Preparation and biodistribution of ⁹⁹Tc^m-PIDP as bone imaging agent

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Abstract A novel zoledronic acid derivative, 1-hydroxy-2-(2-propyl-1*H*-imidazol-1-yl)ethane-1,1-diyldiphosphonic acid (PIDP), was synthesized by three-step reactions from 2-propyl-1*H*-imidazole. It was labeled with ⁹⁹Tc^m in conditions of 0.1 mg SnCl₂·2H₂O at pH 6.0 and ⁹⁹Tc^mO₄⁻ in aqueous solution for 20 min at room temperature. The labeling yield and radiochemical purity of ⁹⁹Tc^m-PIDP are both higher than 95%. The biodistribution results show that the bone uptake is up to 8.47%ID/g which is the maximum of bone uptake at 30 min after injection of ⁹⁹Tc^m-PIDP in mice. The pharmacokinetic parameters can be estimated from the exponential equation of *C*=59.565*e*^{-11.307*t*} + 2.069*e*^{-1.211*t*}. The clear bone image of rabbit was obtained at 120 min after injection of ⁹⁹Tc^m-PIDP. The results indicate that ⁹⁹Tc^m-PIDP has highly selective uptake in the skeletal and low uptake, rapid clearance in soft tissues, so it would be a potential novel bone imaging agent.

Key words Diphosphonic acid, Bone imaging agent, 99Tcm-PIDP, Biodistribution

1 Introduction

Since Fleisch H *et al.*^[1] found in 1968 that diphosphonates have high affinity for bone mineral, they have experienced three generations of development. Zoledronic acid (ZL), a third-generation diphosphonates, under the registered trade name Zometa^[2,3], has been in clinical applications for treatments of patients with tumor-induced hypercalcaemia and osteolytic bone metastases.

⁹⁹Tc^m-labeled diphosphonates have been widely used as bone imaging agent and become an important branch of nuclear medicine^[4], such as ⁹⁹Tc^m-MDP. But diphosphonates with a imidazole ring have higher affinity for bone mineral^[5], instead, zoledronic acid was labeled with ⁹⁹Tc^m, with good bone scan images^[6,7]. In this work, for developing novel bone imaging agent with excellent biological properties, we optimized the imidazolyl group and synthesized 1-hydroxy-2-(2-propyl-1*H*-imidazol-1-yl)ethane-1,1diyldiphosphonic acid (PIDP), a novel diphosphonate in similar structure to ZL with a substitutional group in the imidazole ring. The complex ⁹⁹Tc^m-PIDP was further prepared and its biodistribution in normal mice and bone scan in rabbit was studied, which may be potentially useful as a bone imaging agent.

2 Materials and methods

2.1 Reagents, instruments and animals

Stannous chloride and hydrochloride (analytical grade) were from Sinopharm Chemical Reagent Corp. Ltd. 2-Propyl-1*H*-imidazole (chemical pure grade) was from Shanghai Hanhong Chemical Corp. Ltd. ⁹⁹Tc^mO₄⁻ was supplied by Jiangsu Institute of Nuclear Medicine. Ketamine hydrochloride injection were from Jiangsu Hengrui Medicine Corp. Ltd and diazepam injection from Jiangsu Jumpcan Pharmaceutical Corp. Ltd. Elemental analysis was carried out using an Elementar Vario EL III analyzer.

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Electron spray ion (ESI) mass spectra were measured using a Waters Platform ZMD4000 LC/MS. NMR spectra were obtained on a Bruker DRX-500 spectrometer. Chemical shift values were given relative to internal tetramethylsilane. A Packardmulti-prias γ Counter (USA), Philips SKYLight ECT (USA) were used.

Normal mice (18–20 g) and rabbit (2.4 kg) were supplied by Southern Yangtze Center of Experimental Animals. The animal experiment in this study was approved by the Animal Care and Ethnics Committee of Jiangsu Institute of Nuclear Medicine.

2.2 Synthesis of PIDP

PIDP was synthesized in three-step reactions from the 2-propyl-1*H*-imidazole, in the route in Fig.1.



Fig. 1 The synthetic route of PIDP.

2.2.1 2-(2-propyl -1H-imidazole-1-yl) acetic acid ethyl ester (Compound **2**)

2-Propyl -1*H*-imidazole (11 g , 0.1 mol) was dissolved in CH₂Cl₂ (75 mL). KOH (8.4 g , 0.15 mol), K₂CO₃ (13.8 g, 0.0835 mol), and tetrabutyl ammonium bromide (0.7 g, 0.002 mol) were added and stirred at room temperature for 30 min. The solution was treated dropwise with ethyl bromoacetate (11.2 mL) for about 30 min. The mixture was heated to reflux for approximately 7 h on a water-bath. The resulting precipitate was filtered and washed with CH₂Cl₂ (40 mL). The filtrate was washed with saturated NaCl solution, the aqueous layer was separated. The organic layer was dried over Na₂SO₄. Finally, the mixture was evaporated to yield Compound **2** as a brown gum (yield: 57%), which was used without any further purification for the next step.

2.2.2 2-(2-propyl-1H-imidazole-1-yl)acetic acid (Compound **3**)

Compound 2 (9.8 g, 46 mmol) was placed in a

two-neck flask. H_2O (100 mL) and concentrated HCl (1 mL) were added. The mixture was heated to reflux on an oil-bath. Stirring for about 7 h, the solution was concentrated and recrystallized from ethanol to give the colorless crystals (yield: 80%).

M.p.: 139–141°C; ¹H- NMR (500 MHz, DMSO): $\delta7.61$ (s, 2H, CHring), 5.12 (s, 2H, COOHCH₂), 2.9 (t, 2H, CH₃CH₂CH₂), 1.7 (m, 2H, CH₃CH₂CH₂), 0.9 (t, 3H, CH₂CH₃); ESI-MS, *m*/*z* (%): 168 (8) = M, 167(100) = M-H, Anal. calcd for C₈H₁₂N₂O₂: C, 57.13; H,7.19; N, 16.6; Found C, 56.65; H, 7.35; N, 16.84.

2.2.3 1-Hydroxy-2-(2-propyl-1*H*-imidazol-1-yl) ethane-1, 1-diyldiphosphonic acid (Compound **1**)

Compound 3 (4.2 g, 25 mmol) was dissolved in phosphoric (85%, 5 mL) and chlorobenzene (25 mL). Heating to 100°C, the solution was treated dropwise with phosphorus trichloride (6.5 mL) for about 20 min, and kept at 100°C for 3 h. The chlorobenzene was decanted. The yellow residue was put in 9 mol/L HCl (30 mL) and heated to reflux for 5 h. The mixture was treated with charcoal before filtration and concentration. By adding ethanol to the solution crude product appeared, and the recrystallized white crystal was Compound 1 (yield: 35%).

M.p.: 229–231°C; ¹H NMR (250 MHz, NaOH/D₂O): δ 7.4 (d, 1H, CHring), 7.18 (d, 1H, CHring), 4.54(t, 2H, NHCH₂), 3.0 (t, 2H, CH₃CH₂CH₂), 1.7 (m, 2H, CH₃CH₂CH₂), 0.9 (t, 3H, CH₂CH₃); ESI-MS, *m*/*z* (%): 314 (8) = M, 313 (100) = M-H. Anal. calcd for C₈H₁₂N₂O₂: C, 30.58; H, 5.61; N, 8.92 Found C, 30.75; H,5.33; N, 9.13.

2.3 Preparation and stability of ⁹⁹Tc^m-PIDP

The solution containing 5 mg of PIDP and 0.1 mg of stannous chloride was adjusted to pH 6.0 with 0.2mol/L phosphate buffered solution to a volume of 1.8 mL. The 18.5 MBq (0.5 mCi) of freshly prepared eluate $^{99}\text{Tc}^{\text{m}}\text{O}_{4}^{-}$ was added, and the mixture was reacted for 20 min at room temperature to prepare $^{99}\text{Tc}^{\text{m}}\text{-PIDP}$.

The radiolabeling yield and the radiochemical purity (RCP) were evaluated by thin-layer chromatography (TLC) with developing systems of (1) acetone: water = 2:1 and (2) purified water. The $R_{\rm f}$ values were 0.9–1.0 for ${}^{99}{\rm Tc}^{\rm m}{\rm O_4}^{-}$, 0.0–0.1 for

⁹⁹Tc^m-PIDP and ⁹⁹Tc^mO₂ in System (1); while $R_{\rm f}$ values were 0.8–1.0 for ⁹⁹Tc^m-PIDP and ⁹⁹Tc^mO₄⁻, 0.0–0.1 for ⁹⁹Tc^mO₂ in System (2).

The RCP for the freshly prepared 99 Tc^m-PIDP was evaluated every hour within 6 h at room temperature (25 ± 2°C), in order to determine whether it was stable enough under ambient condition.

2.4 Biodistribution and pharmacokinetic studies

Biodistribution studies of ⁹⁹Tc^m-PIDP were performed with 35 mice, which were divided into seven groups and sacrificed at 5, 10, 15, 30, 60, 120 and 240 min (five mice at each time point) after injection of 1.85MBq (0.05 mCi) ⁹⁹Tc^m-PIDP in a volume of 0.2mL via the tail vein. Samples of heart, liver, spleen, lung, kidney, bone, muscle, gonad, intestine, stomach, brain and blood were collected and weighed. The samples were counted by a well-type gamma counter to calculate residual activity in different organs. The radioactivity of organs and tissues were expressed as percentage uptake of injected dose per gram of organ (%ID/g). Bone-to-organ uptake ratios were determined from the %ID/g values.

Blood samples (0.1 mL) were collected in microcapillary tubes by nicking the tail with a needle at 10, 30, 60, 90, 120, 180, and 240 min after the ⁹⁹Tc^m-PIDP administration. The data were fitted with the two- compartment model, and the pharmacokinetic parameters were analyzed by the DAS2.1.1 code. Radioactivity as a function of time was given by $C=Ae^{-\alpha t}+Be^{-\beta t}$.

2.5 Rabbit bone-imaging of ⁹⁹Tc^m-PIDP

155 MBq (4.2 mCi, 2 mL) ⁹⁹Tc^m-PIDP was injected through ear intravenous of rabbit 20 min after intramuscular injection of 1 mL Ketamine hydrochloride injection (0.05 g) and 2 mL Diazepam injection (10 mg). Bone scan was carried out with the Philips SKYLight ECT. The whole-body image was observed for 6 h. Scans of bone and soft tissues (heart, liver, kidney, muscle and stomach) were obtained every 300 s under the conditions of low energy, high resolution, and 128×128 matrix in the first hour. Regions of interest (ROIs) were directly drawn on the SPECT composite images, and the occipital region drawn in the same way served as a background

radioactivity region. Shapes and sizes of ROIs were kept constant for all subsequent images. The uptakes of femur, muscle close to femur and other soft tissues were taken, and bone-to-soft tissues uptake ratios were calculated from the same ROI. The bone scanning images were collected at 90, 120, 150, 180, 210, 240, 300 and 360 min, respectively.

3 Results and discussion

3.1 Synthesis of PIDP

The total yield of PIDP is 15.96%, and its melting point is $229-231^{\circ}$ C. PIDP and intermediate Compound **3** are identified by elemental analysis, IR, MS and ¹H-NMR, and the results agree well with the chemical structures (Fig.1).

3.2 Preparation and stability of ⁹⁹Tc^m-PIDP

The thin-layer chromatography of ⁹⁹Tc^m-PIDP shows that the radiolabeling yield and radiochemical purity are both larger than 95%. And ⁹⁹Tc^m-PIDP has good stability in vitro (Fig.2).



Fig. 2 Stability of ⁹⁹Tc^m-PIDP in vitro.

3.3 Biodistribution of ⁹⁹Tc^m-PIDP in mice

The biodistribution of ${}^{99}\text{Tc}^{\text{m}}\text{-PIDP}$ (Table 1) shows that ${}^{99}\text{Tc}^{\text{m}}\text{-PIDP}$ has high affinity for bone mineral. The bone uptake was up to 3.55 at 5 min and increased continuously to a peak of 8.47%ID/g at 30 min after injection of ${}^{99}\text{Tc}^{\text{m}}\text{-PIDP}$. Meanwhile, the blood uptake decreased quickly from 7.11 to 1.46%ID/g in 30 min.

Compared with ⁹⁹Tc^m-MDP^[8,9], the bone uptake of ⁹⁹Tc^m-PIDP were respectively 8.47, 6.55 and 5.94%ID/g, whereas the bone uptake of ⁹⁹Tc^m-MDP^[8,9] were 3.26, 4.79 and 3.87%ID/g at 30, 60 and 120 min after injection. In addition, the bone-to-liver uptake ratios of ⁹⁹Tc^m-PIDP were 15.81, 20.44, and 22.20, respectively, while the uptake ratios of ⁹⁹Tc^m-MDP

were 0.85, 1.26, and $2.96^{[8,9]}$. Besides, at the same time point, the bone-to-spleen uptake ratios of $^{99}\text{Tc}^{\text{m}}$ -PIDP were 25.72, 36.65, and 43.50, respectively, while those of $^{99}\text{Tc}^{\text{m}}$ -MDP were 1.01, 2.63 and 1.82^[8,9],

respectively.	These	indicated	that	bone	absorption,
bone-to-liver	and bo	ne-to-splee	en rat	ios of	⁹⁹ Tc ^m -PIDP
were higher t	han thos	se of ⁹⁹ Tc ^m	-MD	P.	

Table 1Biodistribution of "Tc"-PIDP in mice (mean \pm SD , $n = 5$, %ID/g)								
Organs	5 min	10 min	15 min	30 min	60 min	120 min	240 min	
Heart	4.08±0.13	1.66 ± 0.05	1.18 ± 0.05	$0.60{\pm}0.08$	0.39 ± 0.06	$0.14{\pm}0.01$	0.09 ± 0.01	
Liver	3.47 ± 0.04	1.86 ± 0.09	0.97 ± 0.05	$0.54{\pm}0.04$	0.32 ± 0.03	0.27 ± 0.01	0.23 ± 0.00	
Spleen	1.79 ± 0.06	0.86 ± 0.09	$0.59{\pm}0.06$	0.33 ± 0.02	0.18 ± 0.01	0.14 ± 0.01	0.09 ± 0.01	
Lung	3.85 ± 0.19	3.40±0.15	2.05 ± 0.14	1.31±0.05	0.65 ± 0.03	$0.24{\pm}0.01$	0.09 ± 0.00	
Kidney	8.60±0.39	6.76±0.20	5.33±0.07	3.76±0.13	1.67 ± 0.05	1.45 ± 0.09	1.13±0.03	
Bone	3.55 ± 0.60	5.52±1.26	6.59±1.05	8.47±0.44	6.55±0.71	5.94±0.75	4.04±1.05	
Muscle	1.46 ± 0.11	1.24±0.09	0.56±0.11	0.40 ± 0.04	0.23±0.01	$0.14{\pm}0.02$	0.08 ± 0.00	
Gonad	1.66 ± 0.03	1.61 ± 0.06	1.29±0.08	0.63 ± 0.03	0.21±0.03	0.17 ± 0.01	0.08 ± 0.00	
Intestine	2.14±0.07	1.67 ± 0.08	0.65 ± 0.01	0.53±0.12	0.30 ± 0.03	0.22 ± 0.01	0.09 ± 0.01	
Stomach	1.83±0.16	1.58 ± 0.06	0.74 ± 0.10	0.68±0.13	0.48 ± 0.08	$0.19{\pm}0.02$	0.10 ± 0.00	
Brain	0.33 ± 0.03	0.17 ± 0.01	0.11 ± 0.02	0.05 ± 0.00	0.03 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	
Blood	7.11±0.65	4.52 ± 0.98	2.74±0.60	1.46±0.42	0.75±0.35	0.21±0.07	0.08 ± 0.04	
Uptake ratio (bo	one: organs, tissu	e or blood)						
Heart	0.87	3.33	5.58	14.04	16.92	42.02	43.53	
Liver	1.02	2.97	6.77	15.81	20.44	22.20	17.36	
Spleen	1.99	6.39	11.25	25.72	36.65	43.50	46.09	
Lung	0.92	1.62	3.21	6.45	10.05	24.39	45.74	
Kidney	0.41	0.82	1.24	2.25	3.93	4.10	3.57	
Muscle	2.42	4.44	11.71	21.23	28.86	41.54	52.88	
Gonad	2.14	3.44	5.10	13.51	30.83	34.21	49.81	
Intestine	1.66	3.30	10.18	15.87	22.11	26.50	43.18	
Stomach	1.94	3.50	8.95	12.48	13.55	31.34	39.70	
Brain	1.73	32.16	58.31	158.60	222.78	294.73	331.49	
Blood	0.50	1.22	2.41	5.80	8.73	28.29	50.50	

For a better understanding of the biodistribution and excretion of ⁹⁹Tc^m-PIDP in vivo, we compared it with ⁹⁹Tc^m-ZL further. The clearance in heart and blood of ⁹⁹Tc^m-PIDP are more rapid than ⁹⁹Tc^m-ZL in the same period (30-120 min) with the values of 0.46, 1.25%ID/g for ⁹⁹Tc^m-PIDP and 0.37, 0.44 %ID/g for ⁹⁹Tc^m-ZL respectively. In addition, the resorption of ⁹⁹Tc^m-PIDP in soft tissues of mice including the heart, liver, spleen, lung, kidney and muscle are all smaller than ⁹⁹Tc^m-ZL. Therein, the uptake in liver, spleen and kidney are especially low. From the comparison, we can see that from ^{99m}Tc-ZL to ^{99m}Tc-PIDP, when the imidazole ring was substituted by a propyl group, the resorption in soft tissues became lower and the clearance in soft tissues became more quickly. Therefore, one can conclude that the ^{99m}Tc-PIDP is fit for bone imaging.

3.4 Pharmacokinetics of ⁹⁹Tc^m-PIDP

Mean plasma concentrations in normal mice are shown in Fig.3. The data were analyzed by the DAS2.1.1 code and clearance curve of radioactivity in the mouse blood was obtained. The curve conformed to a two-compartment pharmacokinetic model. Pharmacokinetic parameters were listed in Table 2. The exponential equation was $C_p=59.565e^{-11.307t} + 2.069e^{-1.211t}$.

Table 3 shows uptake ratios of bone-to-soft tissues in the first 50 min for dynamic state collection in rabbit. The ratios of bone-to-heart, liver, kidney, muscle and stomach got bigger. The results indicate that ⁹⁹Tc^m-PIDP has rapid clearance in soft tissues. Fig.4 shows a whole-body image of rabbit obtained at different time after injection of ⁹⁹Tc^m-PIDP. The bone scan image in the first hour showed that ⁹⁹Tc^m-PIDP had a high uptake in bone and low uptake in soft

tissues in normal rabbit. From ⁹⁹Tc^m-ZL to ⁹⁹Tc^m-PIDP, the bone imaging of the latter was better due to the more quick clearance in soft tissues. The bone scan image revealed that ⁹⁹Tc^m-PIDP had highly selective skeletal uptake in normal rabbit.

In summary, ⁹⁹Tc^m-PIDP displays highly selective uptake in the skeletal system and has low uptake and rapid clearance in soft tissues. The preparation and primary biological evaluations suggest that the novel ⁹⁹Tc^m-PIDP is a promising radio-pharmaceutical for targeted bone imaging. Future studies will aim at systematic comparisons of technetlum-99m-labeled zoledronic acid derivatives and ⁹⁹Tc^m-MDP to obtain the most excellent bone imaging agent for clinical applications.



Fig. 3 Clearance curve of radioactivity in mouse's blood.

Table 2	Pharmacokinetic	parameters	of 99Tcm-PIDP
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Parameters	$T_{1/2\alpha}$ /h	$T_{1/2\beta}$ /h	$V_{\rm d}$ /L	K_{12} / h^{-1}	K_{21} /h ⁻¹	$K_{\rm e}/{\rm min}^{-1}$	$CL/L\cdot h^{-1}$
Value	0.17	0.65	0.05	2.09	1.55	13.26	0.11

Ratio	10 min	20 min	30 min	40 min	50 min	
Bone/heart	1.23	1.44	1.68	1.78	1.90	
Bone/liver	0.77	0.91	1.01	1.12	1.18	
Bone/kidney	0.76	0.98	1.24	1.58	1.81	
Bone/muscle	3.37	3.54	3.82	4.00	4.23	
Bone/stomach	3.13	3.73	3.88	4.12	4.57	

 Table 3
 Ratio of bone to soft tissues for dynamic state collection in rabbit



Fig.4 Whole-body image of rabbit at different time after injection of ⁹⁹Tc^m-PIDP.

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