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Preparation and properties of ^{117m}Sn(Ⅳ)-TTHMP in in-vitro and in small animals

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Abstract In this work, TTHMP was synthesized and labelled with ^{117m}Sn. The preparation conditions, stability and lipophilicity of ^{117m}Sn(IV)-TTHMP were investigated. Biodistribution of the complex in rabbits and mice was studied. It was found that the quantity of TTHMP and pH value of the prepararion solution had vital effects on the labeling yield of ^{117m}Sn(IV)-TTHMP. It was also found that ^{117m}Sn(IV)-TTHMP was hydrophilic and stable at room temperature and 37℃ in open air. ^{117m}Sn(IV)-TTHMP showed unexpectedly high bone uptake and bone-to-blood ratio in the animals. This made it potentially useful as an reagent for skeletal scintigraphy and radiotherapy of bone tumors.

Key words ^{117m}Sn, TTHMP, Bone tumor, Biodistribution

CLC numbers R817.8, R738.1

1 Introduction

Tin is an essential ingredient of most technetium-99m radiopharmaceuticals, but its behavior in-vivo is not well understood. On the other hand, Sn can form complexes with most ligands used for labeling with Tc and Re. And what's interested in is not the biobehavior of simple $SnCl_2$ or $SnCl_4$, but Sn-complexes. The aim of investigating new compounds for palliative therapy of painful osseous metastases is to obtain a high concentration of some Sn-compllexes in abnormal bone, with a minimal effect on the red marrow. Studies so far on radionuclide (P, Sr, Re or Sm) -labelled complexes, and their applications, are far from perfection because of marrow depression or inflammation in other tissues[1-5]. Many experiments proved that ^{117m}Sn is an ideal tracer for studying biological behavior of tin compounds and for developing clinically-useful radiopharmaceuticals as well. Srivastava et al.[3,6,7] found that ^{117m} Sn radiopharmaceuticals as therapeutic and diagnostic agents localize in bone after intravenous injection in mammals. The preferred chelates are phosphonate compounds such as PYP, MDP, HEDP and DTPA. Especially ^{117m}Sn (IV) –DTPA may play a far more important role as a radiotherapeutic agent for bone tumors. To the authors' knowledge, however, there are no reports about Sn-labelled alkylamino-phosphonic compound.

Sn-117m exhibits radionuclidic properties acceptable for clinical and therapeutic use, such as a half-life of 14 days, 158keV photons that account for 87% of the emission, and abundance of low energy Auger and conversion electrons (130 and 155 keV). A short half-life is necessary to minimize patient exposure, but it cannot be too short to preclude commercial processing and transport. In addition, a useful radioisotope must yield gamma rays in reasonable abundance that can be imaged with cameras currently available. Internal scatter and absorption of low energy gamma emissions and sensitivity and resolution constraints imposed by the collimators and electronics of scintillation cameras limit useful isotopic emission from 50-60 keV to approximately 350 keV.[6]

In our previous work[8], radioactive Sn was used as a tracer to study the labeling conditions, adsorption on bone model materials and binding to BSA of

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Sn–EDTMP, Sn–TTHMP, Sn–DTPA, Sn–DTPMP and Sn–HEDTMP. In order to seek a new radiotherapeutic reagent, studies have been carried out using ^{117m}Sn (IV) TTHMP.[7,9,10] (See Table 1 and Fig.1).

Table 1 Nomenclature and abbreviation of the ligands in this paper

Abbreviation	Nomenclature
TTHMP	Triethyleneteraamine hexa (methylene phosphonic acid)
HEDP	Ethylidenehydroxydisodium phosphonate
PYP	Pyrophosphate
MDP	Methylene diphosphonate
DTPA	Diethylenetriamine penta(acetic acid)
EDTMP	Ethylenediamine tetra(methylene phosphonic acid)
DTPMP	Diethylenetriamine penta(methylene phosphonic acid)
HEDTMP	*N*-(2-hydroxyethyl)ethylenediame tri(methylene phosphonic acid)

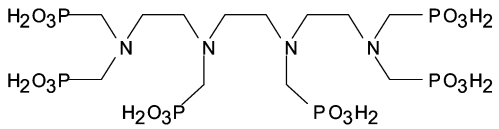


Fig.1 Structure of TTHMP.

2 Experimental

2.1 Materials

^{117m}Sn was supplied by the Institute of Isotopes, China Academy of Atomic Energy. TTHMP and HEDTMP were synthesized in our laboratory and the structure was confirmed by IR, m.p. and elemental analysis, etc. Other reagents were of analytical grade.

FJ-2021γ radio-immunity counter (China No.262 Factory), CAPINTEC CRC-15R dosimeter (Denver Instrument Co.) and Helix Apex SPECT (Elscint Co.) were used for the study.

New Zealand rabbits (five months old, weight 3~4kg, one grade, male) and normal Kunming mice (weight 18±2g, four weeks old, one grade, male and female, 50% each) were supplied by Experiment Animals Center at West China Hospital, Sichuan University.

2.2 Methods

2.2.1 Preparation of ^{117m}Sn(II)

^{117m}Sn was dissolved in concentrated hydrochloric acid at 70-80℃ in N_2. It was dissolved in a minimal volume hydrochloric acid (about 1.0 to 3.0 mL) and diluted with water to form a solution of ^{117m}SnCl(II).

2.2.2 Preparation of ^{117m}Sn(Ⅳ)–ligands

^{117m}Sn(IV)–DTPA was prepared using the method previously described.[7,9] ^{117m}Sn(IV)– TTHMP was prepared similarly. Excess TTHMP (adjusted to pH 6) was added to ^{117m}SnCl(II) chloride solution with whisking, and the pH of the mixture was readjusted to 6 with NaOH. The solution was heated in a boiling water bath for 5 min and allowed to cool to room temperature. Ten equivalents (over tin) of 30% H_2O_2 were added to the solution. The solution was heated again for 5 min in a boiling water bath to remove excess H_2O_2. After cooling, the solution was sterile filtered (0.22 μm filter) and the labeling yield was determined by paper chromatography.

2.2.3 Stability and lipophilicity of ^{117m}Sn(Ⅳ)-TTHMP

The ^{117m}Sn(IV)–TTHMP (labeling yield ≥95%) was placed at room temperature(~25℃) and 37℃, respectively. Changes in labeling yield were checked periodically by paper chromatography.

According to Ref.[11], we set up a water-oil system (N-octanol-water system) and mensurated partitin coefficient $K_{ow}=[C_0)/[C_w]=N_o/N_w$, where C_0 is concentration of ^{117m}Sn(IV)–TTHMP in N-octanol; C_w is concentration of ^{117m}Sn(IV)–TTHMP in water, and N_o and N_w are radioactivity count in N-octanol and in water, respectively.

2.2.4 Studies in small animals

The uptake of ^{117m}Sn(IV)-TTHMP and ^{117m}Sn (IV)-DTPA in New Zealand rabbits was confirmed. Four adult normal New Zealand rabbits were injected through the ear edge vein using 1.0 mL of ^{117m}Sn(IV)-TTHMP or ^{117m}Sn(IV)–DTPA solution containing ~37 MBq activity. The rabbits were imaged 24 h after injection.

Tissue distribution of the radiopharmaceuticals at various time intervals after i.v. injection was performed in normal Kunming mice. Mice of similar age and weight were selected. Injections were made in the tail vein using aqueous solutions (0.1 mL) of ^{117m}Sn (IV)-ligands. Five mice were assayed for each preparation and each time interval. The mice were sacrificed

by cervical dislocation at various times after injection. Tissue samples were collected using standard techniques, placed in pre-weighted counting tubes, weighed and assayed for radioactivity against a known standard. Results were expressed as percentage of injected dose per organ weight (%ID/g).

3 Results and discussion

3.1 Preparation of ^{117m}Sn(IV)-ligands

Labeling yield of ^{117m}Sn(IV) was assessed by paper chromatography carried out using acetone as developing solvents, in which the speed of acetone was faster. It took 20 min to develop 15 cm .The R_f value in acetone was zero for tin(IV) complex.

3.1.1 Effect of ligand quantity on labeling

From the following experiment processes we could find the effect of ligand quantitively on labeling under different pH values (Table 2).

Table 2 Effect of ligand quantity on labeling yield

Quantity of ligand /mg	Quantity of Sn /mg	Appearance of the initial mixture	Appearance of the mixture after pH was adjusted to pH=7
3.55	0.4	Turbid	Turbid
7.10	0.4	Turbid	Turbid
10.65	0.4	Turbid	Clear
14.20	0.4	Clear	Clear

Excessive ^{117m}Sn may cause the solution turbid due to easy hydrolysis of ^{117m}Sn. Further experiment showed once ^{117m}Sn hydrolyzed to colloid, the colloid could not disappear even if excessive ligand was added thereafter or the mixture was heated.

3.1.2 Effect of pH value on labeling yield

Sn(IV)-TTHMP was adjusted to pH 3, 5, 7, 9.5 and 12 using 0.1mol/L NaOH and concentrated HCl, and the labeling yields were 96.1%, 94.0%, 96.2%, 95.6% and 94.5%, respectively. However, when the pH was about 9.5and 12, the solution became turbid because of Sn(IV)-TTHMP hydrolysis. This shows that Sn(IV)-TTHMP is prone to hydrolysis in alkaline solution, especially when the pH is higher than 9. The higher the pH is, the stronger the hydrolysis of the complex becomes. If the hydrolysate congregates into big colloid, the colloid is prone to storing in liver of animals. Curative effect of target-tissue would be affected and non-target tissue would incept unnecessary lesion.

3.1.3 Stability and lipophilicity of ^{117m}Sn(IV)-TTHMP

The labeling yield was still ≥95% 120 h after preparation at room temperature, and 8h after preparation at 37℃.

A proportional relationship was found between K_{ow} and liver uptake of the compound, while an inverse proportion relationship was observed between K_{ow} and blood clearance.[12] The K_{ow} of ^{117m}Sn(IV) and ^{117m}Sn(IV)-TTHMP were 3.72×10^{-2} and 5.72×10^{-4}, respectively.

The results showed that they were hydrophilic, which was in accordance with our original assumption.

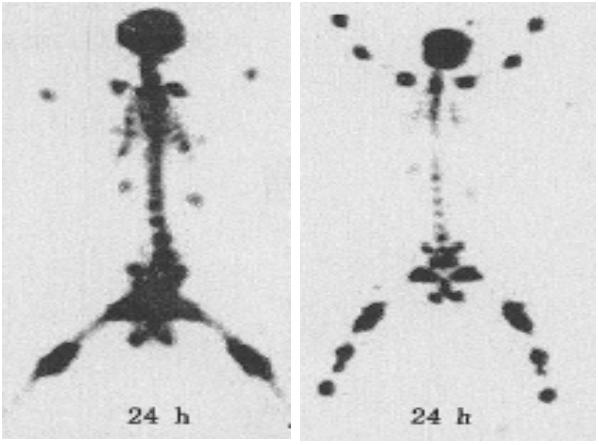
3.2 In-vivo behavior of ^{117m}Sn(IV)-TTHMP in animals

The plane bone scanning of rabbits (Fig.2) showed ^{117m}Sn(IV)-TTHMP was principally absorbed by skeletal system. The skull, spine and legs could be observed clearly, which was similar to the images of ^{117m}Sn(IV)-DTPA in clarity. It appeared that these two Sn-complexes had good bone-seeking properties with very little blood, liver, or lung background. Other specific organ uptake was invisible, except faint renal activity following radionuclide administration. Scintiphoto of ^{117m}Sn(IV)-DTPA was more vivid than ^{117m}Sn(IV)-TTHMP, but much more intense accumulation at joints was seen in the case of ^{117m}Sn(IV)-TTHMP.

Biodistribution of ^{117m}Sn(IV)-TTHMP in mice shows that ^{117m}Sn(IV)-TTHMP is a good overall bone localizing agent with very low blood, muscle, kidney, or liver uptake. The uptake of ^{117m}Sn(IV)-TTHMP in bone was very fast and persisted for a long time

(Fig.3), and this would be advantageous for bone scanning and therapy.

The bone uptake of ^{117m}Sn-DTPA, ^{117m}Sn-HEDTMP and ^{117m}Sn(IV)-TTHMP in normal mice were monitored and compared (Table 4). ^{117m}Sn(IV)-TTHMP showed the highest bone uptake compared with ^{117m}Sn-DTPA and ^{117m}Sn-HEDTMP. And bone uptake of ^{117m}Sn(IV)-TTHMP steadily increased to 24.41±2.11% ID/g 48 h after administration.



(a) ^{117m}Sn(Ⅳ)-TTHMP (b) ^{117m}Sn(Ⅳ)-DTPA

Fig.2 Scintiphotos of the rabbit at 24h after injection.

The binding of the three complexes to bone was expected (Table 5). However, the binding of ^{117m}Sn-HEDTMP and ^{117m}Sn(IV)-TTHMP to bone were surprising. The difference among the three Sn-complexes may be due to the ligand portion of the complexes, because long-term research indicates that inorganic composition of bone is mainly phosphate and hydroxy phosphorite，phosphonic acid's ligands (or compounds) are prone to binding to bone.[13] The biodistribution of alkylamino phosphonic acids is perhaps similar to their compound. This will be testified in a further study.

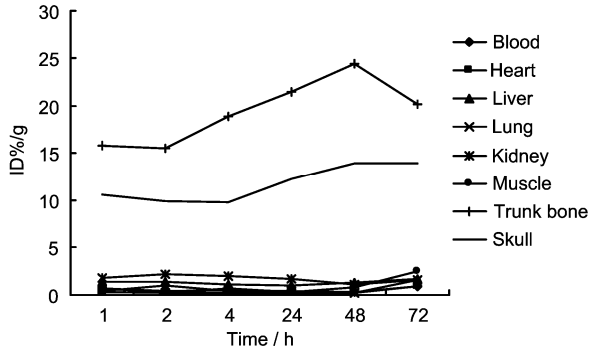


Fig.3 Tissue distribution of ^{117m}Sn(Ⅳ)-TTHMP in mice (n=5).

Table 4 Bone uptake in mice of various ^{117m}Sn(Ⅳ)-labelled compounds (n=5) (%ID/g)

Ligands	Time after injection/h				
	1	2	4	24	48
TTHMP	15.82±1.18	15.51±0.19	18.89±2.35	21.46±1.84	24.41±2.11
HEDTMP	13.95±0.98	16.49±1.53	20.20±2.17	18.73±1.30	19.75±1.82
DTPA	8.55±0.92	12.72±1.28	12.29±1.42	6.625±0.67	5.336±0.73

Table 5 Normalied tissue distribution in mice of various ^{117m}Sn(Ⅳ)-labelled compounds, %injected dose per organ (%ID/organ) (n=5, t=24h)

Ligands	Bone	Blood	Liver	Kidneys	Muscle	Whole body
TTHMP	38.63±2.13	0.0052±0.001	0.0109±0.002	0.0036±0.000	0.004±0.001	38.65±2.134
HEDTMP	33.71±2.04	0.6576±0.12	0.5522±0.104	0.4591±0.092	0.5387±0.211	35.92±2.557
DTPA	11.93±1.28	0.0187±0.007	0.492±0.201	0.168±0.001	0.249±0.082	12.86±1.570

*: Data for bone, blood and muscle were calculated using the % dose per gram values (normalized for 20g body weight) using the following factors: muscle 43, bone 10, blood 7 percent, respectively, of body weight.

4 Conclusions

^{117m}Sn-labelled compounds were prepared and the in vitro properties such as stability, lipophilicity and the in-vivo distribution in rabbits and mice were investigated. The labeling yield of ^{117m}Sn(IV)-TTHMP is ≥95% at room temperature, but pH and quantity of ligand exert a great influence on appearance of reaction solution. Sn(IV)-TTHMP is prone on hydrolysis in alkaline solution, especially when the pH is over 9. The complex is very stable at room temperature and 37℃. The value of K_{ow} of Sn(IV)-TTHMP shows that ^{117m}Sn-TTHMP is hydrophilic.

Despite the chemical differences among the ligands, the bone uptake is high for either ^{117m}Sn-HEDTMP, ^{117m}Sn-TTHMP or ^{117m}Sn-DTPA in mice

and rabbits. And the binding of ^{117m}Sn-TTHMP to trunk bone and skull is particularly noteworthy. ^{117m}Sn-TTHMP is the best overall bone localizing agent with very low blood, muscle, kidney, or liver uptake. Thus, ^{117m}Sn-TTHMP may play a far more important role as bone scanning agent or radiotherapeutic agent for bone tumors. This needs further investigation.

References

1 Kaplan E, Fels I G, Kotlowski B R. J Nucl Med, 1960, **1**: 1-13.

2 Maxon H R, Schroder L E, Thomas S R, *et al*. Radiology, 1990, **176**: 155-159.

3 Srivastava S C, Suresh C, Meinken G E, *et al*. Formulation for tin-117m diethylenetriaminepentaacetic acids. United States patent: 6,604,532,1999.

4 Laing A H, Ackery D M, Bayly R J, *et al*. Br J Radiol, 1991, **64**: 816-822.

5 Lewington V J, McEwan A J, Ackery D M, *et al*. Eur J Cancer, 1991, **27**: 954-958.

6 Srivastava S C, Suresh C, Meinken G E, *et al*. Radiopharmaceutical stannic Sn-117m chelate compositions and methods of use. United States patent: 6,231,832,2001.

7 Atkins H L, Mausner L F, Srivastava S C, *et al*. Radiology, 1993, **186**: 279-283.

8 YANG Yuqing, LUO Shunzhong, PU Manfei, *et al*. Appl Radiat Isot, 2005, **62**: 597-603.

9 Krishinamurthy G T, Swailem F M, Walsh T K. The Journal of Nuclear Medicine, 1995, **117**: 30.

10 Oster Z H, Som P, Srivastava S C, *et al*. Int J Nucl Med Biol, 1985, **12**(3): 175-184.

11 WANG Liansheng, HAN Shuokui. Molecular structure, properties and activities. Beijing: Chemistry Industry Publishing Company, 1997.

12 Abrams M J, Murrer B R. Advances in metals in medicine. London: JAI Press Inc, 1993: 134-135.

13 Francis M D. Calcified Tissue International, 1969, (3): 151.