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Synthesis and biological results of the technetium-99m-labeled 4-nitroimidazole for imaging tumor hypoxia

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Abstract—1-(4-Nitroimidazole-1-yl)-propanhydroxyiminoamide (N4IPA) was synthesized. The biodistribution of ^{99m}Tc-N4IPA in mice bearing S180 tumor demonstrated that the complex showed accumulation in tumor and slow clearance from it. The tumor-to-tissue uptake ratios increase with time. These results suggest that ^{99m}Tc-N4IPA would be a marker for imaging tumor hypoxia.

1. Introduction

Tumor hypoxia is believed to be an important factor in resistance of tumors to radiation and some other forms of therapy. The identification and quantification of tumor hypoxia may predict outcome and may identify patients who might benefit from concomitant radiosensitizing therapy to overcome the hypoxia effect. Current electrode measurement techniques are cumbersome and not readily available to most investigators. Nuclear medicine offers a non-invasive method for demonstrating tumor hypoxia.[1] Compounds that are selectively accumulated in hypoxic tissue may be labeled with either positron-emitting radionuclides or single-photon gamma emitters such as ^{99m}Tc, thus allowing wider availability. Although many types of nitroaromatic compounds have shown promise as probes for hypoxic cells, almost all of these have been based on nitroimidazoles.[2–6] Linker et al. found that ^{99m}Tc–BATO complex containing 4-nitroimidazole had the potential for selective retention in hypoxic tissue.[3] And some ^{99m}Tc-labeled metronodazoles, 5-nitroimidazole derivatives showed selectively accumulation in tumors.[4,5]

Recently, Nakayama et al. found that the bidentate ligand hydroxyiminoamides could form highly in vivo and in vitro stable complexes with ^{99m}Tc and might be a useful new chelating moiety for design ^{99m}Tc radiopharmaceuticals.[7] In this study, a hydroxyiminoamide derivative with 4-nitroimidazole was synthesized, ^{99m}Tc complex was prepared, and the behavior of the complex as tumor hypoxia marker was evaluated in vivo (Scheme 1).

2. Synthesis and radiolabeling

Acrylonitrile (53.2 mL, 0.8 mol) was added to the suspension of 4-nitroimidazole (22.6g, 0.2 mol) in triethylamine (160 mL), and the mixture was refluxed over an oil-bath for 12 h. After cooling, the white solid was filtered off and washed with fresh water several times. 1-(2-Cyanoethyl)-4-nitroimidazole was obtained as a white solid by recrystallization from methanol, mp 109–110 °C (lit.[8] mp 110–111 °C). Yield: 21.73 g (65%). ^{1}H NMR δ (DMSO-d_6): 3.17 (t, 2H, CH_2), 4.39 (t, 2H, CH_2), 7.93 (s, 1H, imi-H), and 8.47 (s, 1H, imi-H).

The previous product (8.3 g, 0.05 mol) was refluxed for 17 h with a methanolic solution of hydroxylamine (0.25 mol). Standing at 4 °C overnight gave the white crystalline powder, which was recrystallized from water to give 7.18 g of 1-(4-nitroimidazole-1-yl)-propanhydroxyiminoamide (N4IPA) as pale green crystals, mp 144.5–146.0 °C. Yield 72%. ^{1}H NMR δ (DMSO-d_6): 2.50 (overlap between CH_2 and solvent), 4.26 (t, 2H, CH_2), 5.52 (s, 2H, NH_2), 7.80 (s, 1H, imi-H), 8.37 (s, 1H, imi-H), and 8.98 (s, 1H, OH). ^{1}H NMR δ (acetonitrile-d_6): 2.62 (t, 2H, CH_2), 4.36 (t, 2H, CH_2), 5.21 (s, 2H, NH_2), 7.63 (s, 1H, imi-H), 8.11 (s, 1H, imi-H), and 8.38 (s, 1H,

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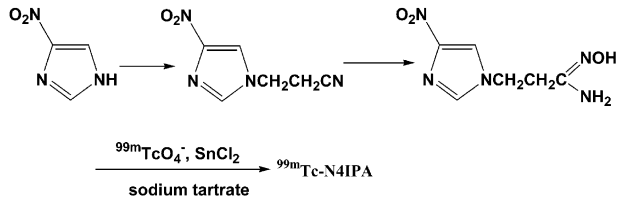

Scheme 1. Synthesis of 1-(4-nitroimidazole-1-yl)-propanhydroxyiminoamide and preparation of ^{99m}Tc–N4IPA complex.

OH). ^{13}C NMR δ (DMSO-d_6): 31.98, 44.51, 121.67, 137.50, 146.77, and 149.40. MS (EI) calculated for $C_6H_9N_5O_3$ (M^+): m/z 199. Found: 199.

Analytical calculated for 1-(4-nitroimidazole-1-yl)-propanhydroxyiminoamide, $C_6H_9N_5O_3$: C, 36.18; H, 4.52; N, 35.18; O, 24.12. Found: C, 36.25; H, 4.52; N, 35.41; O, 23.92.

Sehgal and Agrawal reported that alkylation of 4(5)-nitroimidazole with chloropropionitrile resulted in the formation of 1-(2-cyanoethyl)-5-nitroimidazole and the isomeric 1-(2-cyanoethyl)-4-nitroimidazole in poor yields 3.34 and 1.63%, respectively.[8] We found acrylonitrile reacted easily with 4-nitroimidazole in triethylamine, and the desired product, 1-(2-cyanoethyl)-4-nitroimidazole, was obtained after recrystallization with high yield 65%. And the isomeric 1-(2-cyanoethyl)-5-nitroimidazole was not found in the reaction mixture. Obviously, it is an ideal method for producing 1-(2-cyanoethyl)-4-nitroimidazole.

The action of hydroxylamine on nitriles is the most widely used process for the preparation of hydroxyiminoamides.[9] In this study, 1-(4-nitroimidazole-1-yl)-propanhydroxyiminoamide (N4IPA) was synthesized easily in a similar manner.

The ^{99m}Tc complex was prepared by reconstituting a lyophilization kit containing 200 μg of N4IPA, 25 μg of sodium tartrate, 1.36 mg of KH_2PO_4, 14.39 mg of $Na_2HPO_4{\cdot}12H_2O$, 10 μg of $SnCl_2{\cdot}2H_2O$ and 25 mg of urea with 3.0 mL of generator $^{99m}TcO_4^-$ effluent. The solution was heated to 75 °C for 10–15 min. The yield of ^{99m}Tc–N4IPA complex was determined by TLC in which a polyamide strip was used as the fixed phase and a 0.9% NaCl solution as the developer. The water-soluble complex ^{99m}Tc–N4IPA migrates to the top half of the strip (R_f = 0.4–0.6) while the lipophilic and/or insoluble radiochemical impurities remain near the origin (R_f = 0–0.3). Radiochemical purity was >95%. The complex was observed to be stable for a period of 6 h with retention of radiochemical purity to the extent of ~90%.

3. Biodistribution results

In vivo biodistribution studies were performed in Kunming mice bearing S180. Male mice were inoculated subcutaneously in the left axilla with a suspension of approximately 10^6 S180 cells. Over the course of 7–8 days, tumors reached 10–15 mm in diameter, corresponding to tumor weights of 0.5–1.5 g, each mouse received an intravenous dose of the ^{99m}Tc complex (1×10^5 Bq, 100 μL) via tail vein. The mice were sacrificed by cervical dislocation in groups of five at various time intervals after injection and the organs or tissues of interest were removed, weighed and counted. The counting tubes, including a standard equivalent to 1% of the injected dose, were assayed in a well-type gamma counter and the results were calculated as percentages of injected dose per gram tissue. Tumor-to-organ ratios were calculated from the percentages of the injected dose per gram for tumor and relevant organs. The final results are expressed as mean ± one standard deviation (SD). All experiments were carried out following the principles of laboratory animal care and the China Law on the protection of animals.

In Table 1 are presented the tissue distribution data for five mice bearing S180 tumor at each of five time intervals after injection of ^{99m}Tc–N4IPA. It can be seen that the initial distribution of ^{99m}Tc–N4IPA appears to reflect blood perfusion in each organ or tissue, and there is no indication of selective uptake or binding of radioactivity in the studied normal tissues. The blood clearance data is characterized by an initial very rapid distribution phase and a much slower clearance during

Table 1. Biodistribution of ^{99m}Tc–N4IPA in mice bearing S180 tumor (%/g)[a]

Tissue	30 min	1 h	2 h	4 h	8 h
Blood	0.70±0.05	0.28±0.08	0.22±0.02	0.18±0.04	0.15±0.05
Heart	0.24±0.08	0.14±0.03	0.12±0.05	0.08±0.02	0.06±0.01
Lung	0.39±0.15	0.22±0.03	0.17±0.04	0.12±0.01	0.10±0.02
Liver	0.98±0.21	0.79±0.06	0.46±0.07	0.39±0.09	0.23±0.04
Kidney	5.63±1.22	5.44±0.79	3.80±1.17	3.14±0.88	2.26±0.57
Spleen	0.13±0.02	0.10±0.01	0.09±0.02	0.08±0.01	0.06±0.01
Tumor	0.55±0.08	0.45±0.07	0.39±0.10	0.34±0.06	0.32±0.07
Muscle	0.15±0.05	0.11±0.07	0.05±0.01	0.04±0.01	0.04±0.01
Brain	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.00	0.04±0.01
Tumor/blood	0.78±0.13	1.19±0.39	1.81±0.49	1.88±0.53	2.06±0.82
Tumor/muscle	3.66±1.33	4.25±2.78	8.12±2.64	8.60±2.63	8.83±2.93
Tumor/heart	2.31±0.84	3.22±0.85	3.39±1.66	4.47±1.37	5.39±1.48
Tumor/lung	1.42±0.58	2.06±0.43	2.24±0.78	2.94±0.57	3.09±0.92
Tumor/liver	0.56±0.14	0.57±0.10	0.85±0.25	0.88±0.26	1.41±0.39

[a] Each value is mean ± SD.

the 2–8 h period. Interestingly, the decline in tumor activity can be neglected. This slow decline in radioactivity is in contrast to most other tissues. And the tumor-to-tissue ratios increase with time going. At 2 h after injection, the tumor/muscle ratio is 8.1, tumor/blood 1.8, tumor/heart 3.4, tumor/lung 2.2, tumor/liver 0.9. Moreover, the tumor/liver ratio attains 1.4 at 8 h. The results demonstrate that S180 tumor contains hypoxic cells, and the radiolabeled tracer may be selectively accumulated; meanwhile the tracer may flow out from other tissues.

It is well known that 4(5)-nitroimidazoles, which has a lower electron affinity than 2-nitroimidazole, would not be efficiently reduced and retained in hypoxia cells. However, a small number of studied 4(5)-nitroimidazole-derived radiosensitizers exhibit additional or atypical properties. 2-Methyl-4-nitroimidazole derivative, a less electron-affinity compound, also gives a large in vitro sensitization efficiency ratio (SER) 1.80 ± 0.18. The degree of sensitization achieved is greater than that would be predicted from the drug's electron affinity.[10] Yang et al. developed a ^{99m}Tc-labeled metronodazole, 5-nitroimidazole derivatives to image tumor hypoxia, and biodistribution showed selectively accumulation in tumor.[4] In 2003, Das et al. reported a modified metronidazole, which labeled with ^{99m}Tc by using a cysteine-based bifunctional chelating agent, showed high tumor/muscle ratio (14.7 at 3 h postinjection).[5] Therefore, the group of 4(5)-nitroderivatives of the imidazole may be reduced and bound in hypoxic cell, and the radiolabeled 4(5)-nitroimidazoles can act also as hypoxia-imaging agents.

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