Bone-Seeking Properties of Tc-99m Carbonyl Diphosphonic Acid, Dihydroxy-Methylene Diphosphonic Acid and Monohydroxy-Methylene Phosphonic Acid: Concise Communication

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Carbonyl diphosphonic acid, dihydroxy-methylene diphosphonic acid and monohydroxy-methylene diphosphonic acid were synthesized and labeled with Tc-99m. Their chemical structures were confirmed and their bone-seeking relationships were compared with those of Tc-99m MDP in experimental animals. Monohydroxy-methylene diphosphonic acid was found to have higher bone affinity and faster blood clearance than MDP, whereas carbonyl diphosphonic acid and dihydroxy-methylene diphosphonic acid demonstrated lower bone affinity and slower blood clearance than MDP.

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Technetium-99m-labeled methylene diphosphonic acid (MDP) is generally considered the radiopharmaceutical of choice for skeletal imaging. Analogs of MDP have been studied (*1,2*) in an attempt to improve bone localization relative to the background activity in blood and muscle. As part of a continuing investigation of the effect, on bone localization, of substitutions at the bridge carbon atom of MDP, we designed and synthesized carbonyl diphosphonic acid, dihydroxy-methylene diphosphonic acid, and monohydroxy-methylene diphosphonic acid, and compared them with MDP for bone-seeking properties in the rat.

METHODS

Syntheses (Fig. 1). *Tetrasodium carbonyl diphosphonate (Compound A).* To a solution of 12.5 g (0.05 mole) of dichloromethylene diphosphonic acid* in 75 ml of water was added 32 g (0.80 moles) of NaOH. The solution was heated at reflux for 1 hr and then cooled to room temperature. Methanol (500 ml) was added to precipitate the crude product. The precipitate was redissolved in a minimum amount of water and the pH adjusted to 10.5 with 6 *N* HCl. Yellow crystals were collected and washed, first with acetone and then with ether. Melting point >300°C. The yield was 11.1 g (91%). Infrared spectrum absorption (KBr) showed an absorption band at 1612 cm^{-1} (C = 0 strech). Analyses for C, P, and Na are shown in Table 1.

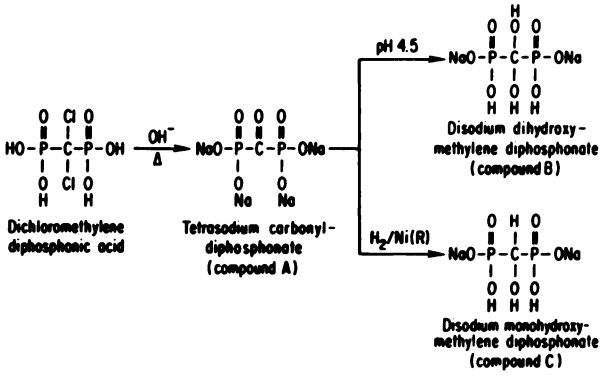

FIG. 1. Syntheses of Compounds A, B, and C.

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TABLE 1. ANALYTICAL RESULTS AND Tc-99m LABELING EFFICIENCIES IN DIPHOSPHONIC ACIDS*

Compound	For-mula	% Carbon Calc'd	% Carbon Found	% Hydrogen Calc'd	% Hydrogen Found	% Phosphorus Calc'd	% Phosphorus Found	% Sodium Calc'd	% Sodium Found	Labeling efficiency
Tetrasodium carbonyldiphosphonate (Compound A)	$CO_7P_2Na_4$	4.32	4.41	—	—	22.29	22.43	33.10	33.01	>96%
Disodium dihydroxy-methylene diphosphonate (Compound B)	$CH_4O_8P_2Na_2$	4.77	4.65	1.60	1.57	24.59	24.68	18.25	18.17	>96%
Disodium monohydroxy-methylene diphosphonate (Compound C)	$CH_4O_7P_2Na_2$	5.09	5.13	1.71	1.73	28.25	28.46	19.49	19.26	>96%

* Microanalyses by Galbraith Laboratories Inc., Knoxville, TN.

Disodium dihydroxy-methylene diphosphonate (*Compound B*). Compound A (4.8 g, 0.02 mole) was dissolved in 25 ml of water, and 2 *N* HCl was added to adjust the pH to 4.5. The bright yellow color of the basic solution faded during the pH change. On standing, 3.9 g was obtained, for a 78% yield. Melting point >300°C. Analyses for C, H, P and Na are shown in Table 1.

Disodium monohydroxy-methylene diphosphonate (*Compound C*). Compound A (4.8 g, 0.02 mole) in 100 ml water was subjected to 300 lb/in^2 of hydrogen for 8 hr at 100°C in presence of Raney nickel catalyst. The reaction mixture was cooled to room temperature and

TABLE 2. TISSUE DISTRIBUTION OF Tc-99m DIPHOSPHONIC ACIDS IN RATS* AT 2 HR AFTER I.V. INJECTION

Organ	MDP	A	B	C
	Percent injected dose per gram tissue			
Blood	0.043 ± 0.018	0.071 ± 0.025	0.062 ± 0.021	0.021 ± 0.014
Spleen	0.035 ± 0.012	0.122 ± 0.061	0.047 ± 0.020	0.054 ± 0.027
Liver	0.027 ± 0.019	0.214 ± 0.084	0.051 ± 0.027	0.098 ± 0.020
Kidney	0.701 ± 0.531	2.693 ± 1.009	3.614 ± 1.278	0.929 ± 0.362
Heart	0.020 ± 0.008	0.043 ± 0.006	0.040 ± 0.006	0.016 ± 0.005
Lung	0.042 ± 0.020	0.093 ± 0.015	0.076 ± 0.009	0.033 ± 0.010
Leg muscle	0.007 ± 0.003	0.013 ± 0.004	0.011 ± 0.004	0.005 ± 0.002
Femur	1.484 ± 0.195	0.961 ± 0.015	1.143 ± 0.126	2.579 ± 0.211
	Percent doses/100 g body weight			
Blood	0.195 ± 0.082	0.323 ± 0.114	0.282 ± 0.095	0.095 ± 0.064
Spleen	0.159 ± 0.054	0.555 ± 0.277	0.214 ± 0.090	0.245 ± 0.123
Liver	0.123 ± 0.083	0.973 ± 0.382	0.232 ± 0.123	0.445 ± 0.091
Kidney	3.186 ± 2.41	12.241 ± 4.586	16.427 ± 5.810	4.223 ± 1.645
Heart	0.091 ± 0.036	0.195 ± 0.027	0.182 ± 0.027	0.073 ± 0.023
Lung	0.190 ± 0.091	0.423 ± 0.068	0.345 ± 0.041	0.150 ± 0.045
Muscle	0.030 ± 0.014	0.059 ± 0.018	0.050 ± 0.018	0.023 ± 0.009
Femur	6.745 ± 0.886	4.368 ± 0.068	5.195 ± 0.573	11.723 ± 0.959
	Target-to-nontarget ratios (g/g)			
Femur/blood	34.49	13.53	18.43	122.81
Femur/liver	54.93	4.49	22.41	26.06
Femur/muscle	211.85	73.92	103.90	515.80

* Six animals, mean ± s.d. A, B, and C see text.

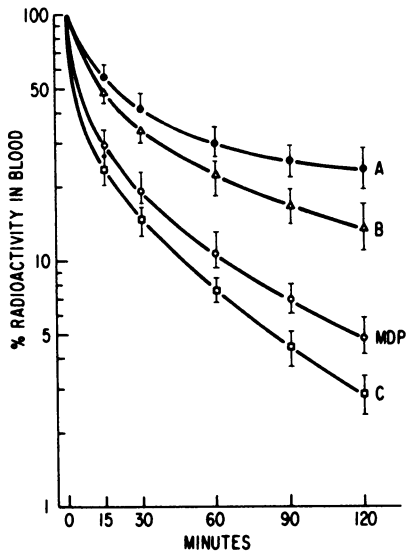

FIG. 2. Blood clearance of Tc-99m diphosphonic acids in rats.

the solution was titrated to pH 5.0 with 2 *N* HCl. A small amount of ethylenediaminetetra acetic acid was added to complex the Ni^{2+} in solution; then methanol was added. The product was collected, crystallized, and recrystallized from methanol/water (6/1). The yield was 3.5 g (75%). Mp 297–300°C; P-31 nmr spectrum: $\lambda = -15.0$ ppm (doublet, j = 15 cps). Analyses for C, H, P, and Na are shown in Table 1.

Tc-99m labeling. Compounds A, B, C, and MDP† were labeled with Tc-99m in the presence of tin (II) as follows: 5 mg (0.02 millimole) of each acid were dissolved in 5 ml of normal saline. To this solution was added 0.01 ml of 0.1% $SnCl_2$/1 *N* HCl solution (5 μmole of $SnCl_2$). Technetium-99m as pertechnetate, 5–6 mCi, was then added and the pH adjusted to 6.5–7.0 with 5% $NaHCO_3$ solution. The solution was then passed through a 0.22-μm sterile Swinnex filter. Labeling efficiencies were determined by instant thin layer chromatography in acetone and saline systems (*4*) (Table 1).

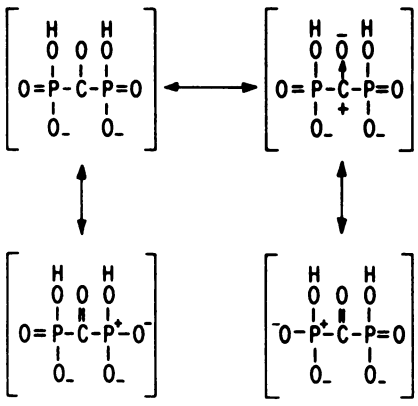

FIG. 3. Resonance structures of carbonyldiphosphonic acid (Compound A).

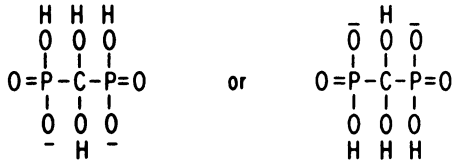

FIG. 4. Ionic structures of dihydroxy-methylene diphosphonic acid (Compound B).

Animal studies. Tissue distribution studies of each compound were performed on Sprague-Dawley male rats (200–220 g), 2 hr after tail-vein injection of 0.3–0.4 ml (300–400 μCi, 2.3 μg of acid) of the labeled radiopharmaceutical. The organs were removed and weighed; radioactivity content was measured in a well scintillation counter. Radioactive counts were corrected for decay. These data are summarized in Table 2. Venous blood samples for clearance studies were obtained in at least six rats at 15, 30, 60, 90, and 120 min after injection.

RESULTS AND DISCUSSION

The tissue distribution studies revealed that relative bone concentrations at 2 hr after injection occurred in the order of C > MDP > B > A (Table 2). The speed of blood clearance was also found in the order of C > MDP > B > A (Fig. 2). These results indicate that Compound C is superior to MDP for bone localization, whereas Compounds B and A are inferior.

The chemistry of Tc-99m MDP and the mechanism of its localization are still not fully understood. It is of interest to quantitate the differences in bone localization resulting from slight modifications of chemical structure. In view of the chemical properties and stereochemical structures of these diphosphonic acids, the following interrelationships between chemical structure and bone localization are suggested.

Chemical structure conformation. A single-crystal structure of MDP has been studied by DeLeMatter (*5*), and the crystal structure of carbonyl-diphosphonic acid (A) was examined by Uchtman (*6*). Both compounds suggested a staggered conformation. However, dihydroxy-methylene diphosphonic acid (B) and monohydroxy-methylene diphosphonic acid (C) were considered to have an eclipsed conformation (*7*). Since MDP with its staggered conformation and Compound C with its eclipsed conformation showed excellent avidity for bone, these structural conformations seem not to be a critical requirement for better bone localization in case of MDP and monohydroxy-methylene diphosphonic acid (C).

Resonance structure and keto-enol tautomerism. The degree of bone localization increased remarkably when the methylene hydrogen was replaced by a hydroxy moiety as in monohydroxy-methylene diphosphonic acid (C), but it decreased substantially when both hydrogens were substituted by dihydroxy groups, as in dihydroxy-

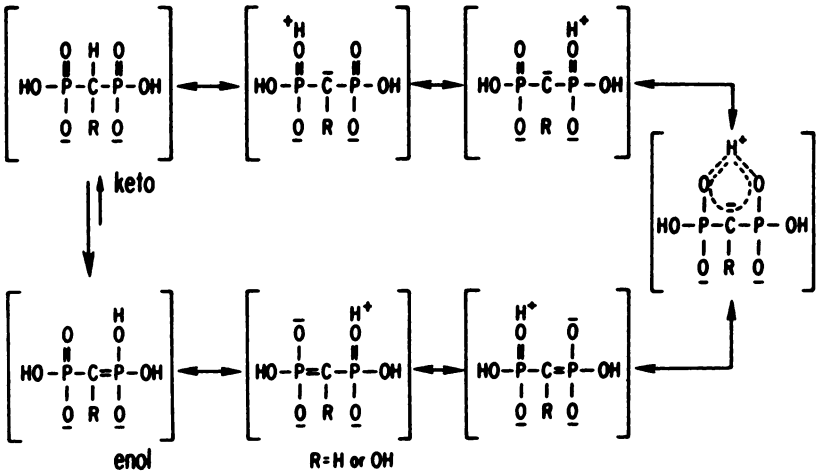

FIG. 5. Resonance structures and keto-enol tautomerism of monohydroxy-methylene diphosphonic acid (Compound C) and MDP.

methylene diphosphonic acid (B). Compounds A and C and MDP can be stabilized by the resonance structures (Figs. 3 and 5). Compound B has no resonance structure (Fig. 4). The stability from the resonance structure may enhance metal chelate formation. Compound C or MDP is likely to exist as a carbanion structure (Fig. 5), which behaves as a nucleophile, whereas Compound A can only exist as a carbonium-ion structure (Fig. 3), which behaves as an electrophile. The difference between these two types of structure should also affect metal binding as well.

The keto-enol tautomerism possible with MDP and Compound C, but not with Compounds A and B, may play another important role in bone localization, as is evidenced by the superior bone-seeking properties of Compound C and MDP. In addition to the use of the phosphoryl oxygen from each phosphonate group to chelate Tc-99m, a second binding site may be created by keto-enol tautomeric equilibrium. Thus, in the case of MDP and Compound C, the molecule could be further stabilized by intramolecular hydrogen binding or by metal chelation (Fig. 6). Compounds A and B could not exist in such a tautomeric structure.

In conclusion, these studies indicate the similarities of these diphosphonic acids. Both MDP and monohydroxy-methylene diphosphonic acid (C) undergo excellent binding with Tc-99m. The active hydrogen of the methylene group, which could contribute to the keto-enol tautomerism in MDP and monohydroxy-methylene diphosphonic acid (C), may play an active role in stabilization of the structure by hydrogen binding or by metal chelation, resulting in improved avidity for bone. Compounds A and B lacked such tautomerism and exhibited poor localization in bone and higher activity in blood and muscle. It is hoped that these results may contribute to the design of better bone-seeking radiopharmaceuticals.

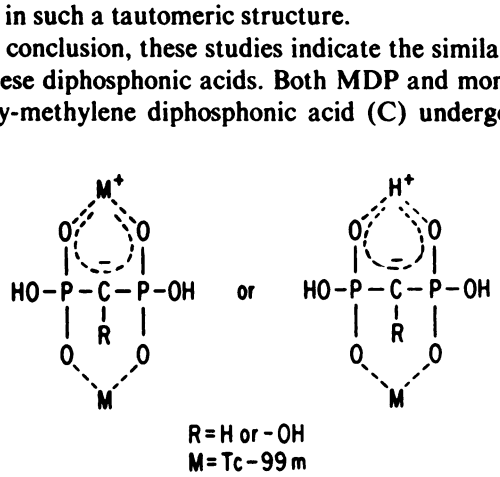

FIG. 6. Proposed structures of MDP or Compound A chelated to Tc-99m.

FOOTNOTES

* Proctor and Gamble Co., Cincinnati, OH.
† P-L Biochemical Laboratories, Inc., Milwaukee, WI.

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