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New amino bisphosphonate compound for skeletal imaging: Comparison study with methylenediphosphonic acid (MDP) and (l-hydroxyethane-l,l-diyl) diphosphonic acid (HEDP)

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Abstract. A novel bisphosphonate derivative (1-aminoethane-1,1-diyl)diphosphonic acid (AEDP) has been prepared and successfully labeled with ^{99m}Tc at high labeling yields. The *in vivo* biodistribution of ^{99m}Tc-AEDP has been investigated and compared with two reference compounds Tc-99m methylene diphosphonate (^{99m}Tc-MDP) and Tc-99m (1-hydroxyethylidene) diphosphonate (^{99m}Tc-HEDP). The biodistribution studies have demonstrated a high uptake of the radiotracer ^{99m}Tc-AEDP in the bone and a rapid clearance from the blood (such as the two technetium-labeled bone imaging agents in current use: ^{99m}Tc-MDP and ^{99m}Tc-HEDP). Additionally, the scintigraphic images of ^{99m}Tc-AEDP in normal rats have revealed high selective skeletal uptake.

Key words: aminoethylidenediphosphonic acid • biodistribution • bisphosphonates • bone imaging agent • radiolabeling

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Introduction

Technetium-99m radiopharmaceuticals play an important role in wide range of applications in nuclear medicine. Bone imaging agents are among the first developed ^{99m}Tc-radiopharmaceuticals and the most widely used radiopharmaceuticals in diagnostic nuclear medicine [1]. Bisphosphonates (BPs) are synthetic organic compounds characterized by a P-C-P backbone structure. They are chemically stable analogues of the endogenous metabolites, inorganic pyrophosphates. The biological effects of BPs on calcium metabolism were originally ascribed to their physicochemical effects to impede the dissolution of hydroxyapatite crystals [2–4].

Because of their inhibitory effect on the bone resorption, various types of BPs are used in bone scanning, and provide an effective way of diagnosis of primary bone cancer, metastatic bone disease, Paget's disease, osteoporosis, bone trauma, etc. Several ^{99m}Tc-labeled BPs have been synthesized, such as methylenediphosphonic acid (MDP) [2], (1-hydroxyethane-1,1-diyl)diphosphonic acid (HEDP) and hydroxyl methylene diphosphonate (HMDP), (Fig. 1), and used in nuclear medicine for both diagnosis and treatment purposes [5–14].

To continue the previous efforts to find better bone-imaging agents, we report in this manuscript the synthesis of a new bisphosphonate compound, AEDP, and investigate its radiolabeling abilities with ^{99m}Tc. Subsequently, we have performed preliminary *in vivo* studies in rats and compared the results with those obtained using two reference

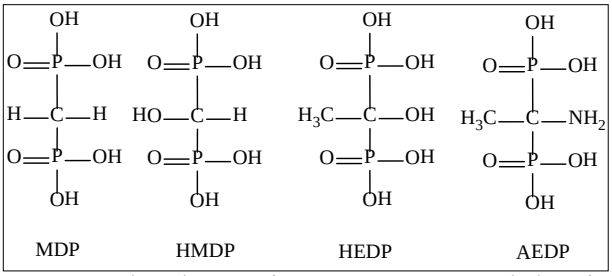


Fig. 1. Bisphosphonate derivatives. MDP: methylenediphosphonic acid; HMDP: hydroxyl methylene diphosphonate; HEDP: (1-hydroxyethane-1,1-diyl)diphosphonic acid; AEDP: (1-aminoethane-1,1-diyl)diphosphonic acid.

compounds, ^{99m}Tc-MDP and ^{99m}Tc-HEDP in order to determine whether ^{99m}Tc-AEDP is convenient as a bone-seeking agent.

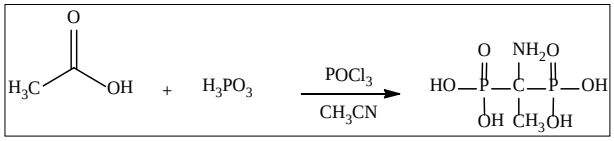
Materials and methods

All chemical reagents and solvents were of commercial quality and used as received. NMR spectra were acquired using a Bruker Bio spin 400 spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C). Chemical shifts (δ) were expressed in ppm relative to tetramethyl silane (TMS) as an internal standard. Melting point (MP) determination was performed using a digital melting point instrument from Stuart model SMP3. Infrared spectra were recorded as KBr pellets in the range 4000–400 cm^{-1} using an FTIR-JASCO 300E. ITLC (instant thin-layer chromatography) measurement was carried out using Whatman No. 3 strips (Sigma Chemical Company, USA) and radioactivity counted in a gamma scanner (Raytest mini GITA, Model BGO-V-detector) equipped with NaI(Tl) detector and single channel analyzer. MDP and HEDP kits were provided as commercial available kits produced by Atomic Energy Commission of Syria. Bone scan was performed with a gamma camera (Siemens Signature, Duel head, Damascus, Syria). Microanalysis was performed using a EURO EA analyzer. X-ray powder diffraction (XRD) patterns were obtained using a Stoe Stadi-P diffractometer with monochromatic Cu$K_{\alpha1}$ radiation (λ = 1.5406 Å) selected using an incident-beam curved--crystal germanium Ge(111) monochromator, using the Stoe transmission geometry (horizontal set-up) with a linear position-sensitive detector (PSD).

Experimental

Synthesis of (1-aminoethane-1,1-diyl)diphosphonic acid (AEDP)

To a mixture of acetonitrile (150 ml), phosphorous acid (16.8 g, 0.2 mol) in glacial acetic acid (10 g, 0.167 mol) was added dropwise phosphoryltrichloride (51.7 g, 0.334 mol) at 55–65°C. The resulting mixture was stirred for 24 h at 70–75°C. The reaction mixture was cooled down to 60–65°C, and then water (150 ml) was added slowly. The reaction temperature was then increased to 90–100°C and



Scheme 1. Synthesis of (1-aminoethane-1,1-diyl)diphosphonic acid (AEDP).

maintained for the next 4–6 h. The reaction mixture was cooled down to 0–5°C and stirred for 3 h. The solid product was separated by filtration, washed with water, and finally, with methanol to produce the corresponding product, in 77% yield. Appearance: white powder (Scheme 1), MP = 279.8–276.6°C.

Spectroscopic data of AEDP: ^{1}H-NMR (D_2O): δ1.46–1.53 (t, 3H, CH_3). ^{13}C-NMR (D_2O): δ17.5 (1C, CH_3), 52.6–55.1 (1C, C-CH_3). ^{31}P-NMR (D_2O): δ13.39 (2P, P-OH). IR (KBr, ν cm^{-1}): 3448–3236 (OH, NH_2), 2338 (P-H), 1602 (O = P-O-H), 1142 (P = O). Analytical data for (AEDP): Found: C, 11.30; H, 4.41; N, 6.48; Calculated C, 11.72; H, 4.42; N, 6.83.

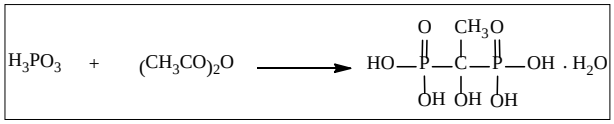
Synthesis of (1-hydroxyethane-1,1-diyl)diphosphonic acid (HEDP)

A mixture of (5.13 g, 62.56 mmol) phosphorous acid and (7.74 g, 75.07 mmol) dried acetic anhydride was refluxed for 4 h at 105°C (Scheme 2). At the same temperature, water vapor was bubbled through the mixture until the distillate became almost free of acid. The reaction mixture was concentrated in a rotary evaporator to yield crystals of HEDP monohydrate. The solid product was separated by filtration and washed with acetone to produce the corresponding product, in 90% yield, MP = 106.3–106.6°C.

Spectroscopic data of HEDP: ^{1}H-NMR (D_2O): δ1.63 (t, 3H, CH_3), δ4.88 (s, H_2O). ^{13}C-NMR (D_2O): δ 19.16 (1C, CH_3), 68.83–71.75 (1C, C-CH_3). ^{31}P-NMR (D_2O): δ19.84 (2P; P-OH). IR (KBr, ν cm^{-1}): 3444–3551 (OH), 2394 (P-H), 1602.6 (O = P-O-H), 1142.4 (P = O).

Powder X-ray diffraction analysis of AEDP

The compound crystallizes as a fine white powder, therefore, a laboratory powder X-ray diffraction data was used for phase identification. The powder X-ray diffraction data were collected at room temperature with a STOE transmission STADI-P diffractometer using Cu$K_{\alpha1}$ radiation (λ = 1.54060 Å) selected with an incident-beam curved-crystal Ge(111) monochromator with a linear PSD. The pattern was scanned over the angular range 5.0–95.0° (2θ). For phase identification, a Crystallography Open



Scheme 2. Synthesis of (1-hydroxyethane-1,1-diyl)diphosphonic acid (HEDP). MDP: methylenediphosphonic acid.

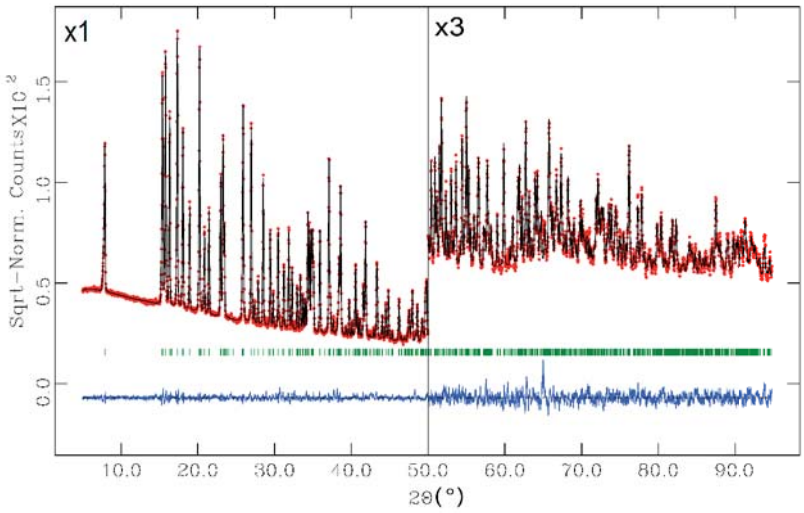


Fig. 2. The final Rietveld plot for (1-aminoethane-1,1-diyl)diphosphonic acid (AEDP).

Database (COD) interfaced by the program QualX [15] was used. This program reports that the crystal structure of this compound was published by Tsaryk *et al*. [16] as a second triclinic polymorph of (1-ammonio-1-phosphonoethyl)phosphonate. Moreover, Rietveld analysis of the powder pattern for this compound (Fig. 2) indicates that the asymmetric unit of this compound, $C_2H_9NO_6P_2$, contains one molecule as a zwitterion. The N atom of the amino group is protonated and one of the phosphonic acid groups is deprotonated. Bond lengths and angles are similar to those obtained from single crystal data [16]. H atoms involved in these hydrogen bonds are located at inversion centers. These bonds and additional O–H...O and N–H...O hydrogen bonds interlink the molecules, giving a three-dimensional supramolecular structure (Fig. 3).

Preparation of kits

375 mg of AEDP or HEDP was dissolved in 15 ml double distilled water in a vial. 312 µl of freshly prepared aqueous solution of $SnCl_2{\cdot}2H_2O$ in nitrogen purged (400 mg $SnCl_2{\cdot}2H_2O$/0.5 ml concentrated HCl/5 ml H_2O) was added into the vial. The pH was adjusted to 7.5 with sodium hydroxide solution. The final solution was adjusted with water to 25 ml and the resulting solution was sterilized by filtration through a cellulose ester filter (0.22 µm). Aliquots of 1 ml were transferred to glass vials and lyophilized for 24 h. The lyophilized vials were sealed under vacuum and stored in a refrigerator at 4°C.

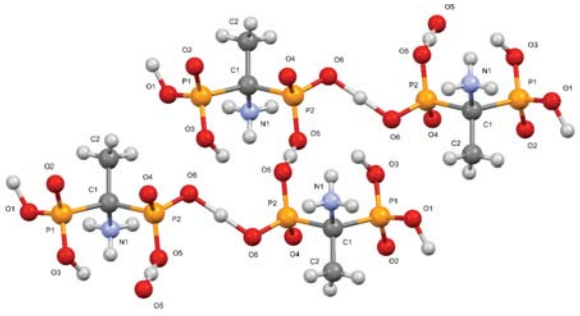


Fig. 3. The molecular structure of (1-aminoethane-1,1--diyl)diphosphonic acid (AEDP) obtained by Rietveld refinement.

Radiolabeling of AEDP, HEDP, and MDP with ^{99m}Tc

Radiolabeling of the kit involves initial warming up of the vial to room temperature followed by the addition of 370–740 MBq (10–28 mCi) of freshly eluted $^{99m}TcO_4^-$ in 2 ml of normal saline and finally, incubation of the vial for 15 min at room temperature. Radiochemical purity was determined by ITLC as follows: 5 µl of the prepared compound was spotted on 10 cm Whatman No. 3 strips (Sigma Chemical Company, USA). The strips were then run with acetone and saline solution as mobile phases. After developing, the radioactivity was counted by a gamma scanner (Raytest mini GITA, Model: BGO-V-detector) equipped with NaI(Tl) detector and a single channel analyzer. By using acetone as the mobile phase, reduced ^{99m}Tc and ^{99m}Tc-complexes remained near the point of spotting with R_f values <0.2, while free $^{99m}TcO_4^-$ moved towards the solvent front. By using saline solution as another mobile phase, ^{99m}Tc-complexes and $^{99m}TcO_4^-$ moved towards the solvent front with R_f values >0.9, whereas reduced ^{99m}Tc remained at the point of spotting (Figs. 4 and 5).

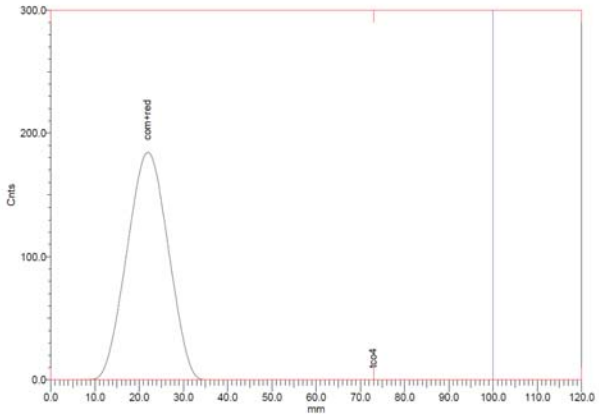


Fig. 4. TLC pattern of ^{99m}Tc-AEDP complex in acetone.

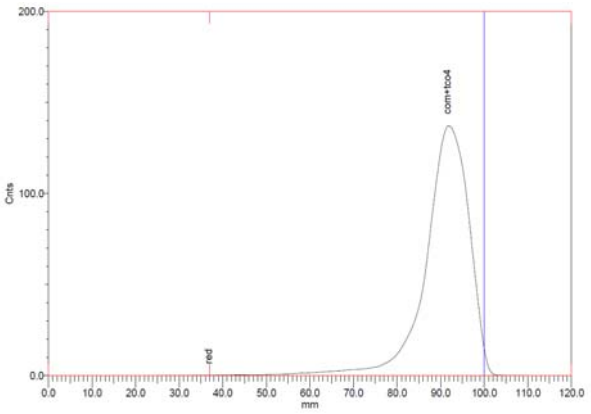


Fig. 5. TLC pattern of ^{99m}Tc-AEDP complex in 0.9% NaCl.

In vivo experiments

Experiments in rats were carried out in accordance with appropriate European Community directive guidelines (86/609/EEC). Biodistribution studies were performed in healthy Wister Han rats (male, 160–220 g). 0.3 ml of ^{99m}Tc-AEDP, ^{99m}Tc-HEDP or ^{99m}Tc-MDP (2.2–3.7 MBq) in saline were administered to rats intravenously via the tail vein. The animals were anesthetized and sacrificed, routinely 1, 3, and 24 h post-intravenous injection (p.i.), and selected organs were taken out. The radioactivity of weighted samples of femur, heart, liver, lungs, kidneys, spleen, stomach, intestine, and blood were measured using a gamma counter CE/SN:03 L 504. The uptakes in the different selected organs, expressed as %ID(±SD)/g, of the organs for all the radiolabeled phosphonate complexes are given in Tables 1–3. Bone-to-blood and bone-to-muscle uptake ratios were determined from the %ID/g values for the organs.

Imaging studies were performed in normal rats (Wistar Han, male, 160–220 g) at 1 h after intravenous injection of 29.6 MBq (0.8 mCi) of ^{99m}Tc-AEDP, ^{99m}Tc-HEDP or ^{99m}Tc-MDP, respectively.

Results and discussion

New product AEDP was synthesized according to the reaction scheme shown in Scheme 1. The synthesis was started with the addition of phosphoryltrichloride to a mixture of acetonitrile and phosphorous acid to obtain crude AEDP. The solid product was separated by filtration, then washed with water and methanol to give pure AEDP in 77% yield.

AEDP was characterized by ^{13}C, ^{31}P, ^{1}H-NMR, XRD, EA, and IR spectroscopic techniques. ^{1}H-NMR spectrum of AEDP showed a triplet peak representing the phosphorus splitting of the methyl hydrogens.

AEDP was labeled with ^{99m}Tc in high labeling yields. Radiochemical purity and radiochemical yields were determined by ITLC and found to be greater than 97% (Fig. 4).

Biodistribution of ^{99m}Tc-AEDP was studied in rats and the results were compared with those obtained using ^{99m}Tc-HEDP and ^{99m}Tc-MDP. One hour post injection, ^{99m}Tc-AEDP, ^{99m}Tc-HEDP, and ^{99m}Tc-MDP showed significant uptake by bone. Femur was taken as a representative of the skeleton and observed uptakes in femur were 5.54%/g, 12.7%/g, and 4.67%/g for ^{99m}Tc-AEDP, ^{99m}Tc-HEDP, and ^{99m}Tc-MDP, respectively, at 1 h post injection. No leaching of the activity from bone was observed for ^{99m}Tc-MDP from 1 to 3 h post injection. In contrast, the activity of bone was decreased after 24 h post injection for ^{99m}Tc-AEDP. Also, the activity of bone was decreased after 3 h for ^{99m}Tc-HEDP. No increase of the uptake in any of the organs and tissues were observed with exception in stomach for ^{99m}Tc-AEDP. The activities in blood were 1.38%/g, 3.65%/g, and 1.7%/g for ^{99m}Tc-AEDP, ^{99m}Tc-HEDP, and ^{99m}Tc-MDP, respectively, at this time point and no significant accumulation of the activity was observed in any of the major organs except in kidneys. However, the uptake observed in kidneys showed an increase up to 3 h, then decreased from 3 to 24 h for both ^{99m}Tc-AEDP and ^{99m}Tc-HEDP. In contrast, the uptake was decreased from 1 to 3 h, then increased from 3 to 24 h for ^{99m}Tc-MDP. From 30 to 50% of the injected activity was cleared via urinary excretion within 3 h post injection for all the complexes. The bone-to-muscle uptake ratios of ^{99m}Tc-AEDP were 167 and 242 at 3 and 24 h, respectively. While the bone-to-muscle uptake ratios of ^{99m}Tc-HEDP and ^{99m}Tc-MDP were 21.4, 367 and 95.6, 211 at 3 and 24 h, respectively, as given in Tables 1–3.

Table 1. Biodistribution pattern of ^{99m}Tc-AEDP complex in Wistar rats. AEDP: (1-aminoethane-1,1-diyl)diphosphonic acid

Organ	Biodistribution of ^{99m}Tc-AEDP [%ID/g]		
	1 h	3 h	24 h
Blood	1.38 ± 0.81	0.51 ± 0.17	0.37 ± 0.06
Liver	0.13 ± 0.06	0.07 ± 0.017	0.44 ± 0.02
Intestine	0.17 ± 0.002	0.50 ± 0.08	0.25 ± 0.07
Kidney	2.00 ± 0.59	3.17 ± 0.28	2.60 ± 0.17
Stomach	0.14 ± 0.003	0.21 ± 0.13	0.36 ± 0.24
Heart	0.15 ± 0.08	0.04 ± 0.01	0.03 ± 0.012
Lungs	0.25 ± 0.08	0.11 ± 0.019	0.06 ± 0.007
Femur	5.54 ± 0.98	5.01 ± 0.76	2.42 ± 0.31
Spleen	0.14 ± 0.01	0.07 ± 0.01	0.45 ± 0.09
Muscle	0.04 ± 0.001	0.03 ± 0.001	0.01 ± 0.001
Femur/blood	4.01	9.8	6.54
Femur/muscle	138	167	242

Table 2. Biodistribution pattern of ^{99m}Tc-HEDP complex in Wistar rats. HEDP: (1-hydroxyethane-1,1-diyl)diphosphonic acid

Organ	Biodistribution of ^{99m}Tc-HEDP [%ID/g]		
	1 h	3 h	24 h
Blood	3.65 ± 2.05	0.74 ± 0.61	0.04 ± 0.01
Liver	0.14 ± 0.03	0.07 ± 0.02	0.01 ± 0.003
Intestine	0.18 ± 0.03	0.13 ± 0.03	0.03 ± 0.009
Kidney	1.53 ± 0.05	1.67 ± 1.20	0.52 ± 0.134
Stomach	0.07 ± 0.004	0.28 ± 0.08	0.06 ± 0.10
Heart	0.20 ± 0.03	0.04 ± 0.009	0.005 ± 0.002
Lungs	0.28 ± 0.04	0.14 ± 0.009	0.01 ± 0.002
Femur	12.71 ± 4.50	4.93 ± 0.22	3.67 ± 0.43
Spleen	0.13 ± 0.02	0.09 ± 0.02	0.03 ± 0.002
Muscle	0.28 ± 0.02	0.23 ± 0.02	0.01 ± 0.001
Femur/blood	3.48	6.5	91.75
Femur/muscle	45	21	367

Table 3. Biodistribution pattern of ^{99m}Tc-MDP complex in Wistar rats. MDP: methylenediphosphonic acid

Organ	Biodistribution of ^{99m}Tc-MDP [%ID/g]		
	1 h	3 h	24 h
Blood	1.70 ± 0.6	0.29 ± 0.023	0.23 ± 0.12
Liver	0.22 ± 0.31	0.05 ± 0.02	0.48 ± 0.15
Intestine	0.31 ± 0.007	0.21 ± 0.16	0.15 ± 0.07
Kidney	1.11 ± 0.83	0.97 ± 0.02	1.86 ± 0.18
Stomach	0.44 ± 0.11	0.22 ± 0.30	0.08 ± 0.04
Heart	0.12 ± 0.04	0.12 ± 0.021	0.029 ± 0.01
Lungs	0.20 ± 0.004	0.13 ± 0.03	0.09 ± 0.016
Femur	4.67 ± 0.03	6.69 ± 0.34	4.22 ± 0.71
Spleen	0.11 ± 0.01	0.08 ± 0.02	1.08 ± 0.19
Muscle	0.08 ± 0.007	0.07 ± 0.006	0.02 ± 0.001
Femur/blood	2.74	23	18.34
Femur/muscle	58.8	95.6	211

Scintigraphic images of ^{99m}Tc-AEDP, in normal rats revealed highly selective skeletal uptake compared with both ^{99m}Tc-HEDP and ^{99m}Tc-MDP (Fig. 6).

Conclusion

A new bisphosphonate analogue as ligand for bone study was developed and evaluated in rats. The labeling efficiency of ^{99m}Tc-AEDP, as determined by ITLC, was greater than 97%. Radioactivity in bone tissue was as high as 5.54 ± 0.98% ID/g (mean ± SD, n = 6) at 1 h after injection and decreased to 2.42 ± 0.31% ID/g at 24 h. Activity in kidneys was the highest at 3 h after injection. The radioactivities in muscle, stomach, small intestine, liver, and blood were all lower than 0.6% ID/g from 1 to 24 h.

The bone-to-blood uptake ratio was 4.01 at 1 h and increased to 9.8 at 3 h post injection, then decreased to 6.54 at 24 h. While, the bone-to-muscle uptake ratio increased from 138 at 1 h to 242 at 24 h. A major part of the injected radiotracer (~54%) was excreted by the urinary system by 3 h.

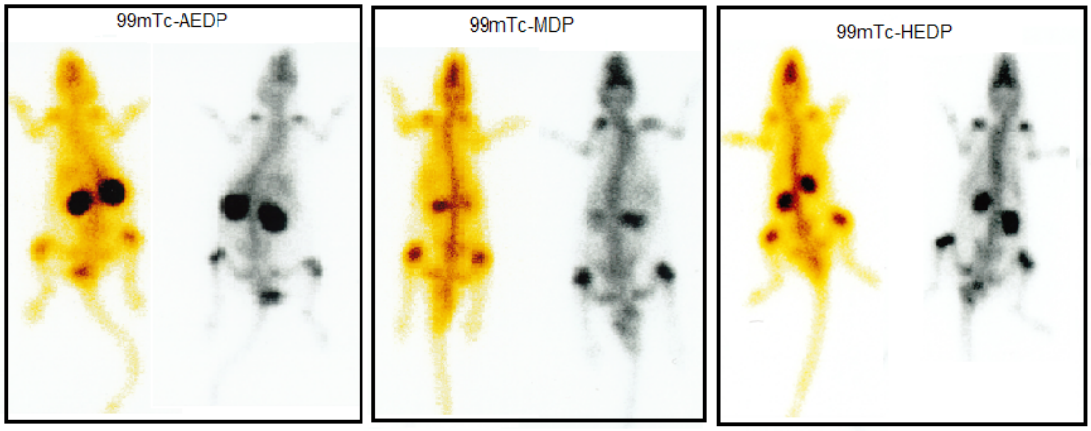


Fig. 6. Whole-body image of rat corresponding to 1 h after injection of 0.8 mCi (29.6 MBq) of ^{99m}Tc-AEDP, ^{99m}Tc-HEDP and ^{99m}Tc-MDP. AEDP: (1-aminoethane-1,1-diyl)diphosphonic acid; MDP: methylenediphosphonic acid; HEDP: (1-hydroxyethane-1,1-diyl)diphosphonic acid.

In conclusion, ^{99m}Tc-AEDP appears to be a good potential candidate for clinical use as a bone-seeking agent, since it displays highly selective uptake in the skeletal system, has low non-target uptake, and rapid clearance in blood. Scintigraphic images of ^{99m}Tc-AEDP in normal rats revealed selective skeletal uptake.

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