A COMPARATIVE STUDY OF THREE ^{99m}Tc-LABELED PHOSPHORUS COMPOUNDS AND ^{18}F-FLUORIDE FOR SKELETAL IMAGING

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The advantages of ^{99m}Tc-labeled bone-imaging agents have stimulated a considerable amount of work toward the development of these radiopharmaceuticals. A comparison was made of the biologic properties of ^{99m}Tc-Sn-polyphosphate, Sn-pyrophosphate, Sn-1-Hydroxyethane-1-diphosphonate (EHDP), and ^{18}F-fluoride.

Three-hour distribution studies were done in rabbits for each radiopharmaceutical. These radiopharmaceuticals were also administered to patients, and scans were obtained between 3 and 4 hr. These scans were evaluated for background activity due to soft-tissue and blood retention. The blood clearance of ^{99m}Tc and ^{18}F was also determined in patients.

On the basis of the above experiments, it was concluded that ^{99m}Tc bone-imaging radiopharmaceuticals available to date leave something to be desired in their blood and soft-tissue clearance and high kidney uptake. While the physical properties of ^{18}F are poor, the biologic properties are still superior for bone-imaging. The biologic properties of polyphosphate are significantly worse than pyrophosphate or EHDP. These latter two agents are more similar to ^{18}F in their blood clearance and soft-tissue uptake.

The physical properties of ^{99m}Tc favor its use in labeling a bone-localizing agent. Its 6-hr half-life and negligible beta-like radiation permit the clinician to administer several millicuries with relatively low radiation burdens. The ability to administer large activities to a patient permits both more rapid scanning and the possibility of total-body scans. Furthermore the 140-keV photon from the ^{99m}Tc isomeric transition is close to ideal for camera imaging. During the last 3 years, a number of ^{99m}Tc-labeled phosphorus compounds have been found to localize in bone and are currently in use as bone-scanning agents (*1–12*). In this report a comparison is made between ^{99m}Tc-Sn-pyrophosphate, ^{99m}Tc-Sn-1-hydroxy-ethane-1-diphosphonate (EHDP), and ^{99m}Tc-Sn-polyphosphate. These compounds are compared with ^{18}F-fluoride.

MATERIALS AND METHODS

The labeled phosphorus compounds used in this study were prepared from sterile pyrogen-free kits supplied by commercial manufacturers. Polyphosphate kits were supplied by Diagnostic Isotopes (DI) and EHDP was supplied by both Procter and Gamble (P & G) and Diagnostic Istopes. Pyrophosphate kits took two forms, lyophilized kits supplied by both CIS and CISR and a nonlyophilized kit also supplied by CIS. The radiopharmaceuticals were prepared and administered according to the protocol suggested by the manufacturer. In general these radiopharmaceuticals were prepared by adding between 2 and 6 ml of sterile pyrogen-free pertechnetate solution to the vials, which contained a lyophilized mixture of Sn(II) and the respective phosphorus compound. The CIS nonlyophilized pyrophosphate was also prepared in this manner. The radiopharmaceuticals were used within 2 hr after pertechnetate was first added to the vial. The ^{99m}Tc-pertechnetate solution used to reconstitute the above kits was prepared by MEK

Received Aug. 14, 1973; revision accepted July 23, 1974.

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extraction of $^{99}MoO_4^-$ in 6 *N* NaOH solution (*13*). Fluorine-18-fluoride was obtained by a procedure described in previous publications (*14,15*).

Distribution studies of each radiopharmaceutical were carried out in four 6-month-old male New Zealand white rabbits. Millicurie amounts of the agent were administered intravenously to the animals, which were sacrificed at 3 hr. After dissection of the animals, the organs were counted in the well of a Searle Radiographics Mediac dose calibrator. This was possible because well-response studies in this laboratory indicated a very low sensitivity to geometry within the lower 6-cm volume of the well. Large organs such as the liver, which did not fit conveniently into the counting region of the well, were counted in two or more parts. Because of this ability to count large samples with uniform counting geometry, it was possible to determine the activity in the skeleton by counting all of the animal's bones rather than relying upon a determination made from the concentration in one type of bone times the weight of the total bone (a value usually estimated by assuming 10% of the animal's body weight). The percentage of the dose per sample was obtained by comparison with a standard. The standard consisted of 5 ml of solution in a 13 × 100-mm test tube. Therefore the standard was distributed in the lower 6 cm of the well with a geometry similar to the bone samples.

The blood clearance of these agents in humans was determined by taking periodic blood samples and comparing the activity in a known volume of blood to a standard. No more than three samples were taken from any one patient; therefore, the blood clearance curves are a composite prepared with data derived from several patients.

The clinical evaluation of these agents was done on scans taken 3–5 hr after the administration of the radiopharmaceutical. The evaluation was done by six individuals, physicians and scientists, to get both a medical and technical point of view. Background due to soft tissue and blood activity was given 3–0 points ranging from no observable to very high background. Renal uptake was rated by comparing the film density over the kidneys to film density over the adjacent spine on scans of the posterior lumbar region. From 3–0 points were given for ratios of kidney/spine $<<1$ — >1. The general appearance and diagnostic potential of the scans was given 3–0 points, rating from excellent to poor.

RESULTS AND DISCUSSION

The 3-hr rabbit distribution studies summarized in Table 1 suggest a reasonable bone uptake for all of the radiopharmaceuticals used in this study.

The CISR lyophilized pyrophosphate kits and ^{18}F-fluoride have somewhat higher bone uptake than the other radiopharmaceuticals. It should also be noted that pyrophosphates from both CIS and CISR have a higher liver uptake than any of their counterparts. When comparing relative tissue uptake, it is more realistic to view the data in terms of the concentration in tissues as opposed to absolute uptakes. Furthermore, because a bone-scanning agent is being studied, a convenient form of presentation is the ratio of the percentage of the dose per gram of bone to the percentage of the dose per gram of the tissue under consideration. The higher the ratio, the better the distribution in favor of bone. The use of a ratio also obviates the need to normalize the data for body weight. Table 2 presents these ratios for several tissues with the different radiopharmaceuticals studied.

A comparison of tissue concentrations within any single radiopharmaceutical category indicates that the muscle distribution is low relative to bone for all of the agents studied. All of the ^{99m}Tc-phosphorus compounds appear to have a substantial kidney

TABLE 1. THREE-HOUR DISTRIBUTIONS OF ^{99m}Tc IN RABBITS

	Percentage of the administered dose					
Organ	Fluoride	DI polyphosphate	CIS lyophilized pyrophosphate	CISR lyophilized pyrophosphate	DI EHDP	P & G EHDP
Blood*	1.63	2.7	1.5	2.1	3.3	0.86
Muscle†	0.63	4.4	3.6	2.2	2.9	0.53
Kidney(s)	0.19	3.9	1.5	1.8	2.7	1.1
Liver	0.38	1.8	8.5	10.3	0.87	0.31
Spleen	0.038	0.03	0.29	0.50	0.006	0.005
Lungs	0.015	0.22	1.5	1.8	0.19	0.04
Skeleton	71.0	34.3	42.2	62.8	37.9	44.7
Urine	26.1	52.7	40.9	18.5	52.1	52.5

* 7% of body weight.
† 43% of body weight.

TABLE 2. RELATIVE CONCENTRATION OF DOSE IN BONE TO DOSE IN SOFT TISSUE

	% dose/gm bone*: % dose/gm organ					
Organ	Fluoride	DI polyphosphate	CIS lyophilized pyrophosphate	CISR lyophilized pyrophosphate	DI EHDP	P & G EHDP
Blood	55.0	9.8	26.3	26.0	12.0	55.8
Muscle	575.0	47.7	57.2	156.0	84.6	666.0
Kidney	39.0	0.9	1.9	3.13	1.4	3.8
Liver	136.0	12.2	3.3	4.5	23.7	87.0
Spleen	15.3	9.3	0.79	0.89	37.0	40.7
Lung	254.0	10.7	19.1	37.8	16.9	57.8

* Averaged values of % dose/gm tibias and % dose/gm femurs.

TABLE 3. RELATIVE CONCENTRATION OF ^{99m}Tc IN BONE AND SOFT TISSUE COMPARED WITH ^{18}F IN BONE AND SOFT TISSUE

	(% dose ^{99m}Tc/gm bone)/(% dose ^{99m}Tc/gm tissue) / (% dose ^{18}F/gm bone)/(% dose ^{18}F/gm tissue)				
Organ	DI polyphosphate	CIS lyophilized pyrophosphate	CISR lyophilized pyrophosphate	DI EHDP	P & G EHDP
Blood	0.18	0.48	0.48	0.22	1.02
Muscle	0.083	0.099	0.27	0.15	1.16
Kidney	0.023	0.049	0.080	0.036	0.097
Liver	0.095	0.024	0.033	0.17	0.64
Spleen	0.61	0.052	0.058	2.42	2.68
Lungs	0.042	0.075	0.149	0.067	0.23

uptake and pyrophosphate appears to concentrate in the liver and spleen. In fact the concentration of pyrophosphate in the spleen is greater than in bone. The fact that neither the spleen nor the liver is visualized on scans of patients is probably the result of species difference. Fluoride appears to have the best overall biologic distribution with a satisfactory ratio of bone to organ for all of the organs studied.

Because of its excellent biologic properties and its history of successful clinical applications (*11*), ^{18}F-fluoride was chosen as the agent to which the other radiopharmaceuticals would be compared. Just as a ratio of tissue concentration to bone concentration was used in comparing tissue uptake, the ratio of a parameter describing one radiopharmaceutical to that same parameter for fluoride could be used to compare radiopharmaceuticals. The parameter chosen was the ratio of the concentrations listed in Table 2. Because scans are set up over bone, this ratio has the advantage of already being normalized for bone. A ratio for a particular tissue that is greater than 1 implies the agent has a biologic distribution superior to fluoride; equal to 1 implies equally good distribution; and less than 1 implies a poorer distribution. Table 3 lists these ratios for the agents used in this study. It is apparent that in rabbits the P & G EHDP is superior to fluoride in its distribution to spleen and is comparable in its distribution to blood, liver, and muscle. This agent is relatively inferior to fluoride in its distribution to lung and kidneys. The P & G EHDP appears to be superior to a certain extent in its soft-tissue distribution to any of the other phosphorus compounds employed in this study. It is of interest to note the large differences in tissue distribution for EHDP from different manufacturers. The only significant difference between the kits is the amount of Sn(II) added. The DI kit contains about 2 mg of $SnCl_2 \cdot 2H_2O$ or ten times more than the P & G kit. Owing to the lack of sufficient animal distribution data for nonlyophilized pyrophosphate kits, this radiopharmaceutical is not compared in the aforementioned tables. There is some suggestion from the clinical study that nonlyophilized pyrophosphate may have a distribution superior to the EHDP.

Unfortunately, it is often difficult and possibly misleading to judge or compare radiopharmaceuticals intended for humans by their responses in animals. The blood clearance of radiopharmaceuticals used for bone imaging is quite important as demonstrated by Sr^{2+}, the rare earth agents, and even fluoride when patients are scanned too early before sufficient blood clearance has occurred. It is also important that the physical half-life of the radionuclide be

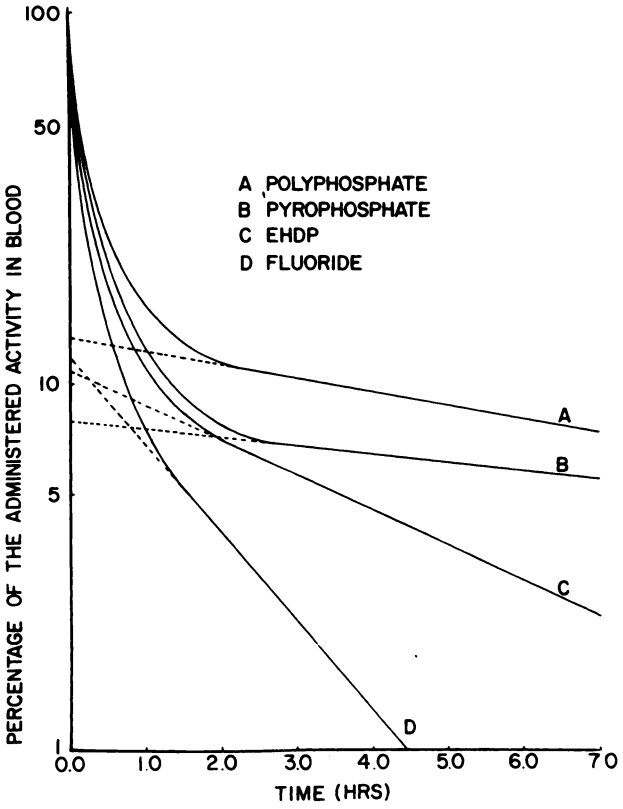

FIG. 1. Blood clearance of ^{99m}Tc-labeled phosphorus compounds and ^{18}F-fluoride. Pyrophosphate (37 patients); EHDP (24 patients); polyphosphate (17 patients); and fluoride (ten patients).

matched to the biologic properties of the radiopharmaceutical. For example, Sr^{2+} requires up to 7 days for satisfactory soft-tissue and bowel clearance prior to imaging. Therefore the 2.8-hr half-life of ^{87m}Sr is a gross mismatch of physical half-life to biologic properties. Likewise the 65-day half-life of ^{85}Sr is far too long. Because soft-tissue and blood clearance are the major limiting factors in scanning bone, a ^{99m}Tc radiopharmaceutical with a clearance closely matched to the 6-hr physical half-life is desirable. Figure 1 is a graph of the blood clearance of several ^{99m}Tc-phosphorus compounds and ^{18}F-fluoride in humans. The long-term clearance was obtained by a least-squares fit to the data. Although not identical, the blood clearances of both sources of EHDP were similar enough to be combined in a single curve. Similarly the three sources of pyrophosphate were also combined. The blood clearance follows a two-component first-order disappearance curve. Table 4 compares the blood clearance of the important long-term component for each of these radiopharmaceuticals. At 3 hr, the proper scanning time for ^{18}F-fluoride and the recommended scanning time for each of the other radiopharmaceuticals, it can be seen that ^{18}F has the lowest blood level, EHDP and pyrophosphate are two to three times greater, and polyphosphate is approximately four times greater than fluoride. Assume on a basis of acceptable past experience with fluoride that a 2% blood level is desired when performing a study; then the fourth column of Table 4 gives the time necessary to attain this level for each of the radiopharmaceuticals. Column five of Table 4 compares the physical half-life of the radionuclide associated with each agent with the time necessary to reach the 2% blood level. EHDP appears to have the closest match of half-life with clearance time at about 8 hr. The above comparison in which 2% is assumed to be ideal for all four radiopharmaceuticals is based upon the assumption that the uptake in the skeleton is also comparable. Since scans are set up over bone, if one radiopharmaceutical has a lower skeletal uptake than another, a greater blood clearance is required for comparable results. This factor may actually play a role in the case of EHDP and the CIS nonlyophilized pyrophosphate. The distinct impression of the individuals evaluating scans for this study was that nonlyophilized pyrophosphate had less background activity than EHDP. However, the blood levels with EHDP are lower by a factor of 2 at the time of scanning. This anomaly might be explained by a higher bone uptake with nonlyophilized pyrophosphate in humans.

The results of the clinical evaluation are summarized in Table 5. The total number of points accumulated by each radiopharmaceutical (method

TABLE 4. KINETIC PARAMETERS OF LONG-TERM COMPONENT OF THE ^{99m}Tc AND ^{18}F BLOOD CLEARANCE IN HUMANS

Radiopharmaceutical administered	Half-time of long-term blood clearance (hr)	Percentage of long-term component at time (0)	3.0-hr blood level (%)	Time to 2% level in blood (hr)	t(2%)/ radionuclide half-life
Fluoride	1.3	11.6	2.3	3.1	1.6
Polyphosphate	8.1	13.3	10.2	22.0	3.7
Pyrophosphate	10.5	8.0	6.8	21.0	3.5
EHDP	3.2	10.8	5.6	8.0	1.3

TABLE 5. EVALUATION OF BONE SCANS USING ^{99m}Tc-LABELED PHOSPHORUS AGENTS

Category	Polyphosphate	EHDP	Nonlyophilized pyrophosphate	Lyophilized pyrophosphate
Renal uptake	0.2	1.1	1.8	0.8
Background activity from soft tissue and blood	0.3	1.5	1.8	1.5
General appearance and diagnostic potential	0.9	2.2	2.4	2.4
No. of studies	12	26	5	28

Total no. of points accumulated in each category normalized to the no. of studies.

for point assignment described before) in each of the categories evaluated were normalized by the number of cases contributing to the total. Because very few clinical studies using DI EHDP and the CISR pyrophosphate were completed at the time of this writing, those studies that were available were combined under the respective general headings EHDP and lyophilized pyrophosphate. Table 5 indicates that renal uptake, soft tissue, blood background, and general appearance are similar in EHDP and lyophilized pyrophosphate. These agents appear to be superior to polyphosphate in all categories rated. From the limited number of cases using nonlyophilized pyrophosphate, there appears to be some indication that this agent is superior to the others with respect to renal and soft-tissue uptake. Further studies with this agent are currently underway.

In general 71 patients were studied utilizing ^{99m}Tc compounds for bone scanning. All patients had a confirmed or suspected diagnosis of malignancy and were being evaluated for skeletal metastases. In selected patients comparative scans were carried out using ^{18}F. On review of the comparative studies, scans were noted to show similar findings.

Overall scan results with ^{99m}Tc-phosphorus compounds were comparable to published figures with ^{18}F. None of the patients showed any adverse reaction to the administered radiopharmaceuticals.

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