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Preparation and preliminary biological evaluation of $^{99}Tc^m$-TADP as bone imaging agent

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Abstract TADP, 2-(1H-1,2,4-triazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid, was synthesized by three step reactions from the raw material 1H-1,2,4-triazole. $^{99}Tc^m$-TADP was prepared with 5 mg TADP at pH 7.0 by joining $^{99}Tc^mO_4^-$ with $SnCl_2 \cdot 2H_2O$ in aqueous solution for 10 min at room temperature. Both labeling yield and radiochemical purity of $^{99}Tc^m$-TADP were more than 95%. The biodistribution in rats and bone scan in rabbits were also studied. The uptake of organ was expressed as %ID/g. The results showed that the bone uptake is up to 17.17%ID/g which is the maximum of bone uptake at 30 min after injection of $^{99}Tc^m$-TADP in rats, bone-to-muscle and bone-to-blood uptake ratios were 61.32 and 13.21, respectively. The clear bone image of rabbit was obtained at 120 min after injection of $^{99}Tc^m$-TADP and clearance in soft tissue was visible. The preparation of $^{99}Tc^m$-TADP was convenient and $^{99}Tc^m$-TADP exhibited high uptake in bone, and it would be a potential new bone imaging agent.

Key words Bone-imaging agent, $^{99}Tc^m$-labeled TADP, Bio-evaluation

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1 Introduction

Since 1970s, $^{99}Tc^m$-labeled bone imaging agent has been used widely and become an important branch of nuclear medicine[1]. The major bone-imaging compounds are $^{99}Tc^m$-labeled phosphate and phosphornate, of which $^{99}Tc^m$-MDP has been in the widest clinical applications.

Diphosphonate has experienced three generations of compounds. Zoledronate, a member of the third-generation bisphosphonate compound, which is extensively used clinically for 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 of Zometa[2,3]. Zoledronic acid[4], MIDP[5] and HMIBP[6] were labeled and the bone scan showed good images especially for $^{99}Tc^m$- HMIBP. Reportedly, bisphosphonates with an imidazole ring have higher affinity for bonemineral[7]. $^{99}Tc^m$-TADP(2-(1H-1,2,4-triazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid) is different from $^{99}Tc^m$-MDP and $^{99}Tc^m$-MIDP. With a ring of three nitrogen atoms, it is therefore considered potentially useful as a bone-imaging agent. In this work, $^{99}Tc^m$-TADP was prepared and experiments were conducted to evaluate the biodistribution in normal rats and bone scan in rabbit.

2 Materials and methods

2.1 Reagents, instruments and animals

$SnCl_2 \cdot 2H_2O$ and hydrochloride, both in analytical grade, were purchased from Shanghai Chemical Reagent Corp. 1H-1,2,4-triazole, purchased from Shanghai Nuotai Chemical Co. Ltd., was of chemical

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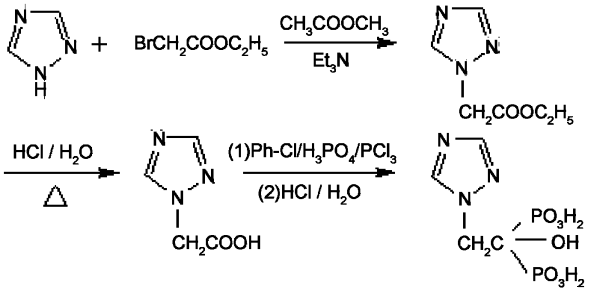
pure. TADP was synthesized at our lab, and had good stability. $^{99}Tc^mO_4^-$ was supplied by Jiangsu Institute of Nuclear Medicine. Ketamine hydrochloride injection was purchased from Jiangsu Hengrui Medicine Co. Ltd. Diazepam injection was purchased from Jiangsu Jumpcan Pharmaceutical Co. Ltd.

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

Normal rats (18~22 g) and New Zealand rabbit (2.2 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 TADP

The synthetic route of TADP is as follows.



2.3 Preparation of $^{99}Tc^m$-TADP

The solution containing 5 mg of TADP and 100μg of stannous chloride was adjusted to pH 7.0 by adding buffer phosphate to a volume of 2 mL. By adding 0.5 mL of freshly prepared eluate $^{99}Tc^mO_4^-$, the labeling reaction for 10 min at room temperature yielded $^{99}Tc^m$-TADP.

2.4 Determination of RLY and RCP

The radiolabeling yield (RLY) and radiochemical purity (RCP) were determined by thin- layer chromatography (TLC) with developing systems of (1) acetone:physiological saline = 2:1 and (2) purified water. R_f for $^{99}Tc^mO_4^-$ was 0.8~1.0 and R_f for $^{99}Tc^m$-TADP and $^{99}Tc^mO_2$ were 0.0–0.1 in System 1, whereas in System 2, R_f for $^{99}Tc^m$-TADP and $^{99}Tc^mO_4^-$ were 0.8~1.0, and R_f for $^{99}Tc^mO_2$ was 0.0~0.1.

2.5 Stability of $^{99}Tc^m$-TADP in vitro

The RCP for the freshly prepared $^{99}Tc^m$-TADP was evaluated every hour at room temperature ((25±2)°C) to determine whether it was stable within 6h, and the result (Fig.1) showed that $^{99}Tc^m$-TADP had good stability *in vitro*.

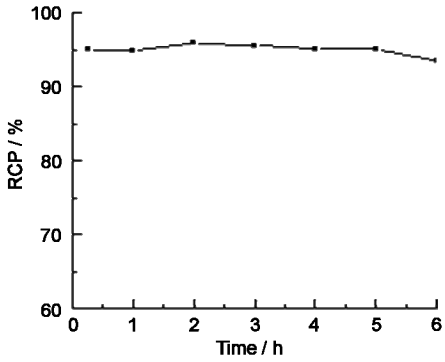


Fig. 1 Stability of $^{99}Tc^m$-TADP *in vitro*.

2.6 Biodistribution of $^{99}Tc^m$-TADP in rats

Thirty five rats, divided into seven groups, were used to determine the distribution of $^{99}Tc^m$-TADP in various organs. The rats were sacrificed at 5, 10, 15, 30, 60, 120 and 240 min (five rats at each time point) after injection via the tail vein of 1 MBq $^{99}Tc^m$-TADP in a volume of 0.2 mL. Samples of blood, brain, heart, liver, spleen, lung, kidney, muscle and femur were taken and weighed. The samples of different organs were counted by a well-type γ-counter to determine residual 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.

2.7 The rabbit bone-imaging of ^{99m}Tc-TADP

A total of 150 MBq of $^{99}Tc^m$-TADP was injected into the rabbit by ear intravenous injection 20 min after an intramuscular injection of anaesthetic, which contained 0.2 g ketamine and 10 mg diazepam. Bone scan was carried out using a Philips SKYLight ECT. The whole-body image was observed for 3 h. The first hour was dynamic state collection time. In this period scans of bone and soft tissues such as muscle, liver, heart, renal and stomach, were obtained every 150 seconds under the condition of feeblemindedness, high resolution, and 128×128 matrix. 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. Then the bone scans

were collected at 75, 90,120,140,160 and 180 min, respectively.

3 Results and discussion

3.1 Synthesis of TADP

TADP was synthesized by three step reactions from the raw material 1H-1,2,4-triazole, and the total yield was 22.5%. Melting point is (254~256)°C (reference 255°C[8]). Determined by IR, HNMR, MS and elemental analysis, the result matches well with the chemical structure.

3.2 Preparation and biodistribution of $^{99}Tc^{m}$-TADP

Thin-layer chromatography (TLC) of $^{99}Tc^{m}$-TADP indicated that both labeling yield and radiochemical purity of $^{99}Tc^{m}$-TADP were more than 95%.

Table 1 showed the result for the biodistribution of $^{99}Tc^{m}$-TADP in rats. $^{99}Tc^{m}$-TADP had high affinity for bone mineral and the bone uptake was up to 9.21 at 5 min and increased continuously to attain a peak value of 17.17%ID/g at 30 min after injection of $^{99}Tc^{m}$-TADP. However, uptake in blood descended quickly to 1.30 %ID/g in 30 min. Compared with $^{99}Tc^{m}$-ZL (2- (imidazol-1-yl)-1-hydroxyethane-1,1-diphosphonic acid)[4] and $^{99}Tc^{m}$-MDP[9], the uptakes of $^{99}Tc^{m}$-TADP in bone was 17.17, 12.58 and 8.53 %ID/g, respectively, whereas the uptakes of $^{99}Tc^{m}$-ZL[4] in bone were 13.45, 9.78 and 10.05%ID/g, and those of $^{99}Tc^{m}$-MDP[9] were 3.26, 4.79 and 3.87 %ID/g at 30, 60 and 120 min after injection. Besides, at the same time point, the bone-to-muscle uptake ratios of $^{99}Tc^{m}$-TADP were 61.32, 96.77 and 106.63, respectively, whereas those of $^{99}Tc^{m}$-ZL[4] were 28.01, 31.55 and 47.84, and those of $^{99}Tc^{m}$-MDP[9] were 20.34, 39.95 and 44.63，respectively.

Therefore, bone resorption and bone-to-muscle uptake ratios of $^{99}Tc^{m}$-TADP were higher than those of $^{99}Tc^{m}$-MDP. From $^{99}Tc^{m}$-ZL to $^{99}Tc^{m}$-TADP, when a carbon atom of imidazole was substituted by nitrogen, that is, triazole instead of imidazole, clearance in soft tissues was more quickly, bone uptake was higher and bone imaging was better.

The bone scan image in the first 60 min showed that $^{99}Tc^{m}$-TADP had a high uptake in bone and the uptakes of soft tissues were low in normal rabbit. Table 2 shows ratios of bone-to-soft tissues for dynamic state collection in rabbit in the first 50 min. The ratios of bone-to-soft-tissues became bigger. Fig.2 shows a whole-body image of rabbit obtained at 120 min after injection of $^{99}Tc^{m}$-TADP.

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

Organ / Uptake ratio	5 min	10 min	15 min	30 min	60 min	120 min	240 min
Blood	6.44±0.18	5.47±0.57	3.45±0.22	1.30±0.07	0.61±0.06	0.32±0.04	0.19±0.01
Brain	0.17±0.00	0.11±0.01	0.08±0.01	0.04±0.00	0.02±0.01	0.02±0.01	0.01±0.00
Heart	1.50±0.09	1.40±0.14	0.81±0.02	0.34±0.02	0.16±0.02	0.10±0.01	0.07±0.00
Liver	1.36±0.09	0.88±0.02	0.69±0.09	0.36±0.03	0.32±0.04	0.15±0.01	0.13±0.02
Spleen	0.86±0.02	0.66±0.01	0.53±0.03	0.23±0.01	0.12±0.01	0.08±0.02	0.06±0.00
Lung	3.70±0.17	1.94±0.08	1.81±0.16	0.75±0.03	0.41±0.04	0.32±0.04	0.14±0.01
Kidney	8.58±0.91	5.30±0.75	4.66±0.10	2.30±0.07	1.43±0.06	1.03±0.04	0.87±0.00
Muscle	1.38±0.01	0.75±0.02	0.72±0.12	0.28±0.02	0.13±0.01	0.08±0.01	0.07±0.00
Bone	9.21±1.60	9.45±2.19	11.14±1.38	17.17±4.17	12.58±3.06	8.53±0.01	7.99±0.79
a_{mbone}/a_{mblood}	1.43	1.73	3.23	13.21	20.62	26.66	42.05
a_{mbone}/a_{mbrain}	54.18	85.91	139.25	429.25	629.00	426.50	799.00
a_{mbone}/a_{mheart}	6.14	6.75	13.75	50.50	78.63	85.30	114.14
$a_{m,bone}/a_{mliver}$	6.77	10.74	16.14	47.69	39.31	56.87	61.46
$a_{mbone}/a_{mspleen}$	10.71	14.32	21.02	74.65	104.83	106.63	133.17
a_{mbone}/a_{mlung}	2.49	4.87	6.15	22.89	30.68	26.66	57.07
$a_{mbone}/a_{mkidney}$	1.07	1.78	2.39	7.47	8.80	8.28	9.18
$a_{mbone}/a_{mmuscle}$	6.67	12.60	15.47	61.32	96.77	106.63	114.14

Table 2 Ratio of bone-to-soft tissue for dynamic state collection in rabbit

Ratio	10 min	20 min	30 min	40 min	50 min
Bone/muscle	4.93	7.27	9.76	11.93	16.67
Bone/liver	0.56	0.77	0.92	0.96	1.13
Bone/renal	0.39	0.52	0.63	0.84	0.94
Bone/heart	0.82	1.14	1.36	1.59	1.62
Bone/stomach	4.00	5.57	6.75	7.45	8.00

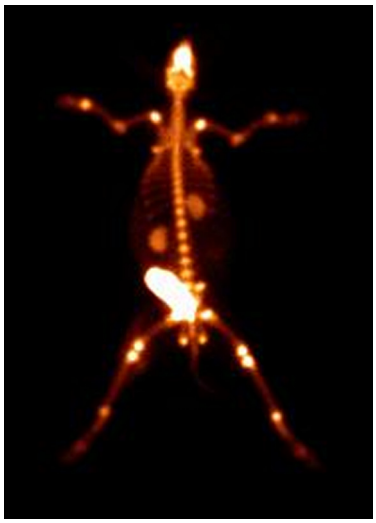


Fig.2 A whole-body image of rabbit obtained at 120 min after injection of $^{99}Tc^{m}$-TADP.

Compared to traditional bone imaging agent, $^{99}Tc^{m}$-TADP is a neotype diphosphonic acid with three nitrogen ring in the molecule, it has not been reported as a bone imaging agent. The finding indicated that $^{99}Tc^{m}$-TADP can be prepared easily. It also shows highly selective uptake in the skeletal system and low uptake in nontarget and rapid clearance in soft tissue. Accordingly, $^{99}Tc^{m}$-TADP seems to be a very good potential bone-imaging agent.

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