# Technetium-99m-Labeled Phosphonic Acid Analog of Serine: Bone Uptake

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A phosphonic acid analog of serine (PAAS), its P-methyl derivative, and an allied compound were labeled with $^{99m}$Tc, and evaluated in experimental animals. These molecules were able to bind $^{99m}$Tc in the presence of stannous ions, but the biologic behavior of the later two labeled compounds was quite different from that of the first one. Technetium-99m-labeled PAAS behaved like $^{99m}$Tc-labeled methylene diphosphonate, but its P-methyl derivative and the third agent showed little bone accumulation. It appears that both hydroxyl groups attached to the phosphorus atom are essential for uptake in bone, but that only one is required for binding $^{99m}$Tc.

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Bone imaging, by means of technetium-99m-($^{99m}$Tc) labeled diphosphonates, has been widely utilized. However, despite studies on the behavior of a number of structural analogs of methylene diphosphonate (MDP), information on the requirement needed for $^{99m}$Tc binding and subsequent uptake in bone is incomplete (*1–3*). We, therefore, analyzed 1-amino-2-hydroxy-ethane phosphonic acid which is the phosphonic acid analog of serine (PAAS), and its P-methyl derivative or 1-amino-2-hydroxy-ethane P-methyl phosphonic acid (P-meth) for their ability to bind cationic species of $^{99m}$Tc. We studied the labeled complexes for their biologic behavior with respect to bone-seeking properties. An allied compound, 2-amino-3-phosphono-propionic acid (2,3-P), was included in the study to supplement the information. Results were compared with $^{99m}$Tc-labeled methylene diphosphonate.

## MATERIALS AND METHODS

### Labeling of Compounds

PAAS and P-meth were obtained from the syntheses reported by Lejczak and co-workers (*4*). A different synthetic route for this group of compounds has also been recently reported (*5*). The third compound (2,3-P) was obtained commercially.* Each compound was dissolved in 0.9% saline (5 mg/ml), and mixed in a ratio of 10:1 with a freshly prepared solution (5 mg/ml) of stannous chloride crystals in 0.1 N HCl. The pH of the resultant mixture was adjusted to 5–6 with 0.5 N NaOH. One milliliter of this solution was mixed with [$^{99m}$Tc]pertechnetate eluate (diluted in isotonic saline) to obtain the desired radiopharmaceutical containing 20–400 MBq/ml (about 0.5–10 mCi/ml). The product was further diluted in isotonic saline (preflushed with nitrogen gas) prior to animal studies. For the sake of comparison, studies were also carried out using commercially available MDP kits.† Binding of $^{99m}$Tc was analyzed by thin layer chromatography (TLC) by estimating presence of pertechnetate ion and colloidal materials. Gelman ITLC-SG plates were used, and separate strips were developed in two systems (either acetone or isotonic saline). Strips were cut into small sections, assayed for radioactivity, and the percent binding was calculated.

### Biodistribution Studies

Biodistribution of the labeled compounds was studied in male CD mice weighing between 30 and 40 g. They were injected with 0.2 ml of tracer doses through tail veins, and killed in groups of 3–5 animals at different time intervals (0.5, 1, 2, and 4 hr after injection of the tracer dose). A blood sample and major organs were obtained from each animal, and the samples were weighed. Radioactivity in these samples was measured using a well-type NaI(Tl) scintillation counter, and compared with a reference standard prepared by diluting a test dose to a known volume. A scintillation counter with a larger crystal was used for the assay of radioactivity in the carcass. Results were expressed as percent dose per g of tissue, and as percent dose per organ. The total blood volume was taken as 7% of the body weight. Activity in the tail (that was <5% of injected dose after 0.5 hr) was included in the carcass.

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**TABLE 1**
Biodistribution of Radioactivity in Mice at Different Times After i.v. Administration of $^{99m}$Tc-Labeled 1-Amino-2-Hydroxy-Ethane Diphosphonate*

| Tissue | % Dose/organ | | | |
|---|---|---|---|---|
| | 0.5 hr | 1 hr | 2 hr | 4 hr |
| Total blood | 1.42 ± 0.30 | 0.54 ± 0.12 | 0.42 ± 0.07 | 0.35 ± 0.10 |
| Heart | 0.04 ± 0.004 | 0.02 ± 0.01 | 0.01 ± 0.003 | 0.01 ± 0.001 |
| Lungs | 0.10 ± 0.02 | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.002 |
| Spleen | 0.02 ± 0.003 | 0.01 ± 0.001 | 0.01 ± 0.003 | 0.01 ± 0.003 |
| Liver | 0.53 ± 0.16 | 0.35 ± 0.08 | 0.34 ± 0.13 | 0.39 ± 0.15 |
| Kidneys | 1.31 ± 0.20 | 1.22 ± 0.34 | 0.74 ± 0.18 | 0.58 ± 0.16 |
| GI tract | 0.89 ± 0.12 | 0.78 ± 0.25 | 1.12 ± 0.14 | 1.06 ± 0.25 |
| Two femurs | 1.58 ± 0.17 | 1.55 ± 0.27 | 1.64 ± 0.18 | 1.53 ± 0.23 |
| Carcass | 28.0 ± 1.1 | 25.3 ± 1.5 | 23.5 ± 2.5 | 24.4 ± 1.9 |

* Phosphonic acid analog of serine (PAAS).

**Radionuclide Imaging**

Rabbits were anesthetized with ketamine, and 0.5 ml of the radiopharmaceutical containing 100–220 MBq (~3–5 mCi) of radioactivity was injected through the ear veins. Rabbits were then imaged between 1 and 2 hr, with attention to the thoraco-abdominal region, using a gamma camera.

## RESULTS AND DISCUSSION

Binding of $^{99m}$Tc, as determined by TLC, was over 95% in each case. All three labeled compounds appeared to be excreted rapidly in the urine. The total amount of radioactivity retained in various tissues and organs of mice fell to a level of about 30% within an hour. However, most of the radioactivity retained after 1 hr with PAAS was localized in the bone, and the pattern of biodistribution remained practically the same between 1 and 4 hr (Table 1). The uptake of PAAS in femurs was ~85% of that of MDP, and 6–10 times that of the other two compounds. Table 2 summarizes the relative biodistribution in mice at 2 hr. The 2-hr period was taken as a good representation of differences in the biologic behavior of these $^{99m}$Tc-labeled complexes. It was also possible to obtain bone images by 1 hr in rabbits with the phosphonic acid analog of serine (Fig. 1). Skeletal bone and the kidneys were clearly visualized. These images were similar to those obtained with $^{99m}$Tc-labeled MDP. The other two compounds predominantly showed the soft tissues, including the kidneys. The longer chain length of 2,3-P might have caused low uptake in bone (*6*).

The most characteristic feature of this study was the observed divergence in bone uptake between two closely related compounds: $HO \cdot CH_2 \cdot CH(NH_2) \cdot PO(OH)_2$ (the PAAS), and $HO \cdot CH_2 \cdot CH(NH_2) \cdot PO(OH)(CH_3)$ (its P-methyl derivative). They are structurally similar except that one of the OH groups in the phosphate moiety of PAAS is replaced with a $CH_3$ group. The bone uptake of PAAS was nearly as high as that with MDP. The uptake in bone was negligible with P-meth. The present

**TABLE 2**
Biodistribution of Radioactivity in Mice at 2 hr After i.v. Administration of $^{99m}$Tc-Labeled Compounds

| Tissue | % Dose/organ | | | |
|---|---|---|---|---|
| | MDP* | PAAS† | P-meth‡ | 2,3-P§ |
| Total blood | 0.39 ± 0.05 | 0.42 ± 0.07 | 1.49 ± 0.26 | 1.66 ± 0.48 |
| Heart | 0.02 ± 0.01 | 0.01 ± 0.003 | 0.02 ± 0.003 | 0.02 ± 0.004 |
| Lungs | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.07 ± 0.02 | 0.08 ± 0.04 |
| Spleen | 0.03 ± 0.01 | 0.01 ± 0.003 | 0.02 ± 0.01 | 0.03 ± 0.01 |
| Liver | 0.67 ± 0.15 | 0.34 ± 0.13 | 1.61 ± 0.33 | 2.12 ± 0.60 |
| Kidneys | 0.91 ± 0.14 | 0.74 ± 0.18 | 2.02 ± 0.39 | 2.81 ± 1.40 |
| GI tract | 1.08 ± 0.36 | 1.12 ± 0.14 | 3.48 ± 0.59 | 4.27 ± 1.17 |
| Two femurs | 1.91 ± 0.20 | 1.64 ± 0.18 | 0.17 ± 0.04 | 0.27 ± 0.13 |
| Carcass | 25.7 ± 2.7 | 23.5 ± 2.5 | 14.7 ± 4.0 | 17.0 ± 3.3 |

* MDP = Methylene diphosphonate.
† PAAS = Phosphonic acid analog of serine.
‡ P-meth = P-methyl derivative of PAAS.
§ 2,3-P = 2-amino-3-phosphono-propionate.

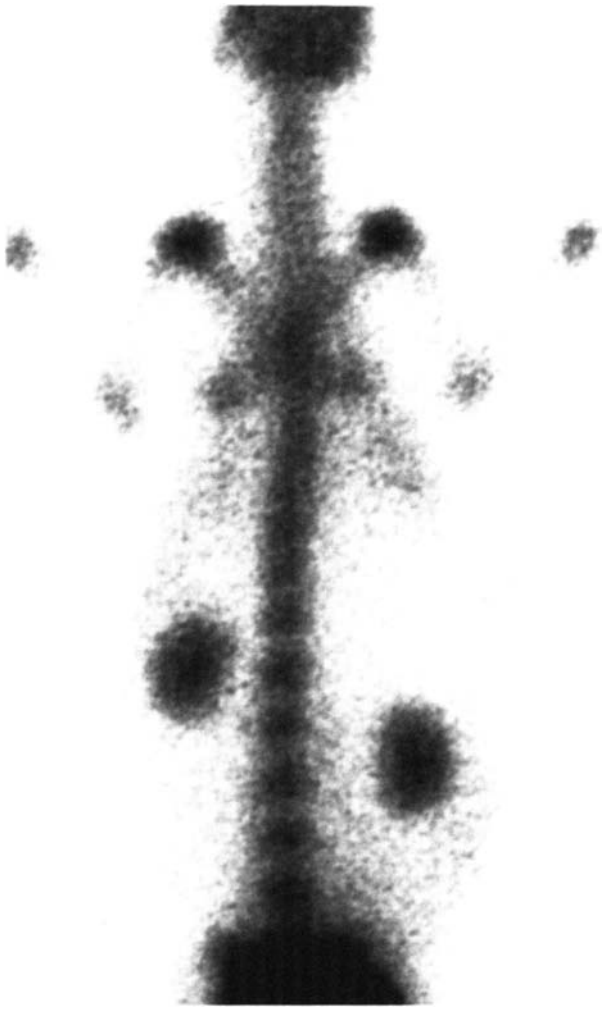

**FIGURE 1**
Posterior image of rabbit, covering thoraco-abdominal region, 1 hr after i.v. administration of $^{99m}$Tc-labeled phosphonic acid analog of serine. Vertebrae and kidneys are well defined

study adds further data to the structural requirements for $^{99m}$Tc-labeled bone-seeking phosphates. It suggests that both hydroxyl groups attached to the phosphorus are not required for binding technetium, but they are needed for significant uptake in bone. Whether this is due to the necessity for the hydroxyl per se, or is related to a changed structural configuration is presently uncertain. However, it is clear that PAAS, despite having but a single phosphate group, can serve as the basis of a suitable $^{99m}$Tc bone imaging agent. In this regard, it resembles monophosphates such as carbamyl phosphate (*7*).

## FOOTNOTES

* Polyscience Inc., Warrington, PA.

† DuPont NEN Medical Products, (Osteolite), North Billerica, MA.

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