New ^{68}Ga-Labeled Skeletal-Imaging Agents for Positron Scintigraphy

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The present skeletal-imaging agents labeled with radiogallium rely upon carrier gallium to augment bone uptake; no gallium-labeled bone-imaging agent free of this disadvantage is available. In attempts to develop such agents, we prepared ^{68}Ga-ethylenediaminetetramethylene phosphonate (^{68}Ga-EDTMP) and ^{68}Ga-diethylenetriaminepentamethylene phosphonate (^{68}Ga-DTPMP) and determined their biologic distributions in rats and dogs. These compounds combine the bone-seeking characteristics of phosphonic acid and the complexing ability of EDTA and DTPA analogs. The chelates are administered without gallium carrier. In rats, 50–60% of the carrier-free dose accumulates in bone at 1 hr after intravenous injection, while 25–30% is excreted through the urine. In dogs, at 3 hr after intravenous injection 35% is found in bone. Although the general patterns of organ distribution of the two ^{68}Ga chelates are similar, ^{68}Ga-EDTMP appears superior because of its faster blood clearance. Bone images obtained with this compound in dogs, using a multidetector positron camera, are presented. The optimum time for imaging was found to be 2.5–3 hr after injection.

J Nucl Med 17: 1003–1007, 1976

The use of radiogallium in ionic form or together with weak complexing agents for bone imaging is restricted by the need to saturate blood-binding sites with stable gallium before sufficient radiogallium is free to provide satisfactory bone images (*1*). The need to administer stable gallium in mg/kg amounts has made this procedure unattractive. We attempted to develop radiogallium-labeled bone-seeking agents that do not require the simultaneous use of carrier gallium. Although ^{68}Ga-tripolyphosphate has recently been reported to provide satisfactory bone localization in mice and rabbits, particularly with the use of stable gallium (*2*), our preliminary work with gallium-labeled polyphosphate, diphosphonate, imidophosphonate, and other weak complexing agents has shown that useful bone uptake in rats and bone images in dogs are not obtained with these agents.

The preparation of ^{113m}In-labeled methylene phosphonates with carrier-free indium for imaging the skeleton (*3,4*) and myocardial infarction (*5*) has been reported. Because the chemical properties of Ga^{3+} and In^{3+} are similar (*6*), we investigated the possibility of employing ethylenediaminetetramethylene phosphonate (EDTMP) and diethylenetriaminepentamethylene phosphonate (DTPMP), labeled by chelation with ^{68}Ga, for skeletal imaging with a multidetector positron camera.

Recently, there has been increasing interest in positron scintigraphy, principally because of its potential advantages, namely, high sensitivity and spatial resolution, over comparable gamma-photon scintigraphic methods. The ability to correct for photon absorption is a particularly useful feature in tomographic reconstruction. Consequently, we are involved in a program to develop radiopharmaceuticals

Received Nov. 26, 1975; revision accepted June 4, 1976.

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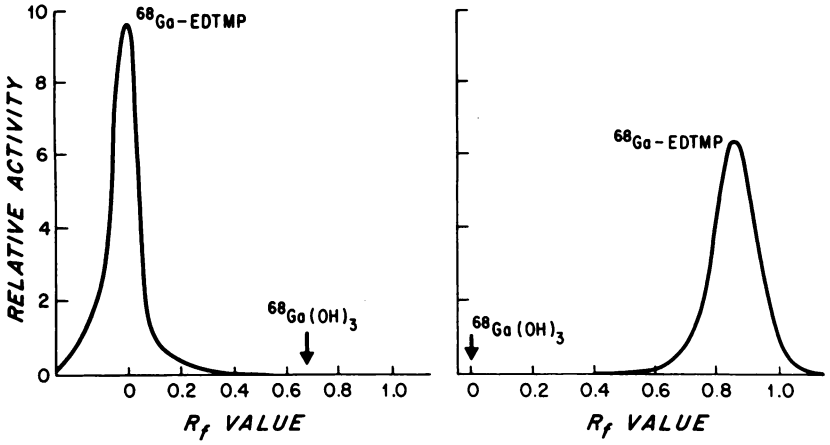

FIG. 1. Radiochromatograms for Ga-EDTMP: (Right) solvent B; (left) solvent C.

labeled with positron-emitting radionuclides and, in particular, with ^{68}Ga. This radionuclide was chosen because it is a short-lived ($t_{1/2}$ = 68 min) positron-emitter and is available as a generator product by decay of its parent ^{68}Ge ($t_{1/2}$ = 287 days).

MATERIALS AND METHODS

Stock solutions of EDTMP and DTPMP (Monsanto Chemical Co., Miamisburg, Ohio) were prepared by neutralizing the phosphonic acids with 6 *M* NaOH solution to pH 7.8 and adjusting the volume with distilled water to a final concentration of 100 mg/ml. The ^{68}Ga activity was obtained as an EDTA complex by eluting a ^{68}Ge–^{68}Ga generator (New England Nuclear Corp., North Billerica, Mass.). The EDTA complex was dissociated with concentrated hydrochloric acid, and the resulting $GaCl_4^-$ complex was separated from the EDTA by absorption on an anion-exchange resin column (7). The $GaCl_4^-$ complex was eluted in acid solution from the column and was mixed with 20–50 mg of Na-EDTMP or Na-DTPMP. After incubation for 2–3 min, the pH was adjusted to 6.5 with dilute NaOH and the solution was sterilized by membrane filtration.

The quality of each preparation was checked by paper radiochromatography using methods described previously (7). Whatman No. 1 filter paper was used with two solvent systems, designated here as B and C. Solvent B is a mixture of isopropanol, trichloroacetic acid, ammonia, and water, while solvent C is a mixture of ethanol, pyridine, and water. The use of two solvent systems helps to ensure that the chelate peaks are adequately separated from those of possible contaminants, such as gallium hydroxide. Gallium hydroxide remains at the origin with solvent C, while with solvent B it migrates with the solvent front, possibly because a soluble species is formed with the solvent constituents. The absence of ^{68}Ga-EDTA contamination in the preparations was also determined by paper chromatography.

Organ distribution in Sprague–Dawley rats was determined by intravenous injection with 1 mg each of EDTMP or DTPMP labeled with ^{67}Ga. The ^{67}Ga was obtained as the citrate complex (New England Nuclear Corp.) and was dissociated from citrate and used as described above for ^{68}Ga. A total of 30 rats were injected and killed at times ranging from 1 hr to 1 day. The tissue samples were counted in a NaI well counter against a standard sample of the injected solution. Total urine and the complete liver, kidneys, and intestines, were removed and counted, along with samples of muscle, bone, and blood. Muscle, bone and blood were assumed to constitute

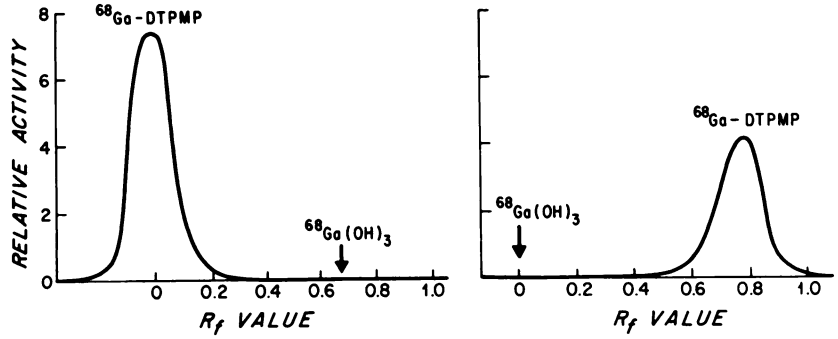

FIG. 2. Radiochromatograms for Ga-DTPMP: (Right) solvent B; (left) solvent C.

TABLE 1. TIME COURSE OF ORGAN DISTRIBUTION OF ^{67}Ga-EDTMP IN RATS (% INJECTED DOSE PER TOTAL ORGAN)

Tissue	Time after injection			
	1 hr	3 hr	5 hr	24 hr
Liver	1.4 ± 0.5	1.0 ± 0.5	1.1 ± 0.2	1.4 ± 0.2
Kidneys	0.7 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.4 ± 0.1
Intestine	1.3 ± 0.4	2.1 ± 0.4	2.4 ± 0.3	1.1 ± 0.3
Urine	24.6 ± 5.5	30.4 ± 7.0	35.2 ± 8.1	40.5 ± 10.8
Muscle	5.7 ± 0.5	2.7 ± 0.2	3.9 ± 0.4	2.1 ± 0.2
Bone	59.2 ± 4.8	57.6 ± 3.5	46.9 ± 3.8	39.5 ± 2.5
Blood	5.5 ± 0.9	2.4 ± 0.6	2.7 ± 0.5	0.7 ± 0.2
Ratio (per gram) of bone to:				
Blood	6.5	13.9	10.4	33.9
Muscle	41.5	85.3	48.1	75.2

TABLE 2. TIME COURSE OF ORGAN DISTRIBUTION OF ^{67}Ga-DTPMP IN RATS (% INJECTED DOSE PER TOTAL ORGAN)

Tissue	Time after injection			
	1 hr	3 hr	5 hr	24 hr
Liver	2.2 ± 0.5	3.2 ± 0.6	0.6 ± 0.1	1.1 ± 0.1
Kidneys	3.3 ± 0.6	0.8 ± 0.2	0.5 ± 0.1	0.3 ± 0.1
Intestine	2.4 ± 0.5	2.1 ± 0.4	1.6 ± 0.3	1.1 ± 0.4
Urine	12.3 ± 8.5	25.5 ± 8.5	35.5 ± 7.5	38.9 ± 6.5
Muscle	8.9 ± 0.5	7.8 ± 0.4	4.9 ± 0.3	1.6 ± 0.2
Bone	55.6 ± 3.2	45.2 ± 3.6	49.7 ± 5.5	62.6 ± 6.5
Blood	9.9 ± 1.2	5.3 ± 1.2	1.4 ± 0.3	0.6 ± 0.1
Ratio (per gram) of bone to:				
Blood	3.37	5.12	21.3	62.6
Muscle	25.0	23.2	40.6	157.0

40%, 10%, and 6%, respectively, of the animal's total weight. Results were then expressed as percent injected dose per total organ.

The compounds were also evaluated in dogs to explore imaging with a positron camera. About 50 mg of each chelating agent was labeled with 8–10 mCi of ^{68}Ga and administered through a catheter in a femoral vein. Periodic blood samples were obtained through an arterial catheter, and each animal was killed at 1, 2, or 3 hr. The animal's bladder was emptied and the total urine counted in an ionization chamber. The animal was then imaged so that composite pictures of the anterior–posterior and lateral views could be obtained. Each image took approximately 5 min and contains 100,000–200,000 counts. After the imaging, the animal was autopsied and samples of tissue were obtained for weighing and subsequent counting in a NaI well counter against a standard. Samples of rib, tibia shaft, and tibia joint bone were collected. Bone marrow was carefully separated from the shaft bone and counted separately. Bone uptake is expressed (in percent dose per gram) as the average for the three bone samples.

Imaging procedures with the positron camera have been described elsewhere (*8*). The images were obtained by placing the animal in one of the focal planes and collecting counts during computer-controlled one-dimensional motion of the camera faces. The camera motion dispersed patterning artifacts that would otherwise appear. The resulting images were processed by performing a nine-point running average to reduce random variations.

RESULTS

Figure 1 shows typical radiochromatograms of ^{68}Ga-EDTMP obtained with solvents B and C on Whatman No. 1 paper. Figure 2 shows radiochromatograms of ^{68}Ga-DTPMP. In each figure, the position in which $Ga(OH)_3$ colloid is known to appear is indicated. Only one species is apparent in these chromatograms, presumably that of the desired chelate.

Tables 1 and 2 list the tissue distribution results obtained at 1, 3, 5, and 24 hr in rats for ^{67}Ga-EDTMP and ^{67}Ga-DTPMP, respectively. Each datum is the average of 3–4 rats with one standard deviation. The results are expressed as percent injected dose per whole organ. In addition, the ratios of activity per gram of bone to that in one gram of blood and muscle are listed.

Tables 3 and 4 list the results obtained with ^{68}Ga-EDTMP and ^{68}Ga-DTPMP, respectively, in the dog study. A total of ten dogs were injected with 8–10 mCi of one of the compounds and killed at 1, 2, or 3 hr. These results are expressed as the percent in-

TABLE 3. TIME COURSE OF ORGAN DISTRIBUTION OF ^{68}Ga-EDTMP IN DOGS (% INJECTED DOSE PER GRAM × 10^{-3})

Tissue	Time after injection		
	1 hr	2 hr	3 hr
Liver	2.03	1.71	1.33
Kidney	16.1	2.95	7.07
Muscle	1.58	0.96	0.62
Bone	9.1	10.0	21.0
Blood	5.5	3.7	1.6
Marrow	5.4	1.3	3.0
Ratio (per gram) of bone to:			
Liver	4.5	5.8	15.8
Kidney	0.6	3.4	3.0
Muscle	5.8	10.4	33.9
Blood	1.7	2.7	13.1
Marrow	1.7	7.7	7.0

jected dose per gram of tissue. Single animals were used to obtain the 1-hr ^{68}Ga-EDTMP and ^{68}Ga-DTPMP values; the remaining values are averages for 2–3 animals. The average dog weight was 18 kg. Assuming a total bone mass of 10% of total body weight, the uptake in bone is approximately 35% for ^{68}Ga-EDTMP at 3 hr.

Figure 3 shows the blood clearance and urinary excretion for each compound. The results were obtained by counting blood samples taken periodically from the 3-hr dogs and have been expressed as percent of injected dose in total blood by assuming that blood constitutes 6% of the animal's weight. The figure also shows the activity at death in total urine for each compound.

TABLE 4. TIME COURSE OF ORGAN DISTRIBUTION OF ^{68}Ga-DTPMP IN DOGS (% INJECTED DOSE PER GRAM × 10^{-3})

Tissue	Time after injection		
	1 hr	2 hr	3 hr
Liver	4.5	3.0	2.4
Kidney	18.0	4.5	5.0
Muscle	1.3	0.9	0.5
Bone	3.7	7.5	10.3
Blood	7.4	6.6	4.2
Ratio (per gram) of bone to:			
Liver	0.82	2.5	4.3
Kidney	0.21	1.7	2.1
Muscle	2.8	8.3	20.6
Blood	0.50	1.1	2.5

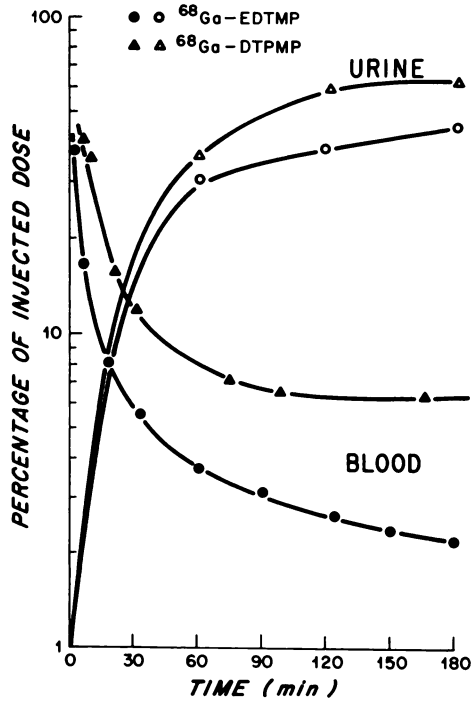

FIG. 3. Blood clearance and urinary excretion for ^{68}Ga-EDTMP and ^{68}Ga-DTPMP. Blood activity is expressed as percent of injected dose in total blood; urinary activity is expressed as percent of injected dose in total urine.

Figures 4 and 5 are composite images obtained in a dog with ^{68}Ga-EDTMP at 2.5–3 hr after injection. The images are superior to those obtained at earlier times after injection. The animal's bladder was emptied prior to imaging.

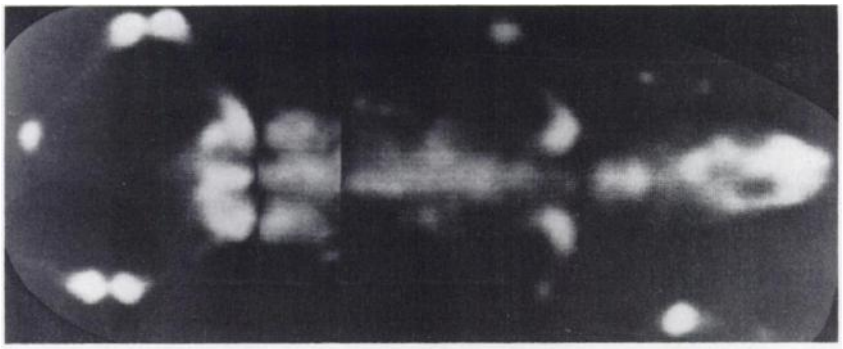

FIG. 4. Composite anterior–posterior skeletal image of dog obtained with ^{68}Ga-EDTMP 3 hr after injection.

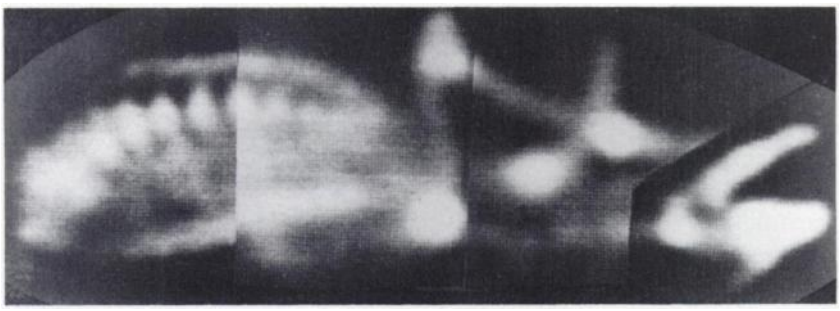

FIG. 5. Composite lateral skeletal image of dog obtained with ^{68}Ga-EDTMP 3 hr after injection.

DISCUSSION

Our initial attempts to develop a gallium-labeled bone-seeking agent involved testing polyphosphate, diphosphonate, and pyrophosphate labeled with ^{68}Ga. The images obtained in dogs with these compounds showed considerable liver activity. Furthermore, ^{67}Ga-labeled pyrophosphate, diphosphonate, and imidophosphate were studied in rats, and activity was found to localize in the reticuloendothelial system (30–50%) and in bone marrow (15–20%), with only slight accumulation (5–10%) in bone at 1 hr. Similar results were obtained when ^{113m}In was used in place of ^{67}Ga as the label. These unfavorable findings are probably due to poor complex stability, such that colloidal forms may be produced and accumulate in the reticuloendothelial system. Accordingly, we have studied compounds with greater stability; EDTMP and DTPMP were chosen since their chelates with gallium may be expected to display stabilities similar to those of their EDTA and DTPA analogs.

The results obtained with gallium-labeled EDTMP and DTPMP show that the general patterns of organ distribution of the two agents are similar. In rats, 50–60% of the injected activity accumulates in bone, while in dogs this value is approximately 35%, the difference presumably being due to species variability. The ratios of bone activity to that of other organs clearly show the preference of both agents for bone tissue. However, the more rapid blood clearance of ^{68}Ga-EDTMP compared to ^{68}Ga-DTPMP, shown in Fig. 3 and Tables 3 and 4, suggests that the former complex is a more successful bone-imaging agent. These observations are supported by the bone images; those obtained with ^{68}Ga-EDTMP appear to be superior.

The bone uptake of these agents in animals at 2–3 hr after injection is lower than that observed for ^{99m}Tc bone-imaging agents at comparable times (*9*). However, because of the shorter half-life of ^{68}Ga, a higher initial activity must be administered to compensate for decay prior to imaging, and this requirement may restrict the use of these agents. Nevertheless, because of the depth-independence of sensitivity and spatial resolution obtainable with positron detection, these ^{68}Ga-labeled bone agents may be particularly useful in imaging deep-seated bone tissue. Furthermore, the ^{113m}In chelates of EDTMP and DTPMP have been shown to localize preferentially in the inorganic matrix of bone, in contrast to the ^{99m}Tc-labeled bone agents, which localize in both the inorganic and organic matrices (R. D. Gantra, personal communication). Presumably, the ^{68}Ga-labeled agents behave similarly, and consequently these agents may be useful in the differential diagnosis of bone disease.

Both EDTMP and DTPMP are essentially nontoxic in mice: the LD_{50} following intravenous injection is 120 mg/kg (*9*). One of us (MKD) is currently conducting clinical bone-imaging trials with ^{113m}In-labeled EDTMP. Preparations containing about 40 mg of EDTMP have been found to provide adequate images; no adverse reactions have been observed in the ten patients studied to date. We think that the ^{68}Ga-labeled compounds may have application as clinically useful bone agents for positron scintigraphy. The localization of these agents in myocardial infarcts is currently under investigation.

ACKNOWLEDGMENTS

The authors would like to thank G. L. Brownell and P. C. Kahn for suggestions and encouragement. We also wish to thank R. E. Brakebill and the Monsanto Chemical Company for supplying quantities of EDTMP and DTPMP. This investigation was supported in part by the Charlton Fund and NIH Grant No. CA-07368.

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