Preparation and bio-distribution of bone tumor therapeutic agent ^{188}Re-TCTMP

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Abstract TCTMP (1,4,8,11-tetraaza cyclotetradecyl-1,4,8,11-tetramethylene phosphonate) was synthesized and coupled with ^{188}Re. The ^{188}Re-TCTMP's coupling condition, stability and bio-distribution in mice were investigated. The results showed that satisfactory yield of ^{188}Re could be obtained under the conditions of media pH=2.0, 0.8~1.6 mg of $SnCl_2$ and 50 mg of ligand. ^{188}Re-TCTMP was stable (complexation yield >95%) in 8 d without protection of N_2. The result of bio-distribution indicated that ^{188}Re-TCTMP had a strong affinity to skeleton and very low non-target tissue's uptake, and the amount of ^{188}Re-TCTMP in blood was (0.06±0.02)%ID/g 6 h after injection, whereas the concentration of ^{188}Re-HEDP (1-hydroxy-ethylidene diphosphonate) in blood was (0.28±0.05)%ID/g 6 h after injection. Compared with ^{188}Re-HEDP, ^{188}Re-TCTMP exhibits better potential for the treatment of metastases.

Keywords ^{188}Re, TCTMP, Bone tumor, Bio-distribution

CLC numbers R979, R817, O628

1 Introduction

Several radionuclides, such as ^{153}Sm, ^{186}Re, ^{177}Lu, ^{212}Bi, ^{211}At and ^{117m}Sn, have been used for bony metastases.[1-7] But the nuclides mentioned above are usually difficult to prepare in spite of their excellent physical and chemical characteristics. Recently, ^{188}Re has been focused because of its excellent nuclear characteristic and easy availability.[8-10] The mechanism of bone uptake of ^{99m}Tc-diphosphonates was believed to be related to the marked affinity of ^{99m}Tc-diphosphonates to the surface of solid-phase calcium phosphate where they bind onto the calcium by chemisorption,[11-13] and the binding can be of two types:[14,15] bidentate or tridentate. The bone uptake mechanism of aminomethylenephosphonate complexes, for example ^{153}Sm-EDTMP (ethylenediamine tetramethylene phosphonate) and ^{117m}Sn-HEDTMP (hydroxyethyl ethylenediamine trimethylene phosphonate), was similar to the mechanism of ^{99m}Tc-diphophonates uptake by the coordination of Ca^{2+} of hydroxyapatite and O atom of phosphonic group. So, ^{188}Re labeled aminomethylenephosphonate would be promising candidates of bone tumor pain palliation therapeuticals. But there was a problem, which is not beneficial to developing ^{188}Re- aminomethylenephosphonate as pharmaceutical that perrhenate is thermodynamic stable and rhenium of lower oxidation state could be re-oxidated to perrhenate when any oxidizing agent exits in the environment, for instance physiologic system, and the instability of ^{188}Re-complexes would enhance non-target radioactivity uptake,[16] which could cause unbearable toxicity. For improving the stability of ^{188}Re-aminomethylenephosphonate, we synthesized a cyclic ligand TCTMP (1,4,8,11-tetraaza cyclotetradecyl-1,4,8,11-tetramethylene phosphonate), evaluated the potential application of ^{188}Re-TCTMP for bone tumor therapy.

2 Experimental

2.1 Materials

1,4,8,11-tetraaza cyclotetrade was provided commercially by Aldrich Inc. USA, ^{188}W-^{188}Re generator was purchased from Kexing Inc. Shanghai, and

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all the other reagents were of analytic grade.

2.2 Synthesis of TCTMP

TCTMP was synthesized according to literature[17] by a one-step reaction of Mannich type procedure, see Fig.1. 35mL of 36% aqueous solution of formaldehyde was added dropwise over 15 min to a refluxing mixture comprising 20 g of 1,4,8,11-tetraaza cyclotetrade, 100 mL of 37% aqueous solution of hydrochloric acid and 32 g of orthophosphorous acid. The reaction mixture was refluxed for 5 h and then the mixture temperature was reduced to 80 ºC and maintained for 2 h. The mixture was cooled and waited for sufficient formation of precipitation over a period of one day. The precipitate was filtered and washed by ice water, then re-crystallized with aqueous methyl alcohol and freeze-dried for use. The results of analysis were: Mp: 210～213 ºC, ^{1}H－NMR (D_2O，δ ppm) (corresponding to H described by italic): 2.01~2.07 (4H) [N-CH_2-*CH_2*-CH_2-N], 2.90~3.60 (24H) [N-*CH_2*-CH_2-*CH_2*-N, N-*CH_2*-*CH_2*-N and N-*CH_2*-PO_3H_2], elemental analyses ($C_{14}H_{36}O_{12}N_4P_4$): found （calculated）%: C, 28.2(29.2); H, 6.1(6.3); N, 9.8(9.7); P, 20.3 (21.5).

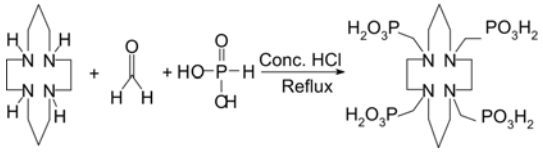

Fig.1 The synthesis schedule of TCTMP.

2.3 Preparation of ^{188}Re-TCTMP

To an aqueous solution mixture containing TCTMP, 0.1 mg of Re ($KReO_4$), ^{188}Re ($NaReO_4$), 0.2 mg of vitamin C and 0.2 mg of gentisate, 2 mol/L of $SnCl_2$ solution was added, and then the pH was adjusted to a certain value. After sealing, the reaction mixture was reacted in boiling water for 30 min. For protecting $SnCl_2$ from oxidization, all the solutions were purged with N_2. The yield was measured using radio paper chromatography developing in two systems. The R_f of ^{188}Re-TCTMP were 0.0~0.1 and 0.7~0.9 when acetone and normal saline were respectively used as developing agent. The R_f values of $^{188}ReO_4^-$ and colloid ^{188}Re ($^{188}ReO_2$) were 0.7~0.9 and 0.0, respectively, in developing systems as described above. A well-type scintillation counter with NaI (Tl) detector was used for the measurement of radioactivity.

2.4 Preparation of ^{188}Re-HEDP

According to literature,[18] to an aqueous solution mixture containing 50 mg of HEDP, 0.1 mg of Re ($KReO_4$), ^{188}Re ($NaReO_4$), 0.2 mg of vitamin C and 0.2 mg of gentisate, 10 mg of $SnCl_2$ was added, and then the pH of solution was adjusted to 2.0. After sealing, the reaction mixture was reacted in boiling water for 30 min and then the yield was measured as described above.

2.5 Stability of ^{188}Re-TCTMP

The pH value of ^{188}Re-TCTMP solution obtained under the optimum condition was changed to 7.0 and then the percentage of yield of ^{188}Re was determined at specific time intervals.

2.6 Bio-distribution of ^{188}Re-TCTMP in mice

Bio-distribution study was performed in normal Kunming mice weighing (20±2) g. 37 kBq of ^{188}Re-TCTMP in ~0.1 mL was injected through tail vein and the mice were sacrificed at specific time intervals by cervical dislocation. The tissues and organs were excised, weighed and counted over a NaI(Tl) scintillation detector. For skeletal uptake studies, femur bone was chosen. The distribution of activity in different organs was calculated as percent-injected dose/g.

3 Results and discussion

3.1 ^{188}Re-TCTMP formation

The main factors affecting the formation of ^{188}Re-TCTMP were studied. The study showed that 0.8~1.6 mg of reducing agent was enough to get satisfied yield (see Fig.2), pH value of reaction solution played important role in the formation of Re-aminophosphonates[8,9,18,19] and the reduction of perrhenate required lower pH value. Study of the influence of solution's pH on the yield of ^{188}Re-TCTMP gave a result in accordance with literatures: at lower pH, it was easy to reach high yield and when the pH was 1~3 the yield was more than 96%, whereas it was only 12% when the pH was 9 (see Fig.3). The ligand

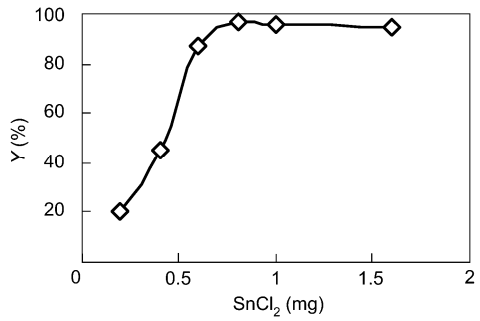

Fig.2 Effect of the amount of $SnCl_2$ on yield of ^{188}Re when TCTMP=50 mg and the media pH=2.0.

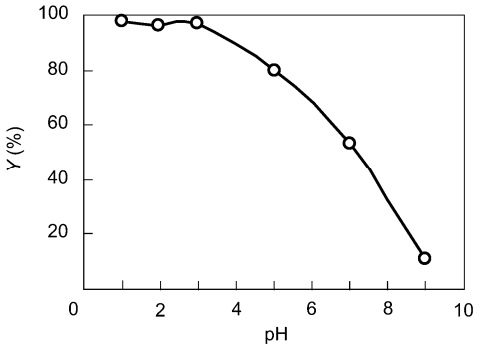

Fig.3 Effect of pH on the formation of ^{188}Re-TCTMP. The reaction was performed with 50 mg TCTMP and 0.8 mg $SnCl_2$.

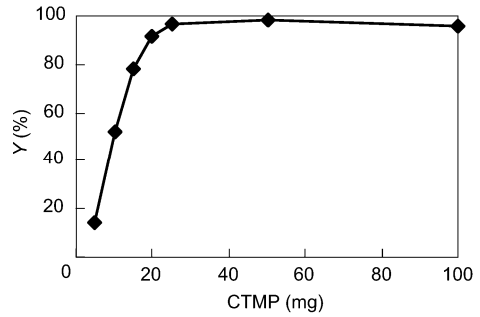

Fig.4 Effect of TCTMP concentration on the formation of ^{188}Re-TCTMP. The reaction was performed with 0.8 mg $SnCl_2$ and the pH of reaction media was 2.0.

concentration was important to ensure the stability of aminomethylene phosphonate,[13,18] so we suggested preparing ^{188}Re-TCTMP with 50 mg of TCTMP although 25 mg of ligand could be enough (see Fig.4).

3.2 Stability of ^{188}Re-TCTMP in vitro

Stability of ^{188}Re-TCTMP was determined for 8 days without protection of N_2 and the result indicated that ^{188}Re-TCTMP was very stable in vitro, as shown in Fig.5. The research by Prakash[19] proposed that ^{188}Re-1,4,8,11-tetraaza cyclotetrade, an analog of TCTMP, was not easy to prepare and was not stable. The high stability of ^{188}Re-TCTMP was partly, if not fully, attributed to the addition of phosphonate group,

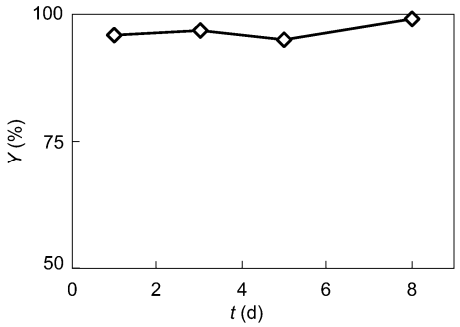

Fig.5 Stability of ^{188}Re-TCTMP in vitro.

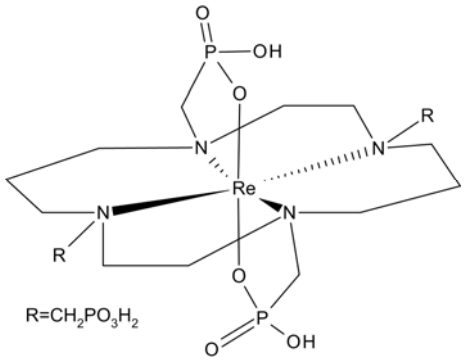

Fig.6 The possible structure of Re-TCTMP.

which provides more protonation site to coordinate rhenium without changing the structure of tetraaza cyclotetrade (see Fig.6).

3.3 Bio-distribution of ^{188}Re-TCTMP in mice

For a comparative study, biodistributions of ^{188}Re-TCTMP and ^{188}Re-HEDP were determined simultaneously. The results showed that both ^{188}Re-cmplexes had rapid bony uptake, and the radioactivities of bone were (23.06±3.66)ID%·g^{-1} for ^{188}Re-TCTMP and (26.06±4.96)ID%·g^{-1} for ^{188}Re-HEDP 15 min after injection. The radioactivity retentions of two complexes were high and the radioactivities of bone were (20.65±4.91)ID%·g^{-1} for ^{188}Re-TCTMP and (23.25±4.67)ID%·g^{-1} for ^{188}Re-HEDP 48 h after injection, which implied strong affinity of two complexes to bone. Through a comparison between Table 1 and Table 2, it was very exciting to find out that the radiation level for non-target uptake of ^{188}Re-TCTMP was very low compared with ^{188}Re-HEDP, although the bone uptake of ^{188}Re-TCTMP was a little lower than that of ^{188}Re-HEDP. The radiation level of blood was (0.06±0.02)ID%·g^{-1} for ^{188}Re-TCTMP but (0.28±0.05)ID%·g^{-1} for ^{188}Re-HEDP 6 h after injection. The considerably low radiation level in non-target tissues was possibly due to the high stability of com-

Table 1 Biodistribution of ^{188}Re-TCTMP in normal mice (n=5)

Tissues	Biodistribution at different time (ID%·g^{-1} tissue)							
	5 min	15 min	30 min	1 h	3 h	6 h	24 h	48 h
Blood	12.27±4.56	5.86±2.36	1.59±0.96	0.68±0.21	0.17±0.09	0.06±0.02	0.03±0.01	0.01±0.00
Heart	3.45±1.11	1.27±0.41	0.63±0.21	0.41±0.11	0.08±0.04	0.05±0.03	0.02±0.01	0.01±0.00
Liver	3.75±0.69	2.84±1.35	0.35±0.20	0.30±0.10	0.34±0.12	0.08±0.03	0.04±0.02	0.02±0.00
Spleen	1.89±0.87	1.05±0.34	0.59±0.33	0.08±0.05	0.12±0.03	0.05±0.01	0.02±0.00	0.00±0.00
Lung	6.98±3.68	3.65±1.43	1.65±0.08	0.61±0.22	0.23±0.03	0.04±0.02	0.02±0.00	0.02±0.00
Kidney	17.95±5.36	16.35±2.35	3.65±1.23	1.26±0.63	1.23±0.32	0.06±0.03	0.06±0.02	0.06±0.01
Muscle	2.35±0.23	2.30±1.03	0.36±0.25	0.22±0.06	0.09±0.06	0.04±0.02	0.03±0.01	0.01±0.00
Skeleton	17.02±3.41	23.06±3.66	21.69±4.58	23.94±5.02	23.56±4.10	21.01±3.64	22.36±4.11	20.65±4.91

Table 2 Biodistribution of ^{188}Re-HEDP in normal mice (n=5)

Tissues	Biodistribution at different time (ID%·g^{-1} tissue)							
	5 min	15 min	30 min	1 h	3 h	6 h	24 h	48 h
Blood	10.52±5.36	4.96±2.17	2.36±1.69	0.59±0.27	0.34±0.11	0.28±0.05	0.30±0.10	0.34±0.09
Heart	2.68±1.27	2.30±1.03	1.23±0.69	0.31±0.09	0.29±0.09	0.26±0.13	0.38±0.15	0.31±0.06
Liver	3.56±1.90	3.05±1.39	1.35±0.61	0.45±0.18	0.56±0.26	0.28±0.11	0.41±0.09	0.26±0.11
Spleen	1.45±0.99	0.96±0.26	1.26±0.32	0.65±0.26	0.43±0.13	0.35±0.13	0.30±0.06	0.35±0.18
Lung	5.69±3.61	3.85±1.65	1.69±0.22	1.02±0.58	0.68±0.25	0.59±0.26	0.36±0.14	0.19±0.03
Kidney	18.62±3.65	15.91±4.00	4.99±2.10	1.68±0.65	1.38±0.06	0.87±0.24	0.55±0.17	0.67±0.26
Muscle	3.08±1.04	2.35±0.62	0.95±0.31	0.69±0.19	0.57±0.24	0.53±0.28	0.53±0.24	0.51±0.17
Skeleton	12.63±4.80	26.06±4.96	27.56±4.35	24.63±5.36	22.91±3.08	23.65±4.87	21.58±6.95	23.25±4.67

plex that could prevent ^{188}Re-TCTMP chemisorbed on the surface of bone from being washed out from bone and ensure only low toxicity to patients.

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