Journal of Radioanalytical and Nuclear Chemistry
https://doi.org/10.1007/s10967-023-08829-0

Evaluation of [^{99m}Tc]Tc-TTHMP as a SPECT imaging agent for skeletal system

Hu Song[1] · Guanquan Wang[1] · Jing Wang[1] · Xia Yang[1] · Hongyuan Wei[1] · Shunzhong Luo[1]

Received: 16 November 2022 / Accepted: 10 February 2023

Abstract
TTHMP (triethylene-tetramine-hexamethylene-phosphonic acid) shows good bone-seeking ability in previous studies. In this research, [^{99m}Tc]Tc-TTHMP was prepared with the radiochemical yield of 98% and the possible application of [^{99m}Tc]Tc-TTHMP in skeletal system imaging was evaluated. The corresponding TTHMP kits have also been developed. [^{99m}Tc]Tc-TTHMP is hydrophilic and stable in saline and serum. The biodistribution data show [^{99m}Tc]Tc-TTHMP has a high uptake in the bone and is excreted rapidly through urinary system. Both micro-SPECT/CT imaging in normal mice and SPECT imaging in a normal Beagle dog confirm the results. It is suggested that [^{99m}Tc]Tc-TTHMP can be a candidate for skeletal system imaging.

Keywords [^{99m}Tc]Tc-TTHMP · Bone-seeking · Biodistribution · SPECT imaging

Introduction

Phosphonate and multidentate aminophosphonate ligands have been extensively studied for their bone uptake characteristics. ^{99m}Tc labeled methylene diphosphonate ([^{99m}Tc]Tc-MDP)[1, 2], ^{153}Sm labeled ethylene-diamine-tetramethylene-phosphonate ([^{153}Sm]Sm-EDTMP)[3, 4] and ^{166}Ho labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetra(methylene phosphonic acid) ([^{166}Ho]Ho-DOTMP)[5, 6] have already been applied in clinics for skeletal system imaging, bone metastases or multiple myeloma therapy.

Kanishi's findings suggest that the accumulation mechanism of [^{99m}Tc]Tc-MDP in bone is by both chemical adsorption onto the surface of the hydroxyapatite and incorporation into the crystalline structure of hydroxyapatite[7]. Besides, it is reported that the affinity of phosphonate complex for calcium in actively growing bones is considered to be the factor responsible for their selective localization into metastatic lesions. And multidentate phosphonates were considered to be more effective candidates compared with diphosphonates[8, 9]. Among the multidentate phosphonates ligands, TTHMP (triethylene-tetramine-hexamethylene-phosphonic acid) has shown high bone-seeking ability in various biodistribution studies complexed with several radionuclides, such as ^{177}Lu[8, 10, 11], ^{117m}Sn[12, 13], ^{188}Re[10], ^{153}Sm[14, 15], ^{175}Yb[16] and ^{166}Ho[17, 18]. The potential application of TTHMP should be further explored.

At present, ^{99m}Tc is still the most convenient and most widely used radionuclide in radiopharmaceutical research and clinics. In this study, [^{99m}Tc]Tc-TTHMP was prepared and the possible application of [^{99m}Tc]Tc-TTHMP in skeletal system imaging was evaluated.

Experimental

General

[^{99m}Tc]$NaTcO_4$ was supplied by the First Affiliated Hospital of Southwest Medical University. TTHMP was prepared in our laboratory as previously reported[12, 13, 15]. MDP kit was provided by Chengdu Yunke Pharmaceutical Co., Ltd. Fetal bovine serum, $SnCl_2$ and other chemicals with analytical purity were purchased from Macklin Inc. Isoflorane was purchased from Hebei Yipin Pharmaceutical Co., Ltd. Radioactivity count was performed with an FJ-2021 γ radioimmune counter. Micro-SPECT/CT imaging of normal mice

Hu Song and Guanquan Wang contribute equally to this work.

✉ Hu Song
caepsh@163.com

[1] Institute of Nuclear Physics and Chemistry, China Academy of Engineering Physics, Mianyang 621999, China

Published online: 01 March 2023

was performed on a small animal U-SPECT^{+}/CT (MILABS Company, Holland). SPECT imaging study of a normal beagle dog was carried out on a GE Infinia Hawkeye4 SPECT which was provided by the First Affiliated Hospital of Southwest Medical University.

Quality control of [^{99m}Tc]Tc-TTHMP

The radiochemical yield of [^{99m}Tc]Tc-TTHMP was determined by paper chromatography. A little portion of [^{99m}Tc]Tc-TTHMP was aspired on Whatman 1$^{\#}$ chromatography paper. The strips were developed in several agents, viz., saline, acetone, pyridine/ethanol/water (1:2:4) and acetic acid/water (1:4), dried, cut into 1 cm segments and the activity was measured. When calculating the radiochemical yield, all data have been corrected for radioactive decay.

Preparation of [^{99m}Tc]Tc-TTHMP and TTHMP kits

Complexation of ^{99m}Tc with TTHMP was realized by reduction of [^{99m}Tc]$NaTcO_4$ with $SnCl_2$. Briefly, in a 0.5mL finger tube, 0.2 mL10 mg/mL TTHMP was added, then 0.02 mL 1 mg/mL $SnCl_2$ (dissolved by concentrated HCl, and protected by 1 mg/mL ascorbic acid) was added. The pH of the solution was adjusted to 6 with 1 mol/L NaOH and HCl. Finally 0.02 mL [^{99m}Tc]$NaTcO_4$ (about 3.7×10^7 Bq) eluent was added and the mixture was incubated at room temperature for about 5 min.

TTHMP kits for ^{99m}Tc labeling were prepared according to the above optimal labeling conditions. First, 10 mg/mL TTHMP, 1 mg/mL $SnCl_2$ (dissolved in 1 mol/L HCl) and 1 mg/mL ascorbic acid were prepared, and then the TTHMP, $SnCl_2$ and ascorbic acid were mixed in a volume ratio of 1:1:1. The pH of the mixed liquid was adjusted to 6 with 1 mol/L NaOH and 1 mol/L HCl, and the volume was recorded. Proper volume of the solution was added into each glass ampoule to ensure that the contents of TTHMP, $SnCl_2$ and ascorbic acid in each ampoule are 5 mg, 0.5 mg and 0.5 mg, respectively. The ampoules were put into the refrigerator at -78℃for 2 h, and then lyophilized in a lyophilizer for 24 h. At last, the ampoules were sealed with rubber stopper under vacuum to obtain the TTHMP kits.

In vitro property of [^{99m}Tc]Tc-TTHMP

The stability of [^{99m}Tc]Tc-TTHMP was studied in PBS and serum. Aliquots (0.1 mL) of [^{99m}Tc]Tc-TTHMP were incubated in PBS and serum for 24 h at 37℃. The incubated mixture was analyzed by paper chromatagraphy using the same systems for quality control. Partition coefficient $P_{o/w}$ of [^{99m}Tc]Tc-TTHMP was also tested by the ''shake-flask'' method as previously reported[19].

Biodistribution of [^{99m}Tc]Tc-TTHMP in normal mice

Normal Kunming mice (18.0 ± 2.0 g, n = 15) were intravenously injected [^{99m}Tc]Tc-TTHMP (0.1 mL, approximate 3.7 MBq) via the tail. At 1 h, 3 h, 6 h, 12 and 24 h post-injection, the mice were sacrificed, various tissues or organs (blood, heart, liver, spleen, lung, kidney, muscle, bone, intestine and brain) of each group were excised, weighed, and counted for ^{99m}Tc activity. Data were corrected for physical decay of radioactivity. The percent injected dose per gram of tissue (%ID/g) was calculated for each specimen.

SPECT imaging of [^{99m}Tc]Tc-TTHMP

[^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP were prepared by TTHMP and MDP kits, respectively. Normal Kunming mice were intravenously injected 0.1 mL (approximate 3.7 MBq) [^{99m}Tc]Tc-TTHMP or [^{99m}Tc]Tc-MDP vial the tail. Before micro-SPECT/CT imaging, the mouse was put in an anesthesia induction chamber, and then isoflorane was sprayed into the chamber. One minute later, the mouse was anesthetized and transferred to the test. At 0.5 h、2 h、3 h、6 and 24 h post-injection, micro-SPECT/CT imaging of the mice was performed. A Beagle dog was intravenously injected 0.1 mL (approximate 1.85×10^8 Bq) [^{99m}Tc]Tc-TTHMP via the left front leg. At 1 h, 2 h, 3 h, 6 and 24 h post-injection, whole-body SPECT imaging of the dog was performed. Anaesthesia of the dog was performed as the previous operation.

Results and discussion

Quality control of [^{99m}Tc]Tc-TTHMP

Because the amount of [^{99m}Tc]Tc-TTHMP is very small and radioactive, directly characterization of [^{99m}Tc]Tc-TTHMP, especially the chemical structure, is not possible by common methods. Paper chromatography combined γ radio-immune counter is a simple and effiective way to monitor the labeling reaction. So in this research, the radiochemical yield of [^{99m}Tc]Tc-TTHMP was determined by paper chromatography.The R_f values of [^{99m}Tc]$TcO_2.nH_2O$, [^{99m}Tc]TcO_4^- and [^{99m}Tc]Tc-TTHMP on Whatman 1$^{\#}$ chromatography paper with different developing systems were shown in Table 1. [^{99m}Tc]TcO_2-nH_2O is a kind of colloid, which is a common species in ^{99m}Tc labeling reaction and will not be developed in the following developing agents. The R_f value of [^{99m}Tc]TcO_2-nH_2O can be difined as 0.0. The R_f value of [^{99m}Tc]TcO_4^- was tested using [^{99m}Tc]$NaTcO_4$. The remaining peak in paper chromatography can be recognized as the product [^{99m}Tc]Tc-TTHMP. It can be seen that the radioactive

Table 1 R_f values of [^{99m}Tc]$TcO_2{\cdot}nH_2O$, [^{99m}Tc]TcO_4^- and [^{99m}Tc]Tc-TTHMP on Whatman 1# chromatography paper with different develop systems

Developing agents	[^{99m}Tc]$TcO_2.nH_2O$	[^{99m}Tc]TcO_4^-	[^{99m}Tc]Tc-TTHMP
Saline	0.0	0.7–0.8	1.0
Acetone	0.0	0.9	0.0
pyridine/ethanol/water (1:2:4)	0.0	0.8–0.9	0.3–0.4
acetic acid/water (1:4)	0.0	0.7–0.8	0.3–0.4

Table 2 The optimized labeling condition and result of [^{99m}Tc]Tc-TTHMP

Factors	pH	$SnCl_2$ (μg)	Temperature	Reaction time (min)	Radiochemical yield
[^{99m}Tc]Tc-TTHMP	6.0	50	Room temperature	5	98%

components were separated well. In consideration of environmental friendliness, saline was selected as the developing agent in subsequent experiments.

Optimization of the labeling condition

The optimized results of labeling conditions are shown in Table 2. The labeling conditions of TTHMP are mild, and the radiochemical yield can reach more than 98% at room temperature in a short time.

The effects of pH, the concentration of TTHMP and the amount of $SnCl_2$ on the formation of the complex were studied by the independent variable method and the results were shown in Fig. 1. The experiment was carried out three times for each condition. It can be seen that pH has a significant influence on the radiochemical yield. When pH is 5–7, the

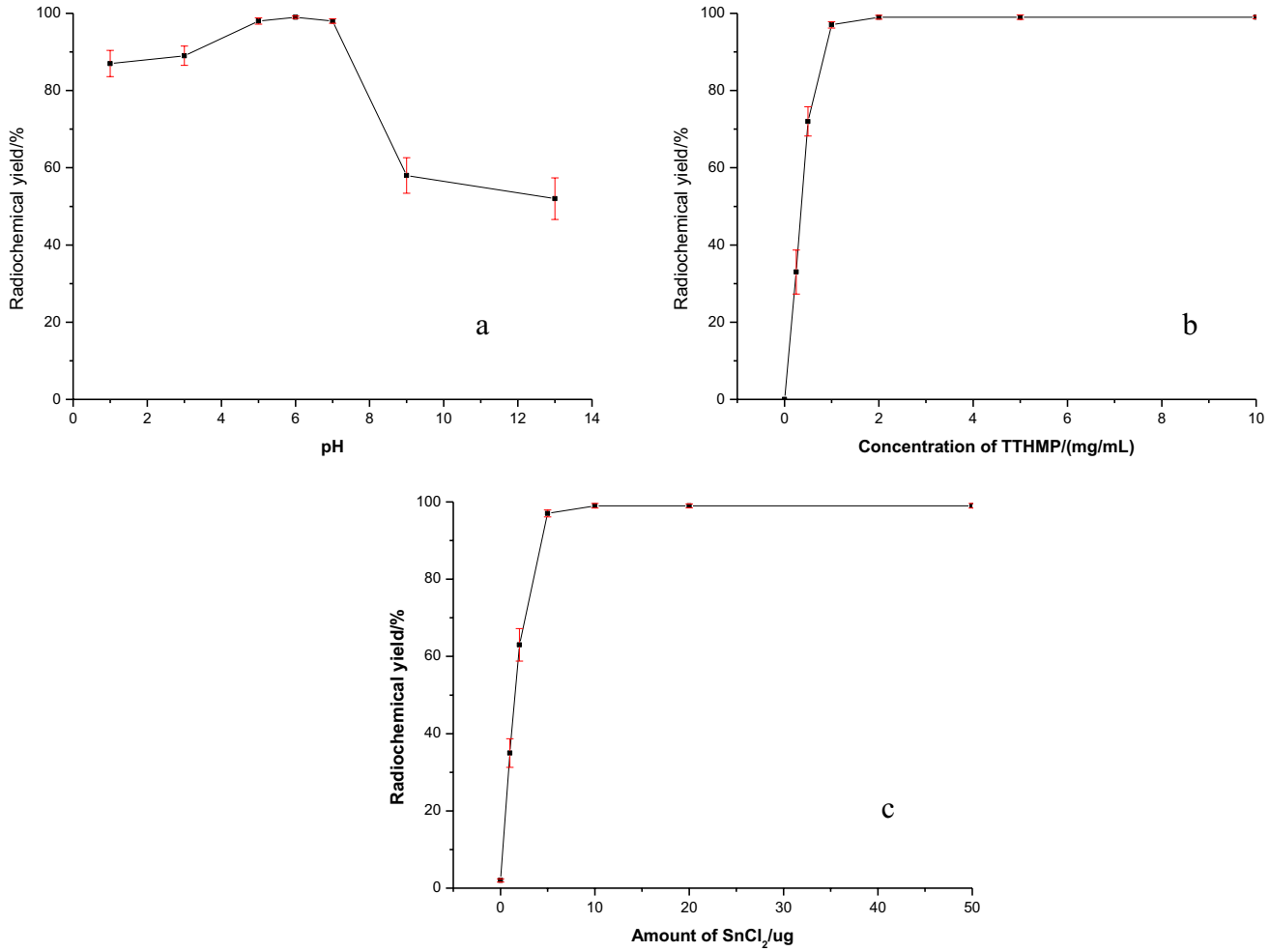

Fig. 1 Influence of pH (**a**), TTHMP concentration (**b**) and $SnCl_2$ amount (**c**) on the radiochemical yield of [^{99m}Tc]Tc-TTHMP

radiochemical yield is more than 98%. The reason may be that when the pH is far less than 5, protonation of TTHMP makes the coordination with ^{99m}Tc very difficult; When pH is alkaline, the reducing ability of $SnCl_2$ is greatly decreased. The radiochemical yield increases with increasing TTHMP concentration. When the ligand concentration was more than 1 mg/mL, the radiochemical yield was more than 98%, and remained constant. The radiochemical yield increases with the increase of $SnCl_2$ content. When the amount of stannous chloride is greater than 50 μg, the radiochemical yield was more than 98%, and the change was not significant.

In vitro property of [^{99m}Tc]Tc-TTHMP

The radiochemical purities of [^{99m}Tc]Tc-TTHMP are 98% both in PBS and serum, which indicate that [^{99m}Tc]Tc-TTHMP are stable. Determination of the partition coefficient in physiological conditions allowed us to conclude that [^{99m}Tc]Tc-TTHMP was hydrophilic ($P_{o/w}$=(4.61 ± 0.46)×10^{-3}).

Biodistribution of [^{99m}Tc]Tc-TTHMP

The results of biodistribution studies in major organs or tissues in normal Kunming mice were summarized in Fig. 2. The data presents that [^{99m}Tc]Tc-TTHMP has a very high and fast bone uptake. The bone uptake reaches 11.5 ± 1.4%/g at 1 h post-injection and decreses gradually with increasing time. The results show [^{99m}Tc]Tc-TTHMP is metabolized quickly. In addition, very low uptake in other organs or tissues was observed except for bone and kidney, which implies [^{99m}Tc]Tc-TTHMP is excreted through kidney.

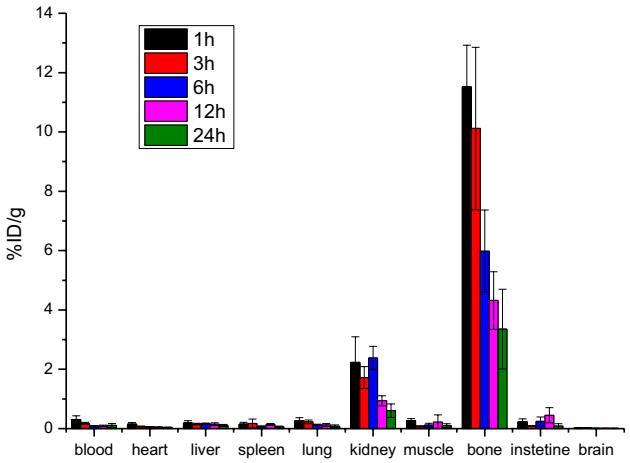

Fig. 2 Biodistribution of [^{99m}Tc]Tc-TTHMP in normal mice (%ID/g; mean ± SD)

SPECT imaging of [99mTc]Tc-TTHMP

The micro-SPECT/CT imaging of [^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP at different time points post-injection in normal mice are shown in Fig. 3. Both [^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP accumulated in the skeletal system rapidly. At 0.5 h post-injection, bone uptake of both [^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP in mice are very high, especially in the skull, spine and joints of legs. During 2 to 3 h post-injection, bone contour can be observed in both subjects administrated with [^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP. In addition, there are obvious radioactivities in mice viscera administrated with [^{99m}Tc]Tc-MDP compared with that administrated with [^{99m}Tc]Tc-TTHMP. A higher contrast of the skeletal system with [^{99m}Tc]Tc-TTHMP than [^{99m}Tc]Tc-MDP can be got during this time interval. At 6 h post-injection, the radio-images of the skeletal system fade compared to early time and there is only high radioactive accumulation in the skull, spine and joints. At 24 h post-injection, [^{99m}Tc]Tc-MDP still existed in the skull, spine and joints. However, [^{99m}Tc]Tc-TTHMP only existed in the leg joints and almost could not be observed in other parts. Besides, the signals of radioactivity in the bladder of both subjects are very high during the micro-SPECT/CT imaging. These results indicate that [^{99m}Tc]Tc-TTHMP has a better

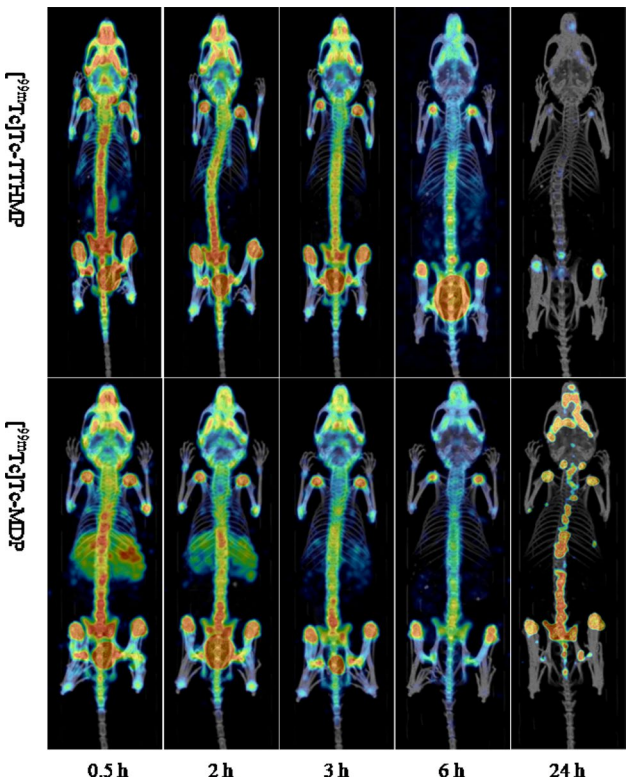

Fig. 3 Micro-SPECT/CT imaging of [^{99m}Tc]Tc-TTHMP and [^{99m}Tc]Tc-MDP in normal mice

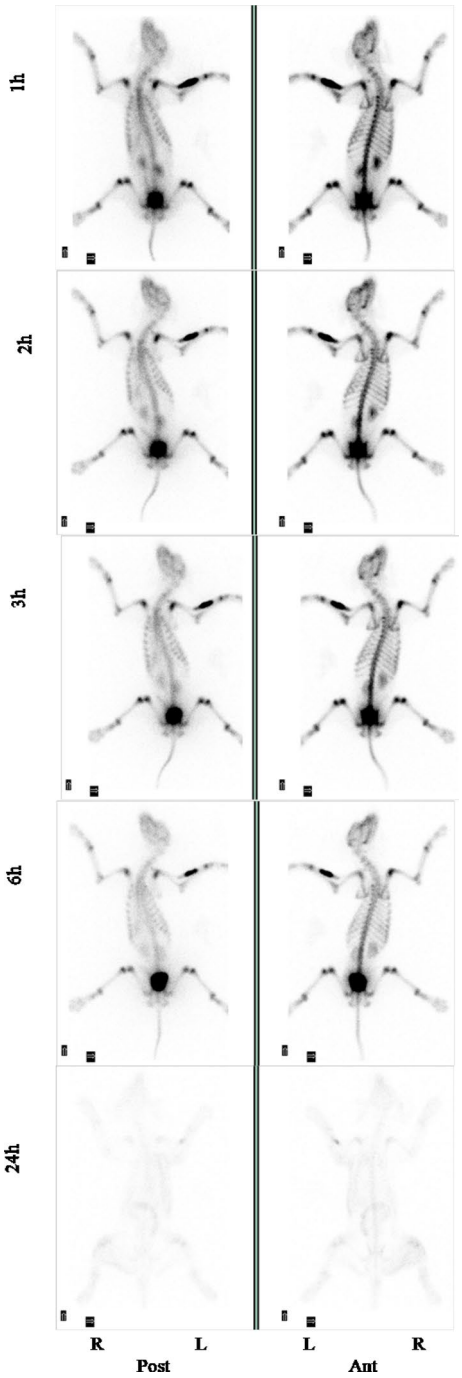

Fig. 4 SPECT imaging of [^{99m}Tc]Tc-TTHMP of in a normal Beagle dog

imaging contrast for the skeletal system during 2 to 3 h post-injection. [^{99m}Tc]Tc-TTHMP was cleared faster than [^{99m}Tc]Tc-MDP and mainly excreted through urinary system, indicating less radiation dose would be accepted by the patients if [^{99m}Tc]Tc-TTHMP could be applied in clinics.

The SPECT imaging of [^{99m}Tc]Tc-TTHMP in a normal Beagle dog are shown in Fig. 4. It can be seen that [^{99m}Tc]Tc-TTHMP displayed excellent bone-seeking property. [^{99m}Tc]Tc-TTHMP has high uptake in the skull, spine and joints of legs. The clear uptake in kidney and bladder demonstrates that [^{99m}Tc]Tc-TTHMP mainly metabolized through urinary system. During 1 to 6 h post-injection, a clear skeletal system could be observed. At 24 h post-injection, the radioactivity is almost excreted, so very low radioactivity signal were recorded.

The biodistribution data and micro-SPECT/CT images in normal mice and SPECT imaging results in a normal Beagle dog show that [^{99m}Tc]Tc-TTHMP has a good bone-seeking ability and [^{99m}Tc]Tc-TTHMP could be a candidate for for skeletal system imaging.

Conclusion

In this study, [^{99m}Tc]Tc-TTHMP and the corresponding TTHMP kits were prepared. The in vitro tests show that [^{99m}Tc]Tc-TTHMP is stable and hydrophilic. The biodistribution and micro-SPECT/CT studies in normal mice and SPECT studies in a normal Beagle dog show that [^{99m}Tc]Tc-TTHMP has a good bone-seeking ability. [^{99m}Tc]Tc-TTHMP is metabolized faster and has a better imaging contrast for the skeletal system during 2 to 3 h post-injection than [^{99m}Tc]Tc-MDP. The results suggest that [^{99m}Tc]Tc-TTHMP could be a candidate for skeletal system imaging.

Acknowledgements We thank the help from Professor Zhenin Li of Nuclear Medicine and Molecular Imaging Center of Soochow University for Micro-SPECT/CT imaging and the help from Professor Yue Chen of the First Affiliated Hospital of Southwest Medical University for SPECT imaging.

Funding This work was supported by the CAEP Innovation and Development Foundation (Grant Number: CX20200003) and the Central Guidance for Local Science and Technology Development Projects (Grant Number: 202138-03).

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All animal experiments were performed in compliance with the guidelines and protocols approved by the Animal Investigation Committee of Southwest Medical University (approval number: 20160796).

References

1. Zhang Y, Lin Z, Li T, Wei Y, Yu M, Ye L, Cai Y, Yang S, Zhang Y, Shi Y, Chen W (2022) Head-to-head comparison of ^{99m}Tc-PSMA and ^{99m}Tc-MDP SPECT/CT in diagnosing prostate cancer bone metastasis: a prospective, comparative imaging trial. Sci Rep 12:15993. https://doi.org/10.1038/s41598-022-20280-x

2. Wang D, Yang Y, Zeng Z, Ye J, Guo C, Huang S, Guo X, Xiao J (2022) Comparison of Bone Metastases between 18F-NaF PET/CT, 18F-NaF PET, and Planar 99mTc-MDP bone scintigraphy in patients with newly diagnosed nasopharyngeal carcinoma. Contrast Media Mole Imaging. https://doi.org/10.1155/2022/5975338
3. Dispenzieri A, Wiseman GA, Lacy MQ, Litzow MR, Anderson PM, Gastineau DA, Tefferi A, Inwards DJ, Micallef INM, Ansell SM, Porrata L, Elliott MA, Lust JA, Greipp PR, Rajkumar SV, Fonseca R, Witzig TE, Erlichman C, Sloan JA, Gertz MA (2005) A phase I study of ^{153}Sm-EDTMP with fixed high-dose melphalan as a peripheral blood stem cell conditioning regimen in patients with multiple myeloma. Leukemia 19:118–125. https://doi.org/10.1038/sj.leu.2403575
4. Dispenzieri A, Wiseman GA, Lacy MQ, Hayman SR, Kumar SK, Buadi F, Dingli D, Laumann KM, Allred J, Geyer SM, Litzow MR, Gastineau DA, Inwards DJ, Micallef IN, Ansell SM, Porrata L, Elliott MA, Johnston PB, Hogan WJ, Gertz MA (2010) A phase II study of ^{153}Sm-EDTMP and high-dose melphalan as a peripheral blood stem cell conditioning regimen in patients with multiple myeloma. Am J Hematol 85:409–413. https://doi.org/10.1002/ajh.21696
5. Giralt S, Bensinger W, Goodman M, Podoloff D, Eary J, Wendt R, Alexanian R, Weber D, Maloney D, Holmberg L, Rajandran J, Breitz H, Ghalie R, Champlin R (2003) ^{166}Ho-DOTMP plus melphalan followed by peripheral blood stem cell transplantation in patients with multiple myeloma: results of two phase 1/2 trials. Blood 102:2684–2691. https://doi.org/10.1182/blood-2002-10-3250
6. Rajendran JG, Eary JF, Bensinger W, Durack LD, Vernon C, Fritzberg A (2002) High-dose ^{166}Ho-DOTMP in myeloablative treatment of multiple myeloma: pharmacokinetics, biodistribution, and absorbed dose estimation. J Nucl Med 43:1383–1390
7. Kanishi D (1993) ^{99m}Tc-MDP accumulation mechanisms in bone. Oral surgery. Oral Pathol 75:239–246. https://doi.org/10.1016/0030-4220(93)90100-I. Oral Medicine
8. Chakraborty S, Das T, Unni PR, Sarma HD, Samuel G, Banerjee S, Venkatesh M, Ramamoorthy N, Pillai MRA (2002) ^{177}Lu labelled polyaminophosphonates as potential agents for bone pain palliation. Nucl Med Commun 23:67–74
9. Goeckeler WF, Edwards B, Volkert WA, Holmes RA, Simon J, Wilson D (1987) Skeletal localization of Samarium-153 Chelates: potential therapeutic bone agents. J Nucl Med 28:495–504
10. Lungu V, Niculae D, Bouziotis P, Pirmettis I, Podina C (2007) Radiolabeled phosphonates for bone metastases therapy. J Radioanal Nucl Chem 273:663–667. https://doi.org/10.1007/s10967-007-0928-0
11. Zolghadri S, Yousefnia H, Jalilian AR, Ghannadi-Maragheh M (2015) Production, biodistribution assessment and dosimetric evaluation of ^{177}Lu-TTHMP as an agent for bone pain palliation. Asia Ocean J nuclear Med biology 3:35–42
12. He J, Luo S, Jiang S, Yang Y, Wang G (2007) Preparation and properties of ^{117m}Sn(IV)-TTHMP in in-vitro and in small animals. Nucl Sci Tech 18:344–348. https://doi.org/10.1016/S1001-8042(08)60005-6
13. Yang Y, Luo S, Pu M, Wang W, Wang G, He J, Liu G, Bing W, Wei H (2005) Labeling conditions, in vitro properties and biodistributions of various Sn-labeled complexes. Appl Radiat Isot 62:597–603. https://doi.org/10.1016/j.apradiso.2004.09.004
14. Naseri Z, Jalilian A, Bahrami Samani A, Ghannadi-Maragheh M (2011) Production, quality control and biological evaluation of ^{153}Sm-TTHMP as a possible bone palliation agent. Iran J Nuclear Med 19:60–68
15. Jiang S, Luo S, Liu G, Bing W, Wang W, Wei H, Deng H, Hu S (2003) Preparation and bio-distribution of bone tumor therapeutic radiopharmaceutical ^{153}Sm-TTHMP. Nucl Sci Tech 14:123–126
16. Safarzadeh L (2014) ^{175}Yb-TTHMP as a good candidate for bone pain palliation and substitute of other radiopharmaceuticals. Indian J nuclear Med 29:135–139. https://doi.org/10.4103/0972-3919.136555
17. Vaez-Tehrani M, Zolghadri S, Yousefnia H, Afarideh H (2016) Estimation of human absorbed dose for ^{166}Ho-PAM: comparison with ^{166}Ho-DOTMP and ^{166}Ho-TTHMP. Br J Radiol 89:20160153. https://doi.org/10.1259/bjr.20160153
18. Yousefnia H, Zolghadri S, Jalilian AR, Tajik M, Ghannadi-Maragheh M (2014) Preliminary dosimetric evaluation of ^{166}Ho-TTHMP for human based on biodistribution data in rats. Appl Radiat Isot 94:260–265. https://doi.org/10.1016/j.apradiso.2014.08.017
19. Song H, Luo S, Wei H, Song H, Yang Y, Zhao W (2010) In vivo biological behavior of $^{99m}Tc(CO)_3$ labeled fullerol. J Radioanal Nucl Chem 285:635–639. https://doi.org/10.1007/s10967-010-0588-3