^{99m}Tc-EHDP: A POTENTIAL RADIOPHARMACEUTICAL FOR SKELETAL IMAGING

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It has recently been reported that ^{99m}Tc-labeled linear polyphosphates with a molecular weight of about 5,000 localize in the skeleton of experimental animals like ^{85}Sr (*1,2*). These new agents have proven useful clinically for detecting both malignant and nonmalignant lesions of the skeleton (*2*). They were developed from the concept that the polyphosphates have an affinity for hydroxyapatite crystals (*3*) and that a stannous chelate of these polyphosphates could form a stable complex with ^{99m}Tc (*2*). Moreover, condensed polyphosphates behaved like pyrophosphates in inhibiting calcium phosphate precipitation in vitro (*3*). Polyphosphates were advocated for the treatment of osteoporosis in man (*4*). However, it was found that the P-O-P linkage in the condensed phosphates was broken down in vivo because of hydrolysis by polyphosphatase enzymes (*4*). More recently, analogs of pyrophosphates called diphosphonates (with P-C-P bonds instead of P-O-P bonds) have shown the same inhibitory effect on calcification in molar concentrations as low as 10^{-6}–10^{-7}. Furthermore, they apparently are completely stable in vivo (*4–8*). This report discusses preliminary results in successfully preparing a complex of ^{99m}Tc with EHDP* using stannous ion as an intermediate chelating agent. This proved to be a good skeletal imaging agent in experimental animals.

MATERIALS AND METHODS

Preparation of an "instant" kit. In 30 ml distilled water dissolve 750 mg of EHDP* and add 50 mg stannous chloride† (0.1 ml of 500 mg/ml in 5 *N* HCl, freshly prepared). Mix well, adjust the pH to 7.5 using dilute NaOH and adjust the volume to 50 ml using distilled water. Pipette 2-ml aliquots into 20-ml empty serum vials and lyopholize overnight in conventional freeze drying equipment. These kits each contain 2 mg $SnCl_2$ $2H_2O$ and 30 mg EHDP (as acid). The ^{99m}Tc-EHDP is prepared by adding 4–6 ml of ^{99m}Tc as pertechnetate (any amount of radioactivity) to the kit and mixing well. The preparation is sterilized terminally by passing through a 0.22-micron-size membrane filter. The final pH ranges from 6.8 to 7.2 and the labeling is quantitative with a negligible amount of free ^{99m}Tc. Alternatively, it is possible to prepare this kit under completely sterile conditions using presterilized solutions and lyopholizing under sterile conditions. Commercial equipment for this purpose is already available‡. To this sterile kit, one may prepare the complex of ^{99m}Tc-EHDP merely by adding 4–6 ml of sterile $^{99m}TcO_4^-$ solution.

The organ distribution of 20–250 μCi of ^{99m}Tc-EHDP injected intravenously was studied in adult New Zealand albino rabbits having an average weight of 3.8 kg, and compared with 10–20 μCi of ^{85}Sr-chloride administered simultaneously. The methods of tissue radioassay used were described previously (*2*). Similar studies at 1 and 4 hr after injection were conducted in rabbits with 3-week-old fractures of the tibia and the concentration of ^{99m}Tc-EHDP in the callus was compared with that of ^{85}Sr.

Imaging of the skeleton of rabbits with 3-week-old tibial fractures was performed using the scintillation

* Ethane-1, hydroxy-1, diphosphonate or 1,hydroxyethylidene diphosphonate or etidronate. The acid form was obtained from Calgon Corp., Pittsburgh, Pa.

† $SnCl_2 \cdot 2H_2O$; Fisher Scientific Co., Rochester, N.Y.

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‡ Virtis Co., Gardiner, N.Y.

camera†. Camera images of the entire body, obtained in three exposures demonstrated the distribution of ^{99m}Tc-EHDP at 1, 2, 4, and 24 hr. A total body scan‡ of a normal dog was obtained 2 hr after injection.

RESULTS

The soft tissue and blood concentrations of the ^{99m}Tc-EHDP at various times were much lower than those for ^{85}Sr, whereas the skeletal concentrations were similar (Table 1). The cumulative urinary excretion for ^{99m}Tc-EHDP was at least three times higher than for ^{85}Sr. The concentration of the ^{99m}Tc compound in the callus was somewhat lower than that of ^{85}Sr but the callus/normal bone concentration ratio was similar. The soft tissue concentrations were much lower for ^{99m}Tc-EHDP so that very good skeletal images were obtained at any time beyond 2 hr after injection (Fig. 1A). The rectilinear scan of the dog (Fig. 1B) also showed good skeletal visualization, although the soft tissue activity seemed to be slightly higher compared with the images in the rabbit.

DISCUSSION

The diphosphonate used in this study (EHDP) is an analog of pyrophosphate with P-O-P bonds replaced by P-C-P linkages. A variety of phosphonates with hydroxyl, methyl, ethyl, and chloryl groups substituted for hydrogens on the carbon atom have been investigated for their effect on calcification by Russell, et al (*4,8*) and Fleisch, et al (*3,7*). They have shown that diphosphonates with P-C-P bonds slow the dissolution of hydroxyapatite crystals in vitro and inhibit PTH-induced bone resorption in tissue culture and in rats (*4*). They have also dem-

† Nuclear Chicago Pho/Gamma HP Camera with high-sensitivity collimator for ^{99m}Tc.

‡ Ohio-Nuclear Model 84-D scanner.

TABLE 1. DISTRIBUTION OF ^{99m}Tc-EHDP AND ^{85}Sr IN RABBITS SIMULTANEOUS STUDY (6 EACH)

	Percent dose in whole organ							
	1 hr		2 hr		4 hr		24 hr	
Organ	^{99m}Tc	^{85}Sr	^{99m}Tc	^{85}Sr	^{99m}Tc	^{85}Sr	^{99m}Tc	^{85}Sr
Blood*	8.61	11.9	2.58	7.08	0.89	3.85	0.49	0.29
Liver	0.80	1.57	0.42	0.98	0.28	0.62	0.43	0.04
Muscle†	6.46	13.4	1.82	8.8	1.25	6.46	0.87	1.00
Kidneys	2.40	0.80	1.22	0.64	0.94	0.34	0.63	0.03
Marrow‡	0.70	0.91	0.38	0.76	0.83	0.50	0.42	0.10
One femur	0.82	1.04	0.84	1.21	0.81	1.25	0.76	0.93
Bone (avg)||	46.6	56.0	48.2	49.4	47.4	68.1	41.5	51.9
Urine	30.9	6.96	51.8	18.0	53.4	22.7	—	—
	Percent dose/1% body weight							
Blood	1.23	1.70	0.37	1.01	0.13	0.55	0.07	0.04
Liver	0.30	0.59	0.18	0.43	0.11	0.26	0.28	0.02
Muscle	0.15	0.31	0.04	0.21	0.03	0.15	0.02	0.02
Kidneys	3.93	1.75	2.72	1.40	1.95	0.71	1.29	0.06
Marrow	0.36	0.41	0.17	0.34	0.38	0.23	0.19	0.05
Bone (avg)	4.66	5.60	4.82	6.44	4.74	6.81	4.14	5.21
Femur	3.85	4.90	3.94	5.74	3.86	6.17	3.42	4.83
Tibia	2.17	3.62	2.49	4.73	2.16	5.13	2.10	4.27
Pelvis	7.15	7.07	7.40	7.70	7.41	8.02	5.70	5.26
Spine	5.87	7.03	5.44	7.59	5.52	7.82	5.30	6.47
Callus	24.7	31.2	—	—	28.9	37.7	—	—
	Ratios							
Bone/blood	4.3	3.4	14	6.4	38	13	81	134
Bone/marrow	14	14	28	19	27	32	29	119
Bone/muscle	35	19	123	32	196	47	292	227
Callus/blood	20	18	—	—	225	81		
Callus/N. tibia	11	9	—	—	12	8		
Callus/N. femur	7	7	—	—	7	7		

* 7% of body weight.
† 43% of body weight.
‡ 2.2% of body weight.
|| 10% of body weight.

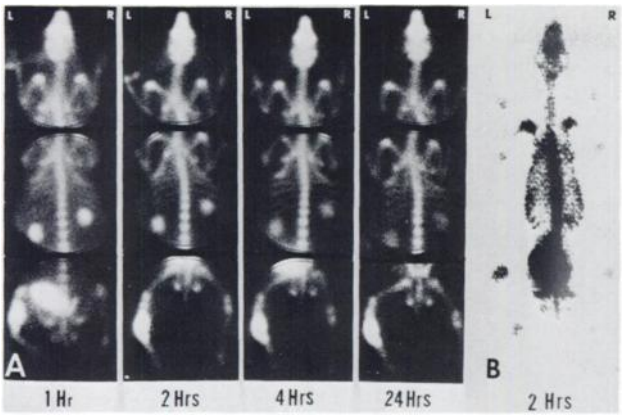

FIG. 1. (A) whole-body posterior images of rabbit with 3-week-old tibial fracture obtained with gamma camera in three exposures after 5 mCi of ^{99m}Tc-EHDP. Notice increased accumulation of radioactivity in callus around fracture site just below left knee. (B) total-body scan (posterior) of normal dog at 2 hr after 5 mCi of ^{99m}Tc-EHDP. Increased activity seen in pelvic area is urine in bladder.

onstrated that diphosphonates, especially EHDP followed by MDP (Methylene diphosphonate), Cl_2MDP (dichloromethylene diphosphonate), and a condensate of EHDP (polyphosphonate), can prevent the formation of calcium phosphate crystals in vitro and also prevent pathologic soft tissue calcification of the aorta and kidney in rats. Cabanela, et al (*5*) found that EHDP was able to prevent local bone loss in immobilization osteoporosis but not the systemic bone loss produced by cortisone administration in rats. They concluded that the appearance of osteoid after EHDP was probably due to an inhibition of calcification rather than a leaching of the mineral out of previously mineralized bone. Autoradiographs in cats (*9*) with the ^{14}C-labeled compound have shown that this material is concentrated chiefly on bone surfaces, but to a lesser extent within calcified bone, especially in the trabeculae. The above results indicate that EHDP and other diphosphonates can localize in areas of bone wherever there is an active transport of calcium and/or phosphate. This action of diphosphonate seems to be very similar to that of polyphosphates. EHDP, however, is resistant to chemical or enzymatic hydrolysis, whereas polyphosphates are believed to undergo enzymatic hydrolysis and be metabolized.

The exact mechanism of the localization of ^{99m}Tc-EHDP in the skeleton is not fully understood. Francis (*10*) described the inhibition of calcium hydroxyapatite crystal growth by EHDP (labeled with ^{14}C). He observed a strong chemisorption (chemical bonding) of EHDP on the microcrystallites of hydroxyapatite and suggested that this prevented further crystal growth. The same mechanism can be invoked to the localization ^{99m}Tc-EHDP (and possibly ^{99m}Tc polyphosphates) for both the normal skeleton and experimental callus.

The metabolic and toxicological properties of EHDP or other diphosphonates are largely unknown. These are new synthetic materials. EHDP has been used unsuccessfully (*6*) for the treatment of osteoporosis in four patients by oral administration. The toxicological data available to date are for oral and subcutaneous administration (*9,11,12*). In man, daily oral doses as low as 10 mg/kg have resulted in hyperphosphatemia, increased total serum calcium, and decreased ionized serum calcium; after 4 months, bone biopsy has shown an increase in unmineralized osteoid (*6*). In animals, only about 3% of an oral dose is absorbed (*7*). Intravenous toxicity has not been studied.

SUMMARY

Excellent skeletal images have been obtained with ^{99m}Tc-EHDP in animals. Its skeletal concentration is comparable to ^{99m}Tc polyphosphates. It has the advantage of a more rapid blood clearance, and relatively lower soft tissue concentrations. However, it is apparently not biodegradable. More information must be obtained about its intravenous toxicity and biological fate before it can be considered for trial in humans. It is likely that other diphosphonates like MDP, Cl_2MDP, and EHDP condensate, labeled with ^{99m}Tc, will also exhibit good skeletal localization. Further work along these lines is in progress.

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