# RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

## Synthesis, Radiotechnetium Labeling, and Comparison of Biologic Behavior of Longer-Chain Analogs of Methylene Diphosphonate

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***Polymethylene diphosphonic acids of different chain lengths (n = 2, 3, and 10) were synthesized and labeled with technetium-99m. Their biologic behavior was compared with that of Tc-99m-labeled methylene diphosphonate (MDP) in experimental animals. With n = 2 (ethylene diphosphonic acid), the compound resembled MDP in bone affinity. The longer-chain analogs had lower bone affinity.***

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Technetium-99m-labeled methylene diphosphonate (MDP) has been reported to be a superior agent for skeletal imaging (*1*). This compound (with linkage P-C-P), as well as imidodiphosphate (linkage P-N-P) and pyrophosphate (linkage P-O-P), have the phosphate groups separated by a single atom. Studies have not appeared on the effects of increasing the number of atoms between the two phosphate components. The objectives of the present study were to determine the Tc-99m binding to, and biologic behavior of, polymethylene diphosphonates [P-$(CH_2)_n$-P]. These compounds can be viewed as longer-chain analogs of MDP.

**Synthesis.** Three different polymethylene diphosphonic acids were synthesized by acid hydrolysis of the corresponding esters (*2*), these esters being obtained commercially*. Tetraethyl (or isopropyl) polymethylene diphosphonate (10 millimol) was added to 40 ml of conc. HCl and heated to reflux overnight. The reaction mixture was cooled to room temperature. The conc. HCl was evaporated under reduced pressure. The solid residue was washed with petroleum ether (30–60°C) and the product crystallized from ethanol-ether (5:1). The synthesized compounds were tested for purity. Thin-layer chromatography (silica gel with 7:3, acetone: ethylacetate) showed a single spot. Infrared spectra indicated the appropriate absorption maxima. The physical properties and analytical data on the acids are summarized in Table 1.

**Radiolabeling.** The three newly synthesized compounds (ethylene diphosphonate, or EDP, propylene diphosphonate, or PDP, and decamethylene diphosphonate, or DDP), and a commercially available MDP†, were labeled with Tc-99m in the presence of tin(II). The acids were dissolved in isotonic saline to obtain a final solution of 0.2–0.4% when the pH was adjusted to between 6 and 7. A tin(II) solution was prepared with 0.1% of crystalline stannous chloride in 0.5 *N* HCl. Phosphonate and tin(II) solutions were mixed in a ratio of 10:1, and the final pH was adjusted to between 5 and 6. The mixture was purged with $N_2$ gas, passed through a 0.22-$\mu$m filter in aliquots of 1 ml for "kits," and stored in the frozen condition. Lyophilized Sn-MDP kits were also obtained as an Investigational New Drug from a commercial source‡. [$^{99m}$Tc] pertechnetate (obtained from a generator) was added to the kit at room temperature (1–30 mCi in 1–2-ml eluate). Paper chromatography of the labeled compounds was carried out with two different systems: 85% methanol, and isotonic saline.

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**TABLE 1. PHYSICAL PROPERTIES AND ANALYTICAL DATA OF POLYMETHYLENE DIPHOSPHONIC ACIDS**

| Name | Chain length (n) | Yield (%) | M.P. (°C) | Carbon (%) | | Hydrogen (%) | | Phosphorus (%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calculated | Found | Calculated | Found | Calculated | Found |
| Ethylene disphosphonic acid (EDP) | 2 | 90 | 223 | 12.63 | 12.45 | 4.24 | 4.19 | 32.60 | 32.63 |
| Propylene diphosphonic acid (PDP) | 3 | 81 | 179 | 17.67 | 17.71 | 5.29 | 5.31 | 30.36 | 30.29 |
| Decamethylene diphosphonic acid (DDP*) | 10 | 74 | 206 | 39.74 | 39.81 | 8.00 | 7.98 | 20.50 | 20.52 |

* DDP was relatively less soluble than EDP or PDP.

**TABLE 2. TISSUE DISTRIBUTION OF RADIOACTIVITY IN IMPORTANT ORGANS OF MICE, 2 HR AFTER I.V. INJECTION OF THE VARIOUS RADIOPHARMACEUTICALS**

| Organs | % dose/organ for different preparations | | | |
|---|---|---|---|---|
| | MDP | EDP | PDP | DDP |
| Total blood | 0.49 ± 0.15 | 0.68 ±0.22 | 0.91 ± 0.27 | 2.04 ± 0.93 |
| Femurs | 1.93 ± 0.12 | 1.89 ± 0.18 | 0.65 ± 0.15 | 0.12 ± 0.08 |
| Liver | 0.33 ± 0.10 | 0.59 ± 0.14 | 1.10 ± 0.46 | 4.41 ± 1.74 |
| Kidneys | 0.48 ± 0.13 | 0.68 ± 0.21 | 1.26 ± 0.36 | 5.16 ± 1.21 |
| G.I. tract | 0.97 ± 0.28 | 0.87 ± 0.34 | 1.40 ± 0.29 | 2.28 ± 0.93 |
| Carcass | 27.40 ± 3.2 | 25.8 ± 4.1 | 25.1 ± 3.9 | 24.9 ± 4.4 |

**Biologic studies.** Biologic evaluations were carried out with the Tc-99m-labeled compounds (EDP, PDP, DDP, and the commercially obtained MDP kits). Tissue-distribution studies were performed in at least five mice per compound. They were sacrificed 2 hr after i.v. injection of tracer doses of the labeled compounds (5–20 $\mu$Ci in 0.2 ml). Organs were removed, weighed, and counted. The carcass was also assayed for radioactivity. Relative distribution of radioactivity in various organs was also assessed in two rabbits per compound by imaging with a gamma camera at different times between 0.5 and 3 hr following i.v. injection of a tracer dose 0.5–1 mCi.

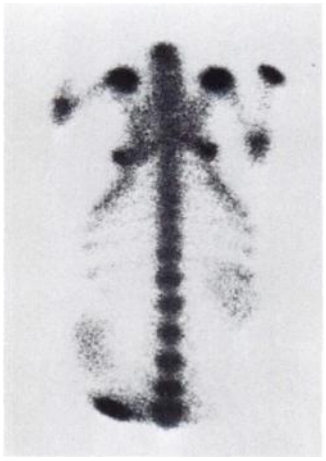

**FIG. 1.** Scintiphoto (posterior view) of thoraco-abdominal region of rabbit, obtained 2 hr after administration of Tc-99m-labeled ethylene diphosphonate (EDP). Gamma camera has high-resolution collimator.

## RESULTS AND DISCUSSION

Chromatographic data did not show any significant amount of radioactive colloid or free pertechnetate. Less than 8% of the radioactivity remained at the origin with saline and over 97% remained with MeOH. The biologic behavior of these labeled compounds could be described by considering the uptake of radioactivity in some of the important organs in mice. This is shown in Table 2. Uptake in femurs was high only with MDP and EDP, and it decreased with further increase in chain length. Moreover, low uptake of radioactivity in the liver and the gastrointestinal tract with all the compounds indicated absence of any significant amounts of Tc-99m-labeled colloidal complex or free pertechnetate, and suggested chelation of reduced Tc-99m with all these compounds. Relatively low activity in the carcass reflected effective excretion, most likely in the urine. Radionuclide imaging of rabbits showed a distribution like that in mice; the urinary bladder accumulated considerable amounts of radioactivity. Ethylene diphosphonate (EDP) appeared to be a good bone-imaging agent; a typical example is shown in Fig. 1. The biologic behaviors of Tc-99m-labeled MDP preparations made in the laboratory, and those from a commercial kit, were essentially the same.

These studies indicate great similarities between

MDP and EDP. They are comparable to other previously used Tc-99m-labeled bone-imaging phosphate complexes (*3*). This is in contrast to the compounds having increased chain length. It appears that the length of the carbon chain between the two phosphorus atoms, as well as the bond angle (*4*), do not critically affect the complexing with Tc-99m; they are important, however, for its uptake in bone. The additional carbon in EDP might permit a wider range of substitutions in an effort to find useful radiopharmaceuticals.

FOOTNOTES

* Organomet Inc., East Hampstead, N.H.
† P-L Biochemicals, Inc., Milwaukee, Wisc.
‡ E. R. Squibb & Sons, Inc., New Brunswick, N.J.

ACKNOWLEDGMENT

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