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NUCLEAR SCIENCE AND TECHNIQUES
Nuclear Science and Techniques, Vol.17, No.5 (2006) 285–288

Study on the preparation and biodistribution of ^{99m}Tc-HMIBP

GUO Xue-Hua[1, 2] LUO Shi-Neng[2, 1,*] WANG Hong-Yong[2] ZHOU Lian[1, 2] XIE Min-Hao[2]
YE Wan-Zhong[2] YANG Min[2] WANG Yang[2]

([1]School of Chemical and Material Engineering, Southern Yangtze University, Wuxi 214036, China;

[2]Jiangsu Institute of Nuclear Medicine, Wuxi 214063, China)

Abstract ^{99m}Tc-HMIBP, a new bone-imaging agent, was prepared by the reduction of ^{99m}Tc-pertechnetate in the presence of $SnCl_2$•$2H_2O$. The effects of the amounts of $SnCl_2$•$2H_2O$ and HMIBP and the pH value on the labeling yield and radiochemical purity of ^{99m}Tc-HMIBP were investigated. When the amounts of $SnCl_2$•$2H_2O$ and HMIBP were more than 10 μg and 2.5 mg, respectively, the pH value was between 2 and 7, and the labeling reaction continued for 10 min, both labeling yield and radiochemical purity of ^{99m}Tc-HMIBP were more than 90%. The biodistribution in rats and bone scan in rabbits were also studied. The results showed that the bone uptake is up to 7.94%ID/g at 30 min after injection of ^{99m}Tc-HMIBP, bone-to-muscle and bone-to-blood uptake ratios were 20.89 and 16.89, respectively. The clear bone image was obtained at 120 min after injection of ^{99m}Tc-HMIBP and clearance in soft tissue was visible. All of the above-mentioned results suggested that ^{99m}Tc-HMIBP may be a potential bone-imaging agent.

Key words Bone-imaging agent, ^{99m}Tc-labeled HMIBP, Biodistribution

CLC number R817

1 Introduction

Since Subramanian's description of the use ^{99m}Tc-polyphosphate for bone imaging in 1971, it has been widely used with radioactive nuclides in the field of nuclear medicine. The major bone-imaging compounds are ^{99m}Tc-labeled phosphate and phosphonate, of which ^{99m}Tc-MDP presently has the widest clinical application. [1]

Zoledronate, a third-generation bisphosphonate compound, which is extensively used clinically for the treatment of patients with tumor-induced hypercalcaemia and osteolytic bone metastases arising from breast cancer or multiple myeloma and for Paget's disease of bone, has been selected for clinical development under the registered trade name Zometa[2,3]. Zoledronic acid[4] and MIDP[5] were labeled and the bone scan was carried out, which showed good images especially for ^{99m}Tc-MIDP. It has been reported that bisphosphonates with an imidazole ring have higher affinity for bone mineral[6]. ^{99m}Tc-HMIBP (1-Hydroxy-2-(1-methylimidazol-2-yl)ethylidene-1,1-bisphosphonic acid), which has a structure similar to that of MIDP with an imidazole ring, is therefore considered potentially useful as a bone-imaging agent. For the above-mentioned reasons, ^{99m}Tc-HMIBP was prepared and experiments were conducted to evaluate the biodistribution in normal rats in order to determine whether ^{99m}Tc-HMIBP is an excellent bone-imaging agent.

2 Materials and methods

2.1 Reagents

1-Hydroxy-2-(1-methylimidazol-2-yl)ethylidene-1,1-bisphosphonic acid (HMIBP) was prepared according to a previous study [7]. $SnCl_2$•$2H_2O$ and hydrochloride were purchased from Shanghai Chemical

*Corresponding author, e-mail: shineng914@yahoo.com.cn
Received date: 2006-08-10

Reagent Company and both were of analytical grade. $^{99m}TcO_4^-$ was supplied by Jiangsu Institute of Nuclear Medicine; Ketamine hydrochloride injection was purchased from Jiangsu Hengrui Medicine Co., Ltd; diazepam injection was purchased from Jiangsu Jumpcan Pharmaceutical Co., Ltd.

2.2 Instruments

A Packard-multi-prias γ Counter (made in U.S.A), and Philips SKYLight ECT (made in U.S.A) were used.

2.3 Animals

Normal rats (weighing 18–20 g) and New Zealand rabbits (weighing 2.4–2.5 kg) were supplied by Southern Yangtze Center of Experimental Animals.

2.4 Preparation of ^{99m}Tc-HMIBP

The solution containing HMIBP and stannous chloride was adjusted to suitable pH by the addition of buffer and made up to a volume of 1.5 mL. After adding 0.5 mL (18.5 MBq) of freshly prepared eluate, $^{99m}TcO_4^-$, the labeling reaction was continued for 10 min at room temperature to yield ^{99m}Tc-HMIBP.

2.4.1 Effect of amount of $SnCl_2•2H_2O$ on the labeling yield.

A total of 5 mg of HMIBP was added to each bottle, and 5, 10, 30, 50, 80, 100, 120, 150, 200, and 250 μg of stannous chloride was added, respectively. After the pH value was adjusted to 4.0, 0.5 mL (18.5 MBq) of the eluate $^{99m}TcO_4^-$ was added to the solution and the reaction was continued for 10 min. As was shown in Fig. 1, the radiochemical purity (RCP) and radiolabeling yield (RLY) of ^{99m}Tc-HMIBP were both more than 90% when the amount of $SnCl_2•2H_2O$ was more than 10 μg.

2.4.2 Effect of pH value on the labeling yield.

HMIBP (5 mg) and stannous chloride (100 μg) were added. When the pH value was between 2 and 7, the RCP and RLY of ^{99m}Tc-HMIBP were both more than 90%, as shown in Fig. 2.

2.4.3 Effect of amount of HMIBP on the labeling yield.

Under the condition of pH 4.0, $SnCl_2•2H_2O$ (100 μg), eluate $^{99m}TcO_4^-$ (18.5 MBq), and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, and 10 mg HMIBP were added, respectively. As can be seen from Fig. 3, both the RCP and RLY of ^{99m}Tc-HMIBP were more than 90% when the amount of HMIBP was more than 2.5 mg.

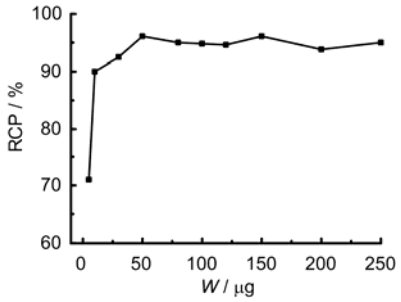

Fig. 1 Effect of amount of $SnCl_2•2H_2O$ on the labeling yield.

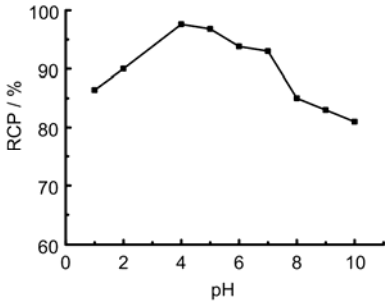

Fig. 2 Effect of pH value on the labeling yield.

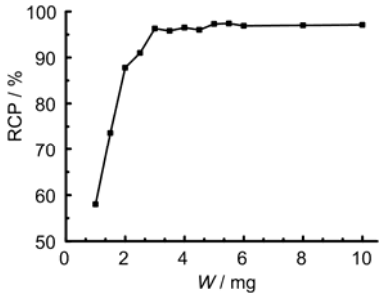

Fig. 3 Effect of amount of HMIBP on the labeling yield.

2.5 Determination of RLY and RCP

The radiolabeling yield (RLY) and the radiochemical purity (RCP) were determined by thin-layer chromatography (TLC) with developing systems of 67% acetone (1) and purified water (2). R_f for $^{99m}TcO_4^-$ was 0.9–1.0 and R_f for ^{99m}Tc-HMIBP and $^{99m}TcO_2$ was 0.0–0.1 in system (1), whereas in system (2), R_f for ^{99m}Tc-HMIBP and $^{99m}TcO_4^-$ was 0.9–1.0 and R_f for $^{99m}TcO_2$ was 0.0–0.1.

2.6 The stability of ^{99m}Tc-HMIBP in vitro

The RCP for the freshly prepared ^{99m}Tc-HMIBP was evaluated every 1 hour at room temperature (30

± 2°C) to determine whether it was stable within 6 h, and the result (see Fig. 4) showed that ^{99m}Tc-HMIBP had good stability in vitro.

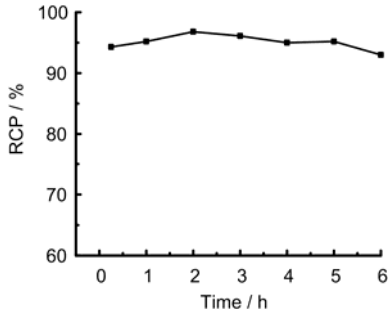

Fig. 4 Stability of ^{99m}Tc-HMIBP in vitro.

2.7 Biodistribution of ^{99m}Tc-HMIBP in rats

Thirty rats, each weighing 18–20 g, were used to determine the distribution of ^{99m}Tc-HMIBP in various organs. The rats were sacrificed at 5, 15, 30, 60, 120, and 240 min (five rats at each time point) after injection via the tail vein of 1.85 MBq (0.05 mCi) ^{99m}Tc-HMIBP in a volume of 0.1 mL. Samples of heart, liver, spleen, lung, kidney, muscle, and bone were taken and weighed. In addition, a 0.1 - mL sample of blood was drawn immediately after sacrifice. Samples of different organs were counted in a well-type γ-counter to determine resident activity in different organs. Tissue concentrations were calculated and expressed as percent uptake of injected dose per gram (%ID/g). Bone-to-organ uptake ratios were determined from the %ID/g values (see Table 1).

Table 1 Biodistribution of ^{99m}Tc-HMIBP in rats ($x \pm \sigma$, $n = 5$, %ID/g)

Time/min	Heart	Liver	Spleen	Lung	Kidney	Muscle	Bone	Blood
5	1.03 ± 0.14	0.77 ± 0.08	0.50 ± 0.07	2.22 ± 0.56	6.01 ± 0.78	0.67 ± 0.12	4.98 ± 1.39	2.40 ± 0.68
15	0.58 ± 0.22	0.49 ± 0.10	0.30 ± 0.06	1.20 ± 0.37	2.24 ± 0.14	0.44 ± 0.10	6.84 ± 0.64	1.07 ± 0.12
30	0.36 ± 0.22	0.21 ± 0.06	0.15 ± 0.02	0.59 ± 0.12	1.20 ± 0.15	0.38 ± 0.12	7.94 ± 1.73	0.47 ± 0.15
60	0.12 ± 0.03	0.14 ± 0.03	0.10 ± 0.05	0.28 ± 0.07	0.95 ± 0.26	0.26 ± 0.21	7.45 ± 1.14	0.12 ± 0.03
120	0.09 ± 0.06	0.13 ± 0.06	0.09 ± 0.09	0.17 ± 0.09	0.68 ± 0.22	0.16 ± 0.08	5.87 ± 2.43	0.05 ± 0.02
240	0.04 ± 0.01	0.08 ± 0.02	0.05 ± 0.03	0.08 ± 0.03	0.53 ± 0.07	0.10 ± 0.13	4.19 ± 0.59	0.02 ± 0.00

Time/min	$a_{m,bone}/a_{m,heart}$	$a_{m,bone}/a_{m,liver}$	$a_{m,bone}/a_{m,spleen}$	$a_{m,bone}/a_{m,lung}$	$a_{m,bone}/a_{m,kidney}$	$a_{m,bone}/a_{m,muscle}$	$a_{m,bone}/a_{m,blood}$
5	4.83	6.47	9.96	2.24	0.83	7.43	2.08
15	11.79	13.96	22.8	5.7	3.05	15.54	6.39
30	22.06	37.81	52.93	13.46	6.62	20.89	16.89
60	62.08	53.21	74.5	26.61	7.84	28.65	62.08
120	65.22	45.15	65.22	34.53	8.63	36.69	117.4
240	104.75	52.38	83.8	52.38	7.9	41.9	209.5

2.8 The rabbit bone-imaging of ^{99m}Tc-HMIBP

A total of 111 MBq (0.5 mL) of ^{99m}Tc-HMIBP was injected into the rabbit 20 min after an intravenous injection of ketamine (1 mL) and diazepam (2 mL). Bone scan was carried out using a Philips SKY-Light ECT. Fig. 5 shows the whole-body image at 120 min after injection of ^{99m}Tc-HMIBP.

3 Results and discussion

The results showed that when the amounts of $SnCl_2 \cdot 2H_2O$ and HMIBP were more than 10 μg and 2.5 mg, respectively, the pH value was between 2 and 7, and the labeling reaction continued for 10 min, both labeling yield and radiochemical purity of ^{99m}Tc-HMIBP were more than 90%.

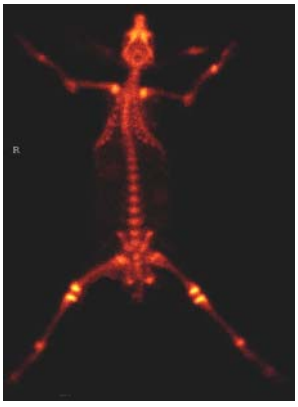

Fig. 5 A whole-body image of rabbit obtained at 120 min after injection of ^{99m}Tc-HMIBP.

^{99m}Tc-HMIBP had high affinity for bone mineral and the bone uptake was up to 4.98 at 5 min and continuously increased to attain a peak value of 7.94 at 30 min after injection of ^{99m}Tc-HMIBP (Table 1). Compared with ^{99m}Tc-MDP, the uptake of ^{99m}Tc-HMIBP in bone was 7.94, 7.45, and 5.87 %ID/g, respectively, whereas the uptake of ^{99m}Tc-MDP [8] in bone was 3.26, 4.79, and 3.87%ID/g at 30, 60, and 120 min after injection. In addition, the bone-to-blood uptake ratios of ^{99m}Tc-HMIBP were 16.89, 62.08, and 117.40, respectively, whereas the uptake ratios of ^{99m}Tc-MDP [8] were 6.91, 17.31, and 26.22, respectively. Besides, at the same time point, the bone-to-liver uptake ratios of ^{99m}Tc-HMIBP were 37.81, 53.21, and 45.12, respectively, whereas those of ^{99m}Tc-MDP [8] were 0.85, 1.26, and 2.96, respectively. The bone scan image showed that ^{99m}Tc-HMIBP had highly selective skeletal uptake in normal rabbits.

To sum up, ^{99m}Tc-HMIBP shows highly selective uptake in the skeletal system and has low nontarget uptake and rapid clearance in nonosseous tissue and it seems to be a very good potential bone-imaging agent.

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