

Synthesis and biodistribution of a novel ^{99m}Tc -DMSA-metronidazole ester as a potential tumor hypoxia imaging agent

Junbo Zhang · Qian Yu · Jinfeng Huo · Yan Pang ·
Sheng Yang · Yining He · Tingting Tang ·
Chenchen Yang · Xuebin Wang

Received: 4 August 2009 / Published online: 28 January 2010
© Akadémiai Kiadó, Budapest, Hungary 2010

Abstract The dimercaptosuccinic acid metronidazole ester (DMSAME) was synthesized and radiolabeled with ^{99m}Tc to form the ^{99m}Tc -DMSAME complex in high yield. The radiochemical purity of the ^{99m}Tc -DMSAME complex was over 90%, as measured by TLC and by HPLC, without any notable decomposition at room temperature over a period of 6 h. Its partition coefficient indicated that it was a lipophilic complex. The tumor cell experiment and the biodistribution in mice bearing S 180 tumor showed that the ^{99m}Tc -DMSAME complex had a certain hypoxic selectivity and accumulated in the tumor with high uptake and good retention. The tumor/blood and tumor/muscle ratios increased with time, suggesting it would be a possible tumor hypoxia imaging agent.

Keywords Dimercaptosuccinic acid · Metronidazole ester · ^{99m}Tc · Hypoxia · Tumor

Introduction

For several years, it has been known that hypoxia plays an important role in the resistance of cancers to treatment by radiotherapy. A number of invasive methods have been

developed for the measurement of hypoxia, but none of these is in routine clinical use because of their invasive nature, inconvenience, and inability to acquire repeated measures. The limitations of these techniques stimulate the development of noninvasive imaging with a hypoxia targeted radiopharmaceutical. In the development of hypoxia imaging agent, nitroimidazole derivatives are enzymatically reduced and accumulated in hypoxic regions, therefore labeled nitroimidazole analogues have received great attention. Currently, the positron emission tomography (PET) tracer [^{18}F]Fluoromisonidazole ([^{18}F]FMISO) has been used to evaluate tumor hypoxia [1]. However, the short half life and high cost of the [^{18}F] isotope restrict its wide application in clinical nuclear medicine. Given the favorable characteristics of technetium-99m (short half-life, optimal γ -energy, inexpensive cost), research has focused on ^{99m}Tc labeled radiopharmaceutical for targeting hypoxia. Recently, several ^{99m}Tc labeled nitroimidazole analogues have been reported [2–7]. However, the relative lower tumor uptakes and slow clearance from blood observed for these hypoxia imaging agents are their common defects. There still exists great interest in the search for an ideal hypoxia imaging agent.

It is known that dimercaptosuccinic acid (DMSA) ligand can be used to form stable $^{99m}\text{Tc}(\text{V})\text{O}$ complex on the basis of efficient binding of the oxotechnetium group to four thiol sulphur atoms. $^{99m}\text{Tc}(\text{V})\text{O}$ -DMSA has been widely used for imaging medullary carcinoma of the thyroid and other soft tissue tumors [8]. Bearing in mind metronidazole has the potential to target hypoxia tumors and $^{99m}\text{Tc}(\text{V})\text{O}$ -DMSA is a tumor imaging agent, we present the synthesis of metronidazole ester using DMSA as a chelator and evaluate its ^{99m}Tc labeled complex as a potential tumor hypoxic imaging agent. To the best of our knowledge, this report constitutes the first of its kind in using the DMSAME in the preparation of ^{99m}TcO complex as targeted agent for hypoxia imaging.

J. Zhang (✉) · Q. Yu · J. Huo · Y. Pang · S. Yang · Y. He ·
T. Tang · C. Yang · X. Wang
Key Laboratory of Radiopharmaceuticals, Ministry of
Education, 100875 Beijing, People's Republic of China
e-mail: zhjunbo@bnu.edu.cn

J. Zhang · Q. Yu · J. Huo · Y. Pang · S. Yang · Y. He ·
T. Tang · C. Yang · X. Wang
College of Chemistry, Beijing Normal University,
100875 Beijing, People's Republic of China

Q. Yu
The Affiliated Hospital of Medical College QingDao University,
QingDao, People's Republic of China

Experimental

Materials

Metronidazole and meso-2,3-dimercaptosuccinic acid (DMSA) were purchased from Fluka, Germany. All other chemicals were of reagent grade and were used without further purification. $^{99}\text{Mo}/^{99\text{m}}\text{Tc}$ generator was obtained from the China Institute of Atomic Energy (CIAE). HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5C, 250×4.6 mm), Shimadzu SCL-10AVP series. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded on a 400 MHz BRUKER AVANCE spectrophotometer.

Synthesis of DMSAMe (Compound 1)

About 0.36 g of DMSA and 1.10 g of metronidazole were stirred vigorously in 15 mL of anhydrous pyridine solution. To the above solution, 0.5 g of N,N' -dicyclohexylcarbodiimide (DCC) dissolved in anhydrous pyridine was then added in an ice bath. The mixture was stirred for 24 h at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in 20 mL of CH_2Cl_2 and filtered off. The filtrate was washed by 5% NaHCO_3 , saturated NaCl solution and water. The organic phase was dried with anhydrous Na_2SO_4 and the solvent was removed under vacuum. The yellow crude product was recrystallized from ethyl acetate to give DMSAMe (compound 1, 0.28 g, 29%).

Preparation of $^{99\text{m}}\text{Tc-DMSAMe}$

1 mL of saline containing $[\text{}^{99\text{m}}\text{TcO}_4]^-$ (15 MBq) was added to a glucoheptonate (GH) kit containing 0.1 mg of stannous chloride dihydrate, 20.0 mg of GH. The mixture was kept at room temperature for 20 min. Successively, 1.0 mg of compound 1 dissolved in 1.0 mL ethanol was then added and the resulting solution was heated at 100°C for 30 min. The TLC was performed on a polyamide strip and eluted with saline. HPLC analysis was carried out with a reversed-phase column, working at a flow rate of 1.0 mL/min. Water (A) and acetonitrile (B) mixtures with 0.1% trifluoroacetic acid were used as the mobile phase and the following gradient elution technique was adopted for the preparation (0–10 min 80%B, 10–40 min 100%B).

Stability study

The stability of the complex was assayed by measuring the radiochemical purity by TLC at different times (3 and 6 h) after preparation.

Determination of the partition coefficient for the complex

The partition coefficient was determined by mixing the complex with an equal volume of 1-octanol and phosphate buffer (0.025 M, pH 7.4) in a centrifuge tube. The mixture was vortexed at room temperature for one min and then centrifuged at 5,000 r/min for 5 min. From each phase 0.1 mL of the aliquot was pipetted and counted in a well γ -counter. Each measurement was repeated three times. Care was taken to avoid cross contamination between the phases. The partition coefficient, P, was calculated using the following equation:

$$P = (\text{cpm in octanol} - \text{cpm in background}) / (\text{cpm in buffer} - \text{cpm in background})$$

Usually the final partition coefficient value was expressed as $\log P$.

Cellular accumulation

The complex was in vitro evaluated following the previous method [7]. S180 cells were suspended in fresh DMEM growth medium at a cell concentration of 2×10^6 cells/mL. Aliquots of 20 mL were placed in glass vials and incubated at 37°C with gentle stirring under an atmosphere of 95% air plus 5% carbon dioxide (aerobic exposure) or 95% nitrogen plus 5% carbon dioxide (hypoxic exposure, oxygen concentration < 10 ppm). After a 30–45-min equilibration period, 0.3 mL of $^{99\text{m}}\text{Tc-DMSAMe}$ was added to each vial at a final activity of approximately 0.25 MBq/mL and a drug concentration of approximately 0.7 $\mu\text{g/mL}$. The 0.9-mL samples were removed at various time intervals. From each sample, the 0.2-mL sample was centrifuged at 1,500 r/min for 5 min. A 90 μL aliquot of the supernatant was removed for counting, and the left sample containing cells and 110 μL medium was also counted. The ratio of the radioactivity in the cell pellet to that in the supernatant medium ($C_{\text{in}}/C_{\text{out}}$) was calculated as (residue counts - supernatant counts)/supernatant counts. At each time point, five samples were determined. During the whole procedure, the cells' viability was more than 90%.

Biodistribution study

Biodistribution studies were performed in Kunming male mice (weighing 18–20 g) bearing S 180 tumor, which grew to a leg diameter of 10–15 mm. 0.1 mL of $^{99\text{m}}\text{Tc-DMSAMe}$ (7.4×10^5 Bq) was injected via a tail vein and the injected radioactivity was measured with a well-type NaI(Tl) detector. The mice were sacrificed at 0.5, 2 and 4 h post-injection. The tumor, other organs of interest and

blood were collected, weighed and measured for radioactivity. The results were expressed as the percent uptake of injected dose per gram of tissue (%ID/g). All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

Results and discussion

Synthesis

The dimercaptosuccinic acid metronidazole ester (DMSAME) (compound 1) was prepared by reacting DMSA with metronidazole in the presence of N,N' -dicyclohexylcarbodiimide (DCC) and anhydrous pyridine [9]. The reaction is schematically shown in Scheme 1.

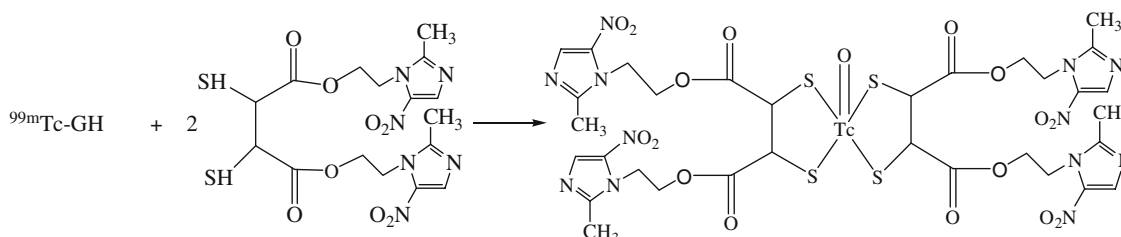
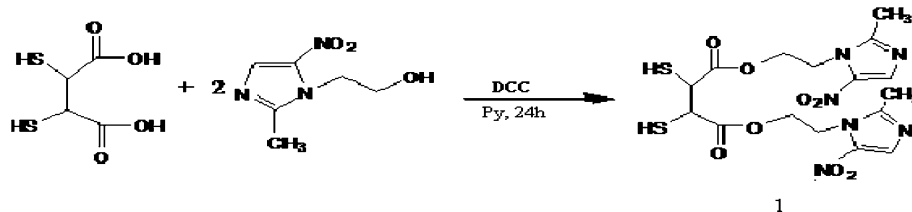
The compound 1 was characterized by $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$. $^1\text{H-NMR}$ (Methanol- d_4) δ : 2.47(s, 3H, CH_3), 2.89 (s, 1H, CHCOOC), 3.75(t, 2H, CH_2), 4.40(t, 2H, CH_2), 7.96 (s, 1H, CH). $^{13}\text{C-NMR}$ (Methanol- d_4) δ : 152.67, 147.98, 143.24, 130.87, 128.69, 61.50, 50.02, 13.97.

Preparation of ^{99m}Tc -DMSAME

The preparation of ^{99m}Tc -DMSAME was carried out using the following procedure in Scheme 2.

For labelling, ^{99m}Tc -DMSAME was prepared by ligand-exchange reaction with ^{99m}Tc -glucoheptonate (GH). ^{99m}Tc -GH is a suitable substrate for the substitution reaction with the compound 1 to give the final complex ^{99m}Tc -DMSAME.

Scheme 1 Synthesis of compound 1



Scheme 2 Preparation procedure and proposed structure of ^{99m}Tc -DMSAME

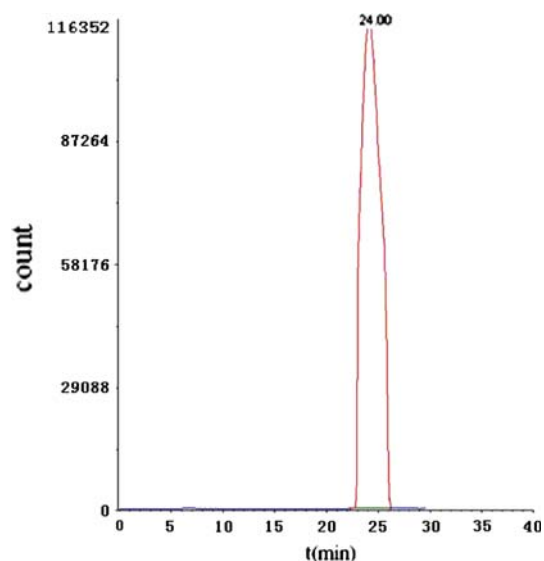


Fig. 1 HPLC pattern of ^{99m}Tc -DMSAME

The radiochemical purity of the complex was routinely checked by TLC and HPLC. By TLC, in saline, $^{99m}\text{TcO}_4^-$, $^{99m}\text{TcO}_2 \cdot n\text{H}_2\text{O}$ and ^{99m}Tc -DMSAME remained at the origin while ^{99m}Tc -GH migrated with the front. The HPLC pattern of ^{99m}Tc -DMSAME is shown in Fig. 1. It was observed that the retention time of $^{99m}\text{TcO}_4^-$ was 6.4 min and ^{99m}Tc -GH was 6.8 min, while that of ^{99m}Tc -DMSAME was found to be 24.0 min. Single peak suggested only one product (^{99m}Tc -DMSAME) was formed. The mean radiochemical purity of the product was over 90% immediately after the preparation.

Based on a previous characterization of the molecular structure of $^{99m}\text{Tc(V)O}$ -DMSA [10], it seems reasonable to presume that the structure of ^{99m}Tc -DMSAME is similar to

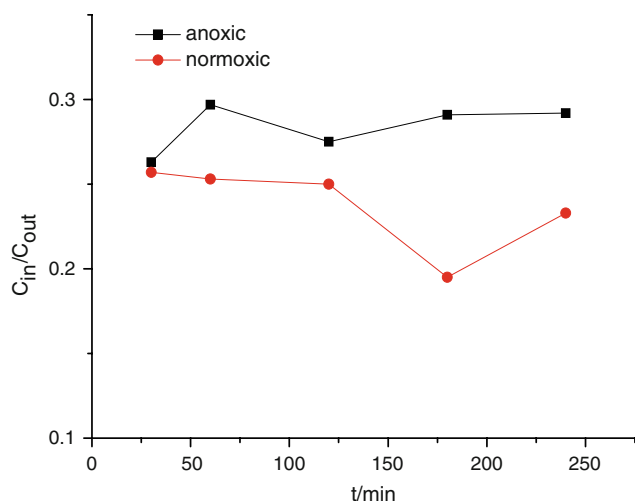


Fig. 2 The accumulation of ^{99m}Tc-DMSAME in anoxic and normoxic S180 cells as a function of time

Table 1 Biodistribution of ^{99m}Tc-DMSAME in mice bearing S 180 tumor (%ID/g)^a

Tissue	0.5 h	2 h	4 h
Heart	1.73 ± 0.21	1.09 ± 0.14	0.89 ± 0.13
Liver	8.60 ± 0.68	6.07 ± 0.91	3.87 ± 0.34
Lung	3.63 ± 0.65	2.17 ± 0.18	1.89 ± 0.24
Kidney	15.50 ± 0.80	17.19 ± 1.17	16.83 ± 3.38
Brain	0.17 ± 0.01	0.10 ± 0.01	0.09 ± 0.01
Bone	3.07 ± 0.19	2.73 ± 0.23	2.33 ± 0.55
Muscle	1.03 ± 0.12	0.62 ± 0.07	0.60 ± 0.10
Blood	4.23 ± 0.40	2.49 ± 0.19	2.00 ± 0.19
Tumor	1.39 ± 0.56	1.54 ± 0.26	1.53 ± 0.10
T/N ratio	1.35	2.49	2.57
T/B ratio	0.33	0.62	0.76

^a All data are the mean percentage ($n = 4$) of the injected dose of ^{99m}Tc-DMSAME per gram of tissue, ± the standard deviation of the mean

T/N tumor-to-muscle, T/B tumor-to-blood

that of ^{99m}Tc(V)O-DMSA, possessing a square pyramidal geometry with an apical Tc = O bond and two DMSAME ligands spanning the four positions in the basal plane

through the four sulfur atoms. Clearly, further studies should be performed, using macroscopic levels of the long-lived ^{99m}Tc, to determine and characterize the structure of ^{99m}Tc-DMSAME.

Stability study

No decomposition of the complex occurred over 6 h at room temperature, suggesting that the complex was stable in the reaction mixture at room temperature.

Partition coefficient

The partition coefficient (log P) value of ^{99m}Tc-DMSAME was 0.30, suggesting it was lipophilic. This value is less than that of ^{99m}Tc-BMS181321 (log P: 1.60) and higher than that of ^{99m}Tc-N2IPA (log P: -1.40) [2].

Cellular accumulation

The effect of anoxic or normoxic conditions on the accumulation of ^{99m}Tc-DMSAME in S180 cells as a function of time is illustrated in Fig. 2. From Fig. 2, it is shown that the uptake of ^{99m}Tc-DMSAME in anoxic cells is constantly more than that in normoxic cells, suggesting ^{99m}Tc-DMSAME has a certain hypoxic selectivity.

Biodistribution of the complex in mice

The results of biodistribution of ^{99m}Tc-DMSAME are shown in Table 1. As described in Table 1, ^{99m}Tc-DMSAME has a high uptake at the site of tumor and good target/non-target ratio. The tumor/blood and tumor/muscle ratios increase with time. The kidney uptake is much higher than the hepatic. Early renal activity reflects urinary elimination of this complex. As seen from Table 2, this is comparable to the results of some radiolabeled hypoxia imaging agents in mice bearing tumor. Among the four complexes, ^{99m}Tc-DMSAME exhibits the highest tumor uptake and this may attribute to its four metronidazoles to possess affinity for hypoxic tumors. ^{99m}Tc-DMSAME has a

Table 2 Comparison of biodistribution of ^{99m}Tc-DMSAME with some reported hypoxia imaging agents

Complex	^{99m} Tc-DMSAME	^{99m} Tc-N2IPA	^{99m} Tc-BMS181321	^{99m} Tc-MNZ-xanthate
Animal	Kunming mice	Kunming mice	C3H mice	Swiss mice
Tumor type	S 180 tumor	S 180 tumor	Fibrosarcoma	Fibrosarcoma
Time p.i. (h)	2	2	2	2
Tumor uptake (%ID/g)	1.54 ± 0.26	0.49 ± 0.07	0.55 ± 0.08	1.36 ± 0.29
T/N ratio	2.49	6.66	2.63	3.32
T/B ratio	0.62	1.00	0.31	0.62
Reference	Present study	2	4	4

good T/N ratio (2.49 at 2 h p. i.), but it is lower than that of the other three complexes. A decrease of T/B ratio in the order is observed: ^{99m}Tc -N2IPA > ^{99m}Tc -DMSAme = ^{99m}Tc -MNZ-xanthate > ^{99m}Tc -BMS181321.

Conclusion

In summary, novel DMSAme ligand was successfully synthesized and ^{99m}Tc -DMSAme was prepared in high yields through a ligand-exchange reaction, which can be easily used for the preparation of a radiopharmaceutical through a freeze-dried kit formulation. ^{99m}Tc -DMSAme showed a certain hypoxic selectivity and a high tumor uptake. However, the tumor/blood ratio of ^{99m}Tc -DMSAme is not high, thus restricting its potential usefulness as a better tumor hypoxia imaging agent.

Acknowledgments The work was financially supported by National Natural Science Foundation of China (20771018) and by Beijing Normal University. The assistance of Ms. Huafan Huang and Prof. Taiwei Chu, College of Chemistry and Molecular Engineering,

Peking University, in cellular accumulation study is gratefully acknowledged.

References

1. Yang DJ, Wallace S, Cherif A, Li C, Gretzer MB, Kim EE, Podoloff DA (1995) *Radiology* 194:795
2. Chu TW, Li RJ, Hu SW, Liu XQ, Wang XY (2004) *Nucl Med Biol* 31:199
3. Kong DJ, Lu J, Ye SZ, Wang XB (2007) *J Label Compd Radiopharm* 50:1137
4. Mallia MB, Mathur A, Subramanian S, Banerjee S, Sarma HD, Venkatesh M (2005) *Bioorg Med Chem Lett* 15:3398
5. Mallia MB, Subramanian S, Banerjee S, Sarma HD, Venkatesh M (2006) *Bioorg Med Chem* 14:7666
6. Mallia MB, Subramanian S, Mathur A, Sarma HD, Venkatesh M, Banerjee S (2008) *Bioorg Med Chem Lett* 18:5233
7. Zhang Y, Chu TW, Gao XG, Liu XQ, Yang Z, Guo ZQ, Wang XY (2006) *Bioorg Med Chem Lett* 16:1831
8. Ohta H, Yamamoto K, Endo K, Mori T, Hamanaka D, Shimazu A, Lkekubo K, Makimoto K, Lida Y, Konishi J, Morita R, Hata N, Horiuchi K, Yokoyama A, Torizuka K, Kuma K (1984) *J Nucl Med* 25:323
9. Bundgaard H, Larsen C, Thorbek P (1984) *Int J Pharm* 18:67
10. Blower PJ, Singh J, Clarke SEM (1991) *J Nucl Med* 32:845