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BIODISTRIBUTION OF THE BONE IMAGING AGENTS ^{99m}Tc-DHPE AND ^{99m}Tc-MDP IN RATS

S.C. Archimandritis*, Al.K. Tsolis**

N.R.C. "Demokritos" Division of Radiodiagnostic Products and Radioisotopes, Aghia Paraskevi Attikis, Athens 15310, Greece

**Department of Chemistry, University of Patras, Patras, Greece

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The self life of the freeze-dried formulation kits utilized for the preparation of the new bone imaging radiopharmaceutical ^{99m}Tc-1,2-dihydroxy -1,2-bis/dihydroxyphosphinyl/ethane /^{99m}Tc-DHPE/ has been investigated as well as the toxicity of the DHPE. In a biodistribution investigation of ^{99m}Tc-DHPE in rats, in comparison to ^{99m}Tc-methylenediphosphanate /^{99m}Tc-MDP/, ^{99m}Tc-DHPE exhibited a certain extent higher skeleton uptake, a higher blood clearance rate, a very low concentration in other organs, a satisfactory biological stability and excretion primarily through the kidneys. These properties demonstrate that ^{99m}Tc-DHPE is a new promising skeleton imaging radiopharmaceutical.

*Author to whom correspondence should be addressed.

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INTRODUCTION

The utilization of technetium diphosphonates as skeletal imaging agents is widely accepted in the field of Nuclear Medicine[1,2]. A number of articles and reviews describing the state of art of this methodology have been published[3-8].

Clinical preparation of technetium diphosphonate radiopharmaceuticals involves the addition of $^{99m}TcO_4^-$ in saline to commercially available kits, which contain an excess of Sn/II/ and diphosphonate ligand usually in the presence of an antioxidant stabilizer. The pertechnetate is reduced by Sn/II/ to lower oxidation states, which in turn are complexed by the diphosphonate ligand.

The most commonly employed diphosphonates are methylene diphosphonate /MDP/[9], hydroxymethane diphosphonate /HDP, HMDP/[10-13], 2,3-dicarboxypropane-1,1-diphosphonate /DPD/[14,15], 1-hydroxyethylidene diphosphonate /HEDP/[16], N,N-dimethylamino methylene diphosphonate /DMAD/[6]. The observed absolute and relative biological behaviours of the ^{99m}Tc skeleton-imaging agents, prepared from the above and other diphosphonate ligands, have been the subject of many biological and clinical investigations[9-18]. It has been found that the ^{99m}Tc diphosphonate preparations contain mixtures of complexes, which can be detected and separated by HPLC and which exhibit various bone uptakes[19,20].

In our search for a more efficient bone-seeking radiopharmaceutical we have developed a new promising bone-imaging agent, ^{99m}Tc-1,2-dihydroxy-1,2-bis/dihydroxyphosphinyl/ ethane /^{99m}Tc-DHPE/[21,22]. This new radiopharmaceutical can be easily prepared in a label-

ling yield better than 95% by addition of a $^{99m}TcO_4^-$ solution in saline to a freeze-dried formulation kit. In preliminary biodistribution experiments using rats, ^{99m}Tc-DHPE demonstrated a very good skeleton uptake, which was to a certain extent higher than the uptake of ^{99m}Tc-MDP, a high blood to bone clearance rate, a relatively low concentration in muscles and other organs, and a satisfactory biological stability. It has been found that ^{99m}Tc-DHPE is excreted primarily through the kidneys[21,22].

In the present article the results of a more extensive biological distribution study of ^{99m}Tc-DHPE are described. Also the results of a parallel biodistribution study, under the same conditions, of the most widely used bone-imaging radiopharmaceutical, ^{99m}Tc-methylene diphosphonate /^{99m}Tc-MDP/ and the comparison of the biological behaviour of these two radiopharmaceuticals are presented. The self life of the DHPE formulation kit as well as the toxicity of the DHPE have been determined.

MATERIALS AND METHODS

DHPE was synthesized according to our published method[23-25]. It was checked by elemental microanalysis prior to its use. All other reagents were of analytical grade. Sodium pertechnetate was obtained either by the methylethylketone extraction process of irradiated MoO_3 or it was eluted with normal saline from a generator and diluted as required.

MDP freeze-dried kits were commercial products of N.R.C. "Demokritos" Athens, Greece.

DHPE freeze-dried formulation kits were prepared according to our published method[21,22]. Each kit vial contained 2.22 mg of DHPE, 50 μg of tin/II/ chloride and 50 mg of sodium acetate.

The ^{99m}Tc-DHPE solution was prepared adding to each kit vial 4.0-6.0 ml of saline solution of sodium pertechnetate /radioactive concentration range 55.5-1184 Bq ml^{-1}, 1.5-32 mCi ml^{-1}/ depending on the requirements. The vial was vigorously shaken for 5 min, it was then allowed to stand at ambient temperature for 5 min and it was ready for analysis and use. The ^{99m}Tc-MDP solution was prepared in a similar way following the instructions of the manufacturer.

The determination of the radiochemical purity of the solution batches of each radiopharmaceutical was carried out by ascending paper chromatography on Whatman No. 1 paper strips with a mixture of methanol and water 95:15 v/v and with a mixture of aqueous phosphoric acid and water 15:85 v/v /Ref. 26/.

Self life of the freeze-dried DHPE formulation kit

The self life of the freeze-dried DHPE formulation kit was determined for kits stored in a refrigerator /4 ^{o}C/, as well as at room temperature in normal light and in the darkness. Three groups of twelve vials each were used for the determination. Each group was stored under the conditions mentioned above. Two vials from each group were used for the preparation of the ^{99m}Tc-DHPE solution 1,2,3,4,5 and 6 months after the production date of the formulation kits. The labelling yield was determined in each case by ascending paper chromatography[26]. A rat was injected in the tail vein with a sample of radiopharmaceutical from each kit

vial. 2 h later scintigrams of these rats were obtained in a Berthold small animal scanner and the quality of the imaging was examined.

DHPE toxicity

The toxicity of DHPE to rats was studied. The solution of DHPE used for injections was prepared by dissolving the required amount of DHPE in an aqueous solution of sodium chloride /0.9% w/v/ and adding to it, under stirring, the required volume of an aqueous 6N sodium hydroxide solution to pH 6.5. Since the room temperature solubility of DHPE under these conditions is 50.0 mg ml^{-1}, this concentration was the highest one injected to the animals. The rats weighed 180 to 250 g. Five groups of four animals each were injected via the tail vein with doses in the range of 5 to 25 mg DHPE per kg of animal body weight /the step increments between groups were 5 mg per kg of animal body weight/. Nine more groups of four animals were injected with doses in the range of 25 to 250 mg per kg of animal body weight /the step increments between groups were 25 mg per kg of animal body weight/. Another group of four animals was injected every 20 h for a period of 10 days with 1 ml of a pH 6.5 saline solution of 50 mg of DHPE per milliliter, corresponding to a dose of 240+40 mg per kg of animal body weight. The injected animals were observed in comparison with controls for a period of 4 weeks after injection.

Biodistribution of ^{99m}Tc-DHPE and ^{99m}Tc-MDP in rats

Biological distribution studies were conducted on random breading closed colony female Hooded rats weighing on the average 160±10 g. 300 microliters of

solution containing ^{99m}Tc-DHPE or ^{99m}Tc-MDP were administered via the tail vein to the anesthetized /diethylether/ animals. The level of the injected radioactivity estimated for the time of the killing of each animal varied from 18.5 MBq to 29.6 MBq /500 μCi to 800 μCi/. Six animals in each case were sacrificed at 1,4,6 and 12 h post injection by direct sampling, with a syringe, of blood from the heart of the anesthetized animal. Samples of femur, muscle and bone were excised and put in preweighed containers. The other organs: spleen, stomach, liver, lungs, heart, kidneys, adrenals, small and large intestine were removed intact. Urine and feces were also collected. These organs and tissues were weighed and assayed by γ-scintillation counting, on a NaI/Tl/ well-type detector using appropriate standards prepared from the initial dose. For blood, muscles and bone the calculation was based on the activity of the sample and the body composition data, 7%, 43% and 10% of the animal body weight, respectively.

RESULTS AND DISCUSSION

The radiochemical purity of the ^{99m}Tc-DHPE radiopharmaceutical obtained from the freeze-dried formulation kits, which were stored in the refrigerator for up to 6 months, was better than 95% for all the tested kits. Radiochemical purity better than 95% was determined for the radiopharmaceutical obtained from the kits, which were stored up to 3 months at room temperature in darkness. For longer storage times the observed radiochemical purity was lower and steadily decreasing in time. The self life of the kit vials

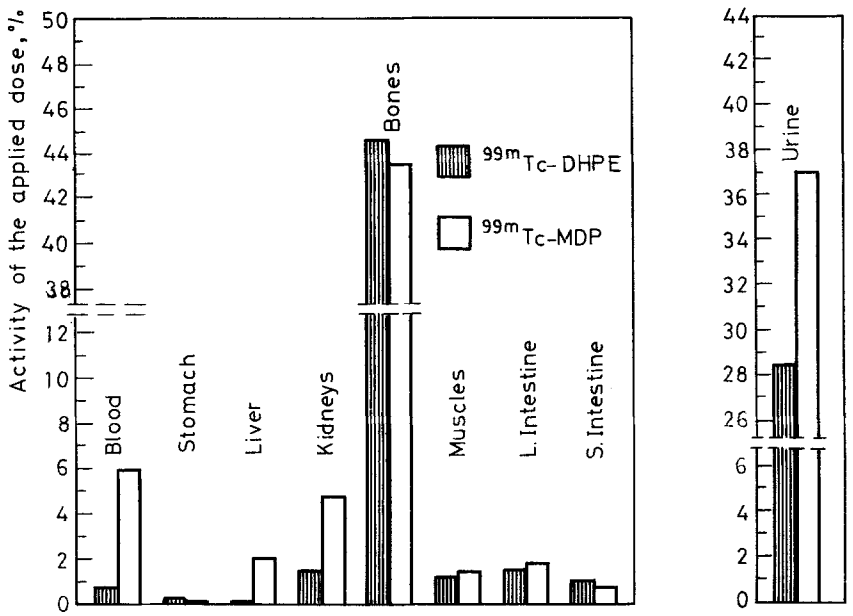

Fig. 1. Organ activities in percent of applied dose 1 h p.i.. Spleen, lungs, heart, adrenals and feces activity 0.1% or smaller

which were kept without any precaution was limited to 2 months.

In the DHPE toxicity investigation, all the caged rats behaved normally in all respects during the post injection period of observation. They ate well and maintained their weights. No death was observed.

The results of the comparative biodistribution investigation of ^{99m}Tc-DHPE and of ^{99m}Tc-MDP are shown in Figs 1-4. These results verify our previously published conclusions. Namely the results show that the ^{99m}Tc-DHPE exhibits a very good and selective skeleton uptake. In fact the percent doses of ^{99m}Tc-DHPE localized in the skeleton are a certain extent higher than the percent doses of ^{99m}Tc-MDP for all post injection periods except for the case 12 h.

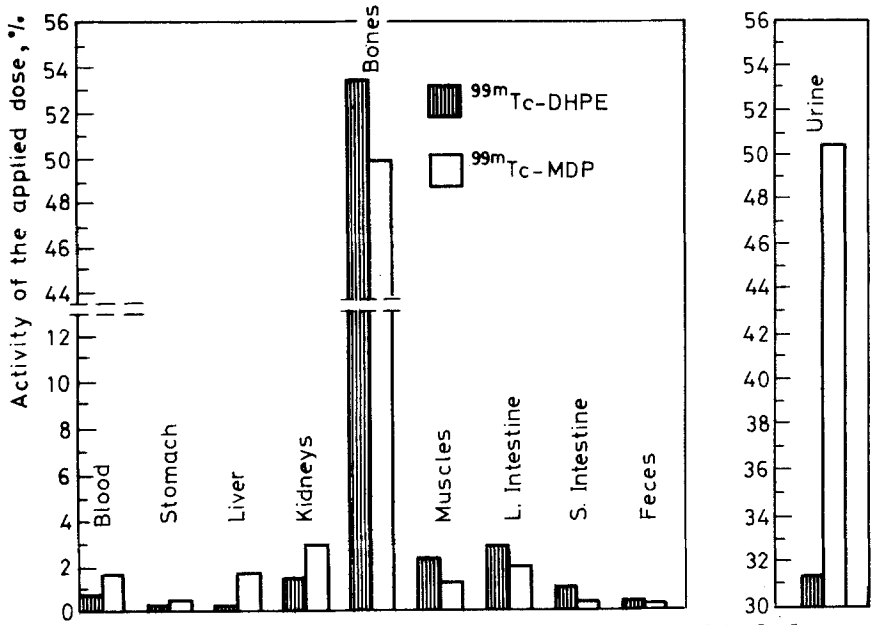

Fig. 2. Organ activities in percent of applied dose 4 h p.i.. Spleen, lungs, heart and adrenals activity 0.1% or smaller

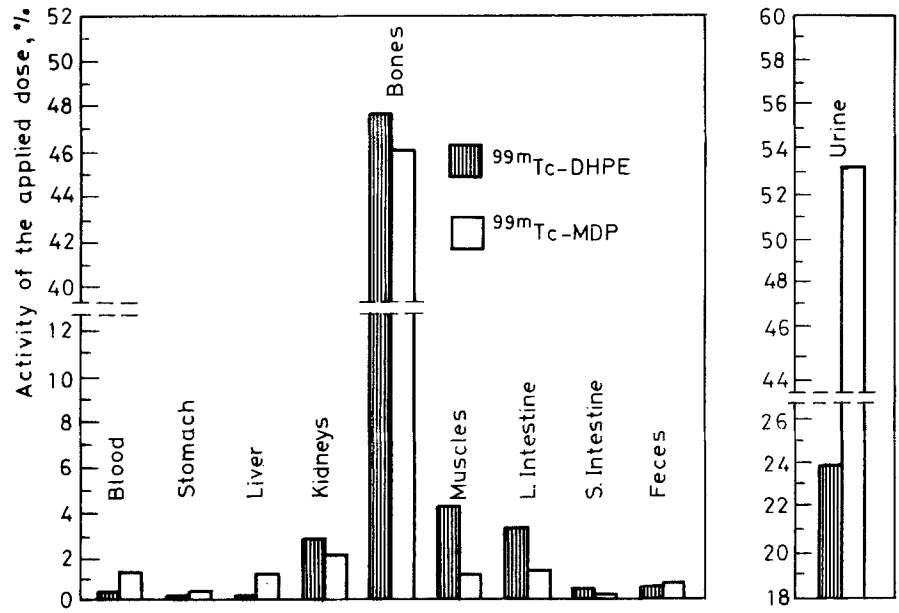

Fig. 3. Organ activities in percent of applied dose 6 h p.i.. Spleen, lungs, heart, and adrenals activity smaller than 0.02%

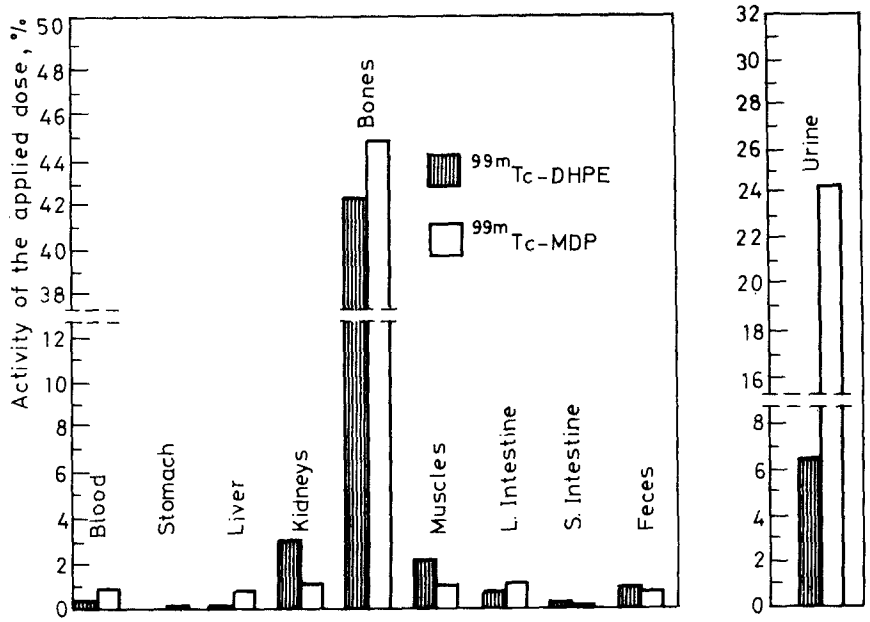

Fig. 4. Organ activities in percent of applied dose 12 h p.i.. Spleen, lungs, heart, and adrenals activity smaller than 0.02%

The percent dose of ^{99m}Tc-DHPE in blood is much lower than that of ^{99m}Tc-MDP especially for the earliest post injection periods, showing a much higher blood clearance rate of ^{99m}Tc-DHPE. The bone vs. blood percent dose ratios are higher for ^{99m}Tc-DHPE, showing a higher blood to bone clearance rate of ^{99m}Tc-DHPE. This was also verified by the quality of the skeleton images of a rabbit injected with ^{99m}Tc-DHPE and photographed with a γ-camera 30, 45, 60 and 90 min post injection. These images are shown in Fig. 5. The percent doses of ^{99m}Tc-DHPE found in muscles are low but a certain extent higher than the doses of ^{99m}Tc-MDP. Liver and stomach localization of ^{99m}Tc-DHPE is negligible for all post injection periods tested, suggest-

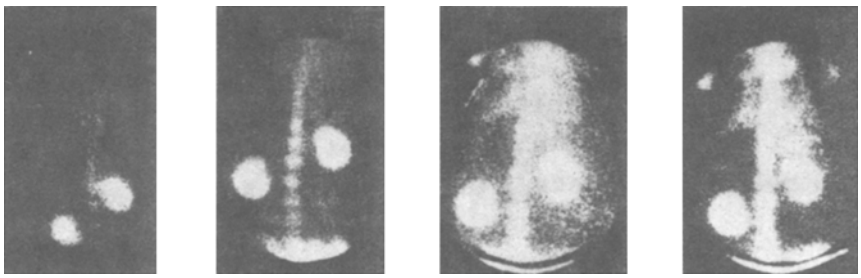

Fig. 5. γ-camera photographs of a rabbit injected with ^{99m}Tc-DHPE at various p.i. times

ing a satisfactory biological stability of this radiopharmaceutical. The percent doses of ^{99m}Tc-DHPE found in other organs are also very low to negligible. The secretion of ^{99m}Tc-DHPE occurs primarily through the kidneys.

CONCLUSION

It can be concluded that the performance of the radiopharmaceutical ^{99m}Tc-DHPE in rats and rabbits is very satisfactory, if not better than that of ^{99m}Tc-MDP. It is planned to be further investigated by additional biological and clinical experimentation.

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