Uptake of Tc-99m Monophosphate Complexes in Bone and Myocardial Necrosis in Animals

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Bidentate monophosphates—phosphonoacetate (PAA), 2-phosphonopropionate (PPA), 2-methyl-2-phosphonopropionate (MPPA), and carbamyl phosphate (CAP)—which are pyrophosphate analogs, were successfully labeled with Sn(II)-reduced [^{99m}Tc] pertechnetate in high yield (>95%).

Biodistribution studies show that these Tc-99m-labeled monophosphates do localize in bone. At 2 hr after injection, Tc-99m CAP has average femur uptakes of 1.9% in rats and 2.9% in rabbits, which correspond to calculated total-bone uptakes of 38% and 58%, respectively. These are comparable with the femur uptakes for Tc-99m methylene diphosphonate (MDP), which are 1.8% in rats and 2.7% in rabbits. However, the blood clearance rate for Tc-99m CAP was slower than that observed for Tc-99m MDP making the former less desirable for use as a bone-scanning agent. The femur uptakes for Tc-99m PAA are 0.9% in rats and 1.2% in rabbits, corresponding to 18% and 24% in total bone, respectively. The PAA derivatives PPA and MPPA have much lower bone uptake.

Technetium-99m CAP also concentrates in necrotic myocardium in rats, in amounts comparable to Tc-99m pyrophosphate.

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All of the Tc-99m bone-scanning agents in current use are complexes containing at least two phosphate groups (*1–4*). It is not clear whether this is a requirement for localization or merely a reflection of the fact that the first Tc-99m bone-scanning agents were polyphosphates (*5*).

Phosphonoacetic acid (PAA), an analog of pyrophosphate (PPi), contains only one phosphate group and represents the simplest monophosphate that would be expected to complex Tc. It is a bidentate ligand containing phosphate and carboxylate groups that are capable of forming two chelating bonds with metal ions. This compound is a new antiviral agent that specifically inhibits virus-induced DNA polymerase by interacting with the enzyme at the pyrophosphate binding site (*6*). Reports on biologic calcification mechanisms have shown that PAA can interact with hydroxyapatite crystal and inhibit calcification (*7*).

Carbamyl phosphate (CAP) is also a bidentate monophosphate compound. It contains an amide group that is un-ionized at physiologic pH but is capable of forming chelate bonds with metal ions. It is a substrate for several enzymes and a natural metabolic intermediate. Currently, it is used for treating sickle-cell anemia and it has low toxicity in dogs (*8*).

Several Tc-99m monophosphate complexes have been prepared and their biodistribution has been studied (*9,10*), but they show no specific localizing properties. Cognizant of the structural and biochemical similarities between PAA and PPi, we have investigated the bone localization of Tc-99m PAA and structurally related monophosphates: 2-phosphonoproprionate (PPA), 2-methyl-2-phosphonoproprionate (MPPA), and CAP (see Fig. 1).

Since the localization of bone-seeking compounds varies considerably in animals, depending upon age and condition, the distribution data for these new agents were compared with the results obtained for Tc-99m MDP under the same experimental conditions.

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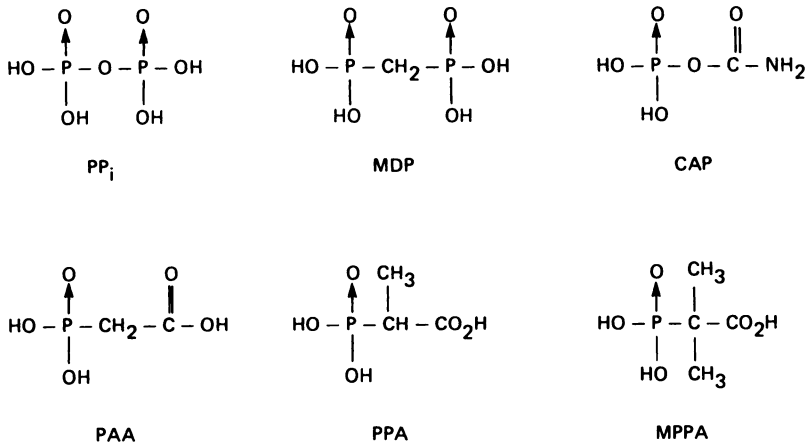

FIG. 1. Structures of phospho compounds.

A heat-damaged rat model for acute myocardial necrosis was first reported by Adler et al. (*11*) and later used by Davis (*12*) to screen radiopharmaceuticals. We have used this rat model to evaluate the localization of these bidentate monophosphates in myocardial necrosis.

MATERIALS AND METHODS

Chemicals. Phosphonoacetic acid (PAA) was prepared by hydrolyzing triethyl phosphonoacetate* in 6*N* hydrochloric acid solution (*13*) (Fig. 2). The recrystallized product showed the correct NMR spectrum and elemental analysis. Two phosphonoacetate derivatives, PPA and MPPA, were kindly provided by Dr. A. F. Isbell†. Carbamyl phosphate (CAP) and MDP were purchased commercially‡.

All of the Tc-99m complexes were prepared by dissolving 10 mg of the phosphate compound in 2–4 ml of saline, adding 100 μg of $SnCl_2 \cdot 2H_2O$ (in 10 μl of 0.1*N* HCl), adjusting the pH to ~6, and finally adding the desired amount of [^{99m}Tc] pertechnetate. For each preparation the radiochemical purity was checked by ascending paper chromatography using Whatman No. 1 paper or Gelman ITLC-SG glass-fiber sheets, with 85% methanol or saline as the solvent (*14*). Chromatograms were cut into 0.5-cm strips and counted in a well counter. All animal studies were carried out within an hour of radiopharmaceutical preparation.

Animal distribution studies. Biodistribution of the Tc-99m phosphate complexes was studied in Sprague-Dawley rats (male, weighing 220–280 g) and New Zealand albino rabbits (male, weighing 1.5–3.0 kg). Under ether anesthesia, rats were injected (into femoral vein) with Tc-99m phosphate complex (1–10 μCi, 0.1–0.8 mg) in 0.2 ml of saline. They were sacrificed after various times and organs of interest were excised and counted in a well counter. Percentage dose per total organ was calculated by comparison of tissue counts to suitably diluted aliquots of the injected material. The distribution studies in rabbits were carried out in a similar manner but using phenobarbital anaesthesia and a larger injected dose (1–5 mCi, 0.5–2 mg). Tissue samples were measured by a dose calibrator. Total activities in blood and muscle were calculated by assuming that they are 7% and 40% of the body weight, respectively.

Localization in acute myocardial necrosis. The method of producing cardiac lesions with a soldering iron reported by Adler (*11*) was used in Sprague-Dawley male rats (200–300 g). Three hours after

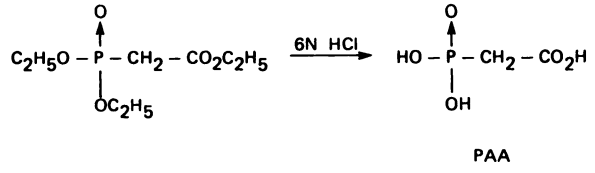

FIG. 2. Synthesis of phosphonoacetic acid.

TABLE 1. CHROMATOGRAPHY* OF Tc-99m-LABELED PHOSPHATES

	% of total activity					
R_f	PPi	MDP	CAP	PAA	PPA	MPPA
0.1	0.76	0.24	0.08	0.79	1.21	0.83
0.2	0.55	0.37	0.03	0.69	0.68	0.55
0.3	0.28	0.47	0.01	0.70	0.44	0.39
0.4	0.39	0.47	0.01	0.59	0.41	0.38
0.5	0.38	0.36	0.01	0.64	0.42	0.36
0.6	0.42	0.42	0.04	0.54	0.53	0.50
0.7	0.78	0.37	0.04	0.92	0.53	0.61
0.8	2.36	0.44	0.49	0.79	0.53	0.38
0.9	4.86	1.22	1.82	0.99	0.95	0.59
1.0	89.7	95.6	97.4	93.3	94.3	95.4

* Gelman ITLC-SG fiberglass sheets developed by saline; average value of 3 chromatograms for each compound.

TABLE 2. DISTRIBUTION OF Tc-99m PAA IN RATS

	% dose/organ (range)*				
	15 min	30 min	1 hr	2 hr	4 hr
Blood	9.06 (8.94– 9.19)	5.18 (4.93–5.34)	3.85 (3.58–4.12)	2.92 (2.43–3.26)	2.06 (1.94–2.25)
Muscle	10.4 (8.99–11.2)	6.89 (6.50–7.60)	5.34 (4.43–5.95)	3.75 (3.63–3.87)	1.63 (1.57–1.66)
Kidney (2)	7.06 (6.57– 7.58)	3.08 (2.52–3.44)	2.07 (1.60–2.45)	1.99 (1.86–2.25)	1.50 (1.46–1.57)
Liver	2.51 (1.83– 3.15)	0.85 (0.82–0.89)	0.74 (0.71–0.76)	0.81 (0.69–1.06)	0.57 (0.53–0.60)
Femur	0.85 (0.71– 0.97)	0.98 (0.95–1.05)	0.95 (0.92–0.97)	0.93 (0.89–0.99)	0.80 (0.70–0.85)
Tibia & fibula	0.69 (0.64– 0.75)	0.75 (0.75–0.76)	0.77 (0.74–0.83)	0.69 (0.58–0.75)	0.70 (0.57–0.78)

* Average value of three rats.

the lesion was produced, the Tc-99m compounds were injected intravenously. The rats were killed 1 hr after the injection. A thin layer of damaged heart muscle was excised for the necrotic (MN) sample, and a larger undamaged area was taken for normal heart muscle. The weights of normal and MN samples were 100–300 mg and 20–60 mg, respectively.

RESULTS AND DISCUSSION

All of the monophosphate compounds formed complexes with Sn(II)-reduced pertechnetate. Chromatograms of these new complexes, using the 85% methanol solvent system and Whatman No. 1 paper, showed a single narrow peak (>98% of the total activity) near the origin, whereas pertechnetate has an R_f value of 0.6. Less consistent results were obtained when saline was used. Generally, 5–30% of the total activity stayed at the origin of a saline chromatogram, 10–40% at the front, and the rest of activity was evenly distributed in between. This is not surprising, since Tc-99m PPi gives the same variable chromatogram with saline. Recently, Zimmer and Pavel (*14*) have reported a chromatographic quality-control procedure for Tc-99m radiopharmaceuticals, in which Gelman instant TLC-SG fiberglass sheets were used, and T-99m PPi was successfully separated from the reduced hydrolyzed technetium (TcO_2). By using the same chromatographic procedure, the percentage of "TcO_2" in these Tc-99m monophosphates was determined to be less than 1.5% (Table 1).

TABLE 3. DISTRIBUTION OF Tc-99m-LABELED PHOSPHATES IN RATS AT 2 HR POSTINJECTION

	% dose/organ (range)*				
	PAA	PPA	MPPA	CAP	MDP
Blood	2.92 (2.43–3.26)	1.48 (1.39–1.59)	21.9 (20.0 –24.2)	3.87 (3.41–4.62)	1.07 (0.85–1.41)
Muscle	3.75 (3.63–3.87)	2.13 (1.84–2.37)	16.2 (14.6 –17.6)	3.76 (2.54–4.16)	1.43 (0.99–2.12)
Kidney (2)	1.99 (1.86–2.25)	2.11 (1.97–2.27)	3.17 (3.12– 3.23)	4.91 (4.52–5.47)	1.70 (1.12–2.55)
Liver	0.81 (0.69–1.05)	0.40 (0.34–0.45)	1.90 (1.82– 1.96)	1.55 (1.26–1.70)	8.10 (6.43–9.79)
Femur	0.93 (0.89–0.99)	0.33 (0.29–0.40)	0.47 (0.44– 0.49)	1.94 (1.80–2.07)	1.77 (1.56–1.96)
Tibia & fibula	0.69 (0.58–0.75)	0.29 (0.22–0.37)	0.40 (0.39– 0.41)	1.63 (1.50–1.72)	1.69 (1.53–1.97)

* Average value of three rats.

TABLE 4. DISTRIBUTION OF Tc-99m-LABELED PHOSPHATES IN RABBITS AT 2 HR POSTINJECTION

	% dose/organ (range)*				
	PAA	PPA	MPPA	CAP	MDP
Blood	5.21 (5.13– 5.29)	4.65 (4.49– 4.81)	6.09 (4.03– 8.15)	4.05 (3.69– 4.40)	0.77 (0.15– 1.47)
Muscle	3.54 (3.30– 3.78)	2.94 (2.54– 3.34)	2.98 (2.64– 3.32)	2.56 (1.90– 3.22)	1.25 (0.66– 1.16)
Kidney (2)	4.16 (3.98– 4.34)	1.86 (1.65– 2.06)	3.93 (2.43– 5.42)	3.99 (3.18– 4.79)	1.31 (0.71– 2.06)
Liver	2.33 (1.68– 2.97)	0.66 (0.65– 0.67)	1.40 (1.38– 1.41)	0.82 (1.76– 1.87)	2.37 (0.34– 6.07)
Femur	1.18 (1.11– 1.24)	0.67 (0.54– 0.80)	0.94 (0.84– 1.03)	2.90 (2.65– 3.14)	2.68 (2.22– 3.31)
Tibia & fibula	1.15 (0.93– 1.27)	0.61 (0.48– 0.73)	0.82 (0.72– 0.92)	2.55 (2.28– 2.81)	2.53 (2.06– 3.02)
Urine	51.2 (47.3 –55.2)	49.6 (40.1 –59.1)	54.0 (53.1 –54.9)	30.0 (29.8 –30.1)	24.7 (15.3 –38.0)

* Average value of two to six rabbits.

TABLE 5. DISTRIBUTION OF Tc-99m CAP IN RATS

	% dose/organ (range)*				
	15 min	30 min	1 hr	2 hr	4 hr
Blood	9.73 (8.54–10.9)	6.84 (6.58– 7.05)	5.99 (5.71–6.46)	3.87 (3.41–4.64)	3.71 (3.56–3.89)
Muscle	11.8 (8.79–14.3)	8.93 (7.81–10.0)	5.55 (5.10–5.99)	3.76 (3.54–4.16)	3.52 (3.26–3.66)
Kidney (2)	4.27 (4.11– 4.58)	4.64 (4.56– 4.70)	4.74 (4.37–5.01)	4.91 (4.52–5.47)	5.16 (4.71–5.46)
Liver	5.45 (4.82– 6.44)	2.21 (1.85– 2.51)	2.47 (2.02–3.05)	1.55 (1.26–1.70)	1.27 (1.16–1.44)
Femur	1.56 (1.35– 1.72)	1.86 (1.63– 1.96)	1.86 (1.67–2.00)	1.94 (1.80–2.07)	1.79 (1.70–1.96)
Tibia & fibula	1.31 (1.25– 1.38)	1.57 (1.32– 1.73)	1.63 (1.44–1.76)	1.63 (1.50–1.72)	1.57 (1.45–1.75)

* Average value of three rats.

TABLE 6. CONCENTRATION OF Tc-99m-LABELED PHOSPHATES IN DAMAGED RAT MYOCARDIUM (MN)*

Agent	% dose/g MN	MN-to-normal ratio	MN-to-muscle ratio	MN-to-blood ratio
PPi	2.76 ± 0.55	21.11 ± 8.33	59.38 ± 3.33	19.49 ± 2.03
CAP	3.05 ± 0.64	26.85 ± 5.61	60.13 ± 9.38	9.33 ± 1.17
PAA	0.60 ± 0.14	7.25 ± 0.52	16.02 ± 4.67	3.42 ± 0.76

* Average value of four to six rats ± S.D.

Table 2 contains distribution data for Tc-99m PAA in rats. The femur uptake at 2 hr postinjection was 0.93% of the dose, which is equivalent to ~18% dose in total bone, showing that this Tc-99m-labeled monophosphate does localize in bone. The femur uptake reached a maximum at 2 hr postinjection and the blood clearance rate was not unusually fast or slow.

The two important criteria for comparing bone-scanning agents are bone uptake and blood clearance. For Tc-99m PAA, these are comparable with Tc-99m PPi but inferior to Tc-99m MDP. In an attempt to improve the bone uptake and/or increase the blood clearance rate, two PAA derivatives, PPA and MPPA, and one monophosphate, CAP, were prepared and studied.

Tables 3 and 4 show the comparative studies of these Tc-99m monophosphates, and Tc-99m MDP, in rats and rabbits at 2 hr. In rats, an unusually high liver uptake was observed for Tc-99m MDP (Table 3). Similar results have been reported by Weber et al. (*15*), who have suggested that rats are poor models for this bioassay.

The femur uptakes of Tc-99m and Tc-99m MPPA in both rats and rabbits are much lower than that of the parent compound, PAA (Tables 3 and 4). This reduction could be the result of the decreased acidity because of the electron-donating effect of the added methyl groups, or it could be due to the changed P-C-C bond angle. We plan to investigate the effect of electron-withdrawing groups—e.g., fluoro- or chloro-—on the distribution of labeled monophosphates. It is difficult to predict the effect of these changes, but only this kind of study of structure-distribution relationship will provide the information needed to design better radiopharmaceuticals.

The femur uptake of Tc-99m CAP at 2 hr postinjection was 1.9% and 2.9% of the dose in rats and rabbits, respectively, which corresponds to 38% and 58% of the dose in total bone, assuming that the femur uptake is 5% of the total-bone uptake (Tables 3 and 4). These values are slightly higher than those observed for Tc-99m MDP under the same experimental conditions. The biodistribution of Tc-99m CAP in rats (Table 5) showed the bone uptake reaching a maximum at 2 hr postinjection, and a blood clearance similar to that of Tc-99m PAA.

In the model of acute myocardial necrosis, CAP showed good necrosis localization (3% dose/g MN and 27:1 MN-to-normal ratio), which is as good as the commonly used PPi (Table 6). By contrast, PAA has a much lower necrosis uptake and the ratio of MN to normal heart was one-third that of PPi. In preliminary studies, PPA and MPPA also showed very little uptake in lesions. Generally, the necrosis-localizing properties for these monophosphates parallel their bone localization.

In summary, bidentate monophosphates can form complexes with Sn(II)-reduced pertechnetate in high yield. They all show bone uptake, and among them Tc-99m CAP is the best, with a total-bone uptake at 2hr of 38% and 58% in rats and rabbits, respectively. Its blood clearance rate is slow, however, making it less attractive than Tc-99m MDP as a

bone-localizing agent. Tc-99m CAP also has good myocardial-necrosis localization in rats, with an uptake similar to that of Tc-99m PPi.

FOOTNOTES

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‡ Sigma Chemical Corporation, St. Louis, Missouri.

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