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Indium-113m complexed with polyfunctional phosphonates EDTMP (an analog of EDTA with carboxylic groups replaced by phosphate groups) and DTPMP (an analog of DTPA) showed preferential skeletal localization in experimental animals. Excellent images of the rabbit skeleton were obtained with both ^{113m}In and ^{111}In complexes using the scintillation camera. In tissue radioassay using ^{85}Sr as a simultaneous biologic standard, ^{113m}In-EDTMP compound showed higher concentration in the skeleton than the DTPMP complex and its bone uptake was comparable to that of ^{85}Sr. Renal excretion was greater for the DTPMP complex (70% vs. 50% for EDTMP at 4 hr) and its blood clearance was faster than EDTMP. EDTMP was found to be the superior agent also to two other polyfunctional phosphonates, NTMP and HMDTMP. Because of the excellent skeletal localization with minimal soft-tissue levels, ^{113m}In-EDTMP may find use in bone scanning in humans wherever ^{99m}Tc bone-imaging agents are not available. These compounds may prove useful also in demonstrating acute myocardial infarcts, particularly for repeat studies after ^{99m}Tc bone agents have already been administered.

Indium-113m is a short-lived radionuclide with excellent physical characteristics that include a monoenergetic gamma emission of 393 keV and a physical half-life of 100 min (*1*). This isotope is available as the daughter nuclide of its long-lived parent ^{113}Sn. Generators for this nuclide have been developed (*2,3*) and are commercially available. This radionuclide has been previously used in nuclear medicine for imaging most major organs in man (*4–9*) except the skeleton.

This report presents methods for the preparation of indium chelates of phosphonate analogs of EDTA and DTPA, which appear to be promising as suitable agents for skeletal imaging.

MATERIALS AND METHODS

Ethylenediaminetetra (methylene phosphonic) acid (an analog of EDTA, herein called EDTMP), hexamethylenediaminetetra (methylene phosphonic) acid (another analog of EDTA with six methylene groups in the center instead of two, herein called HMDTP), and diethylenetriaminepenta (methylene phosphonic) acid (an analog of DTPA, herein called DTPMP) were obtained as sodium, potassium, or ammonium salts in water solution from Calgon Corp., Pittsburgh, Pa. Nitrilotris (methylene) phosphonate [an analog of NTA, herein called NTMP and also known as aminotris (methylene phosphonic) acid] was obtained from Pfaltz & Bauer Chemicals, New York, N.Y. as 50% acid solution in water. Structural formulas for these chemicals are available elsewhere (*10,11*). Diluted solutions of these chemicals were further purified in our laboratory by crystallization methods. All other chemicals used in this study are standard laboratory chemicals.

Indium-113m was obtained from a sterile generator (NEN Pharmaceuticals, North Billerica, Mass.) in 0.05 *N* HCl solution and contained less than 10^{-4}% of ^{118}Sn and 5 μg/ml of zirconia as impurities.

The indium chelates of the above phosphonates were prepared by mixing required amounts of either ^{113m}In or ^{111}In with 25–50 mg of the desired phosphonates in a volume of 4–5 ml. The pH was ad-

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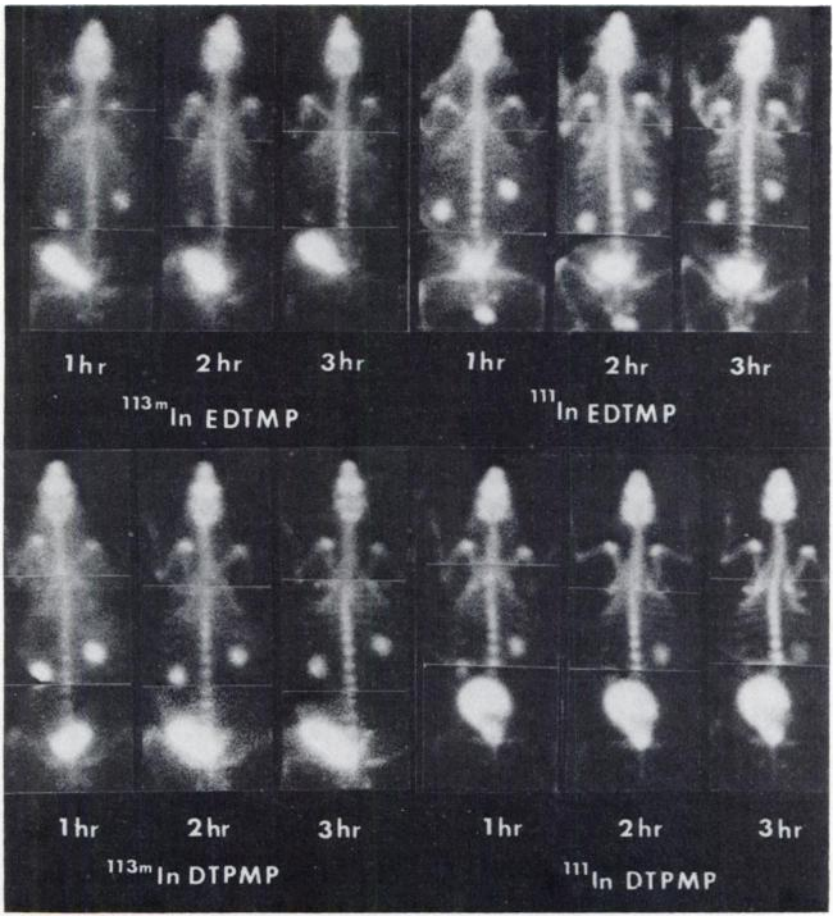

FIG. 1. Composite posterior images of rabbits obtained with both ^{113m}In and ^{111}In chelates of EDTMP and DTPMP after injection of 3–5 mCi of each chelate containing 5–10 mg of phosphonates. A 410-keV parallel-hole collimator was used for ^{113m}In and the 250-keV parallel-hole collimator for ^{111}In. Whole-body image of each rabbit was constructed from three separate images collecting 300,000 counts each.

justed to 7.5–8.0 with dilute (0.1 *N*) sodium hydroxide or dilute (0.1 *N*) HCl as required since the phosphonate salts dissolved in water exhibited high pH as expected and the initial pH of the mixture varied depending on the volume of the acidic indium solution used. The preparation was sterilized by membrane filtration through 0.22-micron-size filters (Millipore Corp., Bedford, Mass.). The amount of free indium in the preparation was determined by paper electrophoresis (Whatman No. 1 paper, barbitol buffer, pH 8.6, Beckman electrophoresis unit). In this system the free indium stayed in the origin while the chelates migrated from the origin toward the anode. In all the above preparations less than 2% of free indium was present as determined by gamma counting of the electrophoretic strips.

Chelates were first prepared with 3–5 mCi of ^{113m}In and 5–10 mg of these phosphonates and injected intravenously into adult albino rabbits weighing 3.5–5 kg. One to three hours after injection the animals were imaged in the posterior projection with a scintillation camera (Searle Radiographics HP, Des Plaines, Ill.) fitted with a 410-keV parallel-hole collimator. Because of the poor imaging characteristics of ^{113m}In with the scintillation camera, the same studies were repeated with ^{111}In and the higher gamma peak of 247 keV was used for these imaging studies. Of the four compounds studied by imaging, only EDTMP and DTPMP were selected for tissue radioassay and organ distribution since they demonstrated better skeletal imaging characteristics compared to the other two compounds. One-half to one millicurie of ^{113m}In labeled with either EDTMP or DTPMP containing 2–5 mg of the phosphonate was administered intravenously to adult albino rabbits weighing 3.5–5 kg along with 10–20 μCi of ^{85}Sr (used as a biologic standard, administered simultaneously). Organ distribution of these compounds was determined after sacrificing the animals from 1 to 4 hr after injection using methods previously described for ^{99m}Tc-labeled bone-imaging agents (*11–13*). No serious problems were encountered between the 514-keV ^{85}Sr peak and the 393-keV ^{113m}In peak during tissue radioassay. Appropriate corrections were made for ^{85}Sr photons in the ^{113m}In window.

RESULTS

Figure 1 illustrates the images obtained with ^{113m}In and ^{111}In chelates of EDTMP and DTPMP. The whole-body image for each rabbit was constructed from three separate posterior view scintillation camera images (300,000 counts/image). These

TABLE 1. INDIUM-113m-LABELED EDTMP AND DTPMP IN RABBITS: SIMULTANEOUS STUDY WITH ^{85}Sr

	EDTMP: Ethylenediaminetetra (Methylene phosphonate)						DTPMP: Diethylenetriaminepenta (Methylene phosphonate)					
	1 hr		2 hr		4 hr		1 hr		2 hr		4 hr	
Organ	^{113m}In	^{85}Sr	^{113m}In	^{85}Sr	^{113m}In	^{85}Sr	^{113m}In	^{85}Sr	^{113m}In	^{85}Sr	^{113m}In	^{85}Sr
Percent dose in whole organ												
Blood	12.6	10.6	8.49	6.58	4.88	4.12	8.61	11.9	3.88	6.66	3.08	5.06
Liver	1.57	1.60	0.769	1.05	0.483	0.593	0.702	1.69	0.453	1.02	0.354	0.675
Muscle	6.75	13.3	4.79	9.96	3.29	6.50	4.61	13.1	1.92	9.59	1.92	8.65
Kidney	1.62	0.681	1.41	0.560	0.812	0.332	2.43	0.860	1.09	0.643	1.03	0.401
Marrow	0.965	0.745	0.645	0.537	0.877	0.641	0.710	1.10	0.340	0.477	0.432	0.616
Urine	34.1	3.43	48.2	14.9	49.1	30.7	31.2	5.58	65.7	17.1	69.9	19.7
One femur	0.94	1.08	1.04	1.37	1.25	1.53	0.782	1.13	0.648	1.27	0.621	1.40
One tibia	0.725	0.836	0.814	1.12	0.951	1.17	0.641	0.918	0.519	1.03	0.471	1.06
Percent dose/1% body weight												
Blood	1.80	1.52	1.21	0.941	0.696	0.589	1.23	1.70	0.555	0.952	0.441	0.723
Liver	0.673	0.711	0.306	0.416	0.216	0.267	0.276	0.665	0.173	0.405	0.184	0.335
Muscle	0.157	0.309	0.111	0.231	0.076	0.151	0.107	0.305	0.044	0.223	0.044	0.201
Kidney	3.85	1.60	2.68	1.08	2.17	0.900	5.62	1.96	2.40	1.38	2.28	0.886
Marrow	0.438	0.339	0.305	0.244	0.399	0.291	0.322	0.500	0.154	0.217	0.196	0.279
Femur	3.73	4.32	4.50	5.95	5.45	6.57	3.57	5.17	3.07	6.00	2.97	6.65
Tibia	3.29	3.82	4.12	5.69	5.16	6.09	3.39	4.86	2.92	5.76	2.76	6.12
Pelvis	6.71	6.09	8.21	8.06	8.86	6.75	7.15	8.41	6.15	8.34	6.37	8.75
Spine	4.96	5.36	5.87	7.11	6.31	7.04	4.80	7.11	4.59	7.61	5.16	9.13
Average bone	5.06	5.17	6.12	6.99	6.82	6.70	5.14	6.83	4.58	7.27	4.79	8.10
Ratios												
Bone-to-blood	2.81	3.40	5.05	7.43	9.80	11.37	4.17	4.01	8.25	7.63	10.8	11.2
Bone-to-marrow	11.55	15.25	20.10	28.65	17.1	23.02	15.9	13.6	29.7	33.5	24.4	29.0
Bone-to-muscle	32.2	16.7	55	30	90	44	48.0	22.3	104	32.6	108	40.2

images demonstrate preferential localization of these complexes in the skeleton with considerable urinary excretion of the radioactivity.

Organ distribution studies are summarized in Table 1 for ^{113m}In-EDTMP and ^{113m}In-DTPMP. For each time interval three animals were used and only average values are shown. The average bone concentrations expressed as percent dose per 1% body weight are the mean of the concentrations in bone samples from femur, tibia, spine, and pelvis. This value was calculated for individual rabbits and only the mean value of these averages is shown. The values of ratios were calculated in a similar manner. The percent dose per 1% body weight is equal to:

$$\text{Percent injected dose in sample or organ} \div \frac{\text{weight of sample or organ}}{\text{body weight}} \times 100$$

$$= (\text{Percent dose/gm}) \times \frac{\text{body weight in gm}}{100}$$

DISCUSSION

The chemical compounds (NTMP, EDTMP, DMDTMP, and DTPMP) used in these studies are analogs of well-known metal chelating agents, nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), hexamethylenediaminetetraacetic acid (HMDTA), and diethylenetriaminepentaacetic acid (DTPA), with the carboxylic group of the acetic acid replaced by a phosphate group. In effect, the phosphorus atom in the polyfunctional phosphonates is linked to the nitrogen through a methylene group, in contrast to the P—O—P bond in pyrophosphate, P—C—P linkage in diphosphonates, and P—N—P structure in imidodiphosphate, all known bone-seeking agents. These multidentate ligands retain the metal chelating ability of their analogs particularly for divalent and trivalent cations. One (EDTMP) was noted for its chelating ability of calcium, copper, manganese, and iron as early as 1956 (*14*). In addition, these polyphosphonates have been shown to inhibit calcification and crystal growth on synthetic hydroxyapatite from supersaturated calcium phosphate solutions (*10*) similar to EHDP (HEDP), a known bone seeker. Meyer and Nancollas (*10*) have also shown that the multidentate ligands EDTMP and DTPMP are more effective than either NTMP or even EHDP in preventing crystal growth on hydroxyapatite, since the former compounds form stronger chelating bonds with calcium on the hydroxyapatite than the latter two.

This phenomenon led us to the development of ^{99m}Tc-labeled bone-imaging agents with these polyfunctional phosphonates using stannous ion (*11,12*).

Since EDTMP also chelates trivalent metals (*14*) these indium complexes were prepared and evaluated. In preliminary studies, divalent metal chelating compounds such as EHDP, MDP, IDP, AEDP, pyrophosphate, and polyphosphate that have been used with ^{99m}Tc for bone imaging (*12*) were tried with indium radionuclides. Even though in vitro chelate formation occurred (as determined by lack of precipitation with nonradioactive indium at neutral or alkaline pH and quantitative passage of ^{113m}In-chelate at pH > 7 through 0.22-micron membrane filter) these compounds disintegrated in vivo and no appreciable bone localization occurred. The same phenomenon was also observed with NTMP probably because of its low chelate stability (*10*). This is understandable from the fact that its parent compound, NTA, has a lower stability constant with metal ions compared to EDTA or DTPA. Therefore, ^{113m}In-NTMP or ^{111}In-NTMP gave very poor skeletal localization in imaging studies in rabbits. On the other hand, ^{113m}In-HMDTP provided excellent skeletal images in rabbits (not shown here) but the count rates were very low for the same injected activity compared to that of ^{113m}In-EDTMP and therefore its quantitative biologic distribution was not studied.

All the imaging studies were performed with the scintillation camera since a rectilinear scanner was not available. Since the 393-keV gamma peak of ^{113m}In is too high an energy for the scintillation camera to give excellent images similar to that of ^{99m}Tc, ^{111}In was chosen and the higher energy peak (247 keV) was used for the imaging studies. The distinct difference in definition of the vertebrae and ribs is obvious between ^{113m}In and ^{111}In for the same chelating agent (Fig. 1). This is mainly due to better imaging characteristics of lower energy gammas of ^{111}In. Since the blood clearance of DTPMP is faster than EDTMP, the DTPMP images are clearer because of lower body background. Count rates for ^{113m}In-EDTMP were approximately twice that for ^{113m}In-DTPMP with the same amount of injected activity for similar areas in the same rabbit (on separate days) indicating EDTMP complex concentrated more in the skeleton. This is further documented in Table 1. Although the scintillation camera images of the ^{113m}In agents are poor, rectilinear scans should produce better images. The large accumulation of activity in the pelvic area of the rabbits reflects urinary excretion of these complexes (Fig. 1). It is significant that larger quantities of the DTPMP complex accumulated in the bladder than the EDTMP did. None of the rabbits urinated during the imaging experiments.

The data in Table 1 are self-explanatory. Strontium-85 was used as a biologic standard to compare the uptake in various organs of individual groups of animals. In the quantification of bone uptake one should not compare just indium concentration from one group to another but also the indium-to-strontium ratios from each group because of the individual variation of bone uptake in animals. With EDTMP, the femur and tibial bones concentrate nine-tenths of strontium activity up to 4 hr whereas this ratio ranged from 0.7 at 1 hr to 0.46 at 4 hr for DTPMP, indicating that ^{113m}In-DTPMP leaves the skeleton after initial concentration. At 4 hr only 49% of the EDTMP complex was excreted in the urine, compared to almost 70% for the DTPMP complex; however, the blood levels of DTPMP were lower than that for EDTMP at all time intervals studied.

The liver and soft-tissue concentrations of the indium complexes paralleled the blood levels and as expected the ^{85}Sr concentration in the soft tissue is much larger than indium complexes. The bone marrow concentration is low for both agents. The bone-to-blood, bone-to-marrow, and bone-to-muscle ratios are comparable to those for ^{85}Sr for both complexes. Because ^{113m}In has a half-life of only 100 min, these studies were carried out only up to 4 hr.

Indium-111 is not recommended for bone imaging since it offers no advantages over ^{99m}Tc. Even in remote locations, it is not more convenient since its physical half-life is so similar to that of ^{99}Mo, the parent radionuclide of ^{99m}Tc.

The above studies indicate that indium-labeled polyfunctional phosphonates, EDTMP and DTPMP, indeed selectively concentrate in the skeleton of experimental animals and appear promising as bone-scanning agents in humans wherever ^{99m}Tc is not available. Of the two compounds, ^{113m}In-EDTMP appears to be a better agent because of its greater concentration and longer retention than the DTPMP complex even though the latter has a faster blood clearance. Another important use of these compounds could be in repeat studies for localizing acute myocardial infarcts in man after the technetium studies. These new indium compounds also localize in experimental infarcts in animals as demonstrated in our preliminary studies.

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