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Synthesis and preliminary biological evaluation of the ^{99m}Tc labeled nitrobenzoimidazole and nitrotriazole as tumor hypoxia markers

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Abstract—1-(3-1,2,4-Nitrotriazole-1-yl)-propanhydroxyiminoamide and 1-(6-nitrobenzoimidazole-1-yl)-propanhydroxyiminoamide were synthesized and radiolabeled with ^{99m}Tc. The ^{99m}Tc labeled complexes continuously accumulated in hypoxic murine sarcoma S180 cells in vitro but not in aerobic cells. Biodistribution results in mice bearing S180 tumor indicated that the tracers could localize in tumor and eliminate from it slowly. These results suggested that the ^{99m}Tc labeled nitrobenzoimidazole and nitrotriazole might be the novel tumor hypoxia markers.

Growth of tumors beyond the capability of their vasculature to supply oxygen can result in hypoxia, the extent of which correlates with aggressive disease, metastasis. It has been recognized that the presence of hypoxic cells in tumors is the major cause leading to their resistance to conventional radiotherapy and chemotherapy. It is important to detect hypoxia within tumors by the radiolabeled markers. Up to now, almost all of the radiolabeled probes for hypoxic cells have been based on nitroimidazoles.[1] It is very interesting and necessary to explore the new leading compounds as the hypoxia markers.

Similar to nitroimidazole, both of nitrotriazole and nitrobenzoimidazole have been used as anticancer bioreductive agents and radiosensitizers, and they may enter hypoxic cells by passive diffusion and undergo a single electron reduction in the absence of adequate supply of oxygen, the reduced species can bind to cell components.[2–4] But they were rarely radiolabeled and evaluated as the hypoxia-imaging agents.[5]

Recently, we reported the hydroxyiminoamide derivatives with the bio-reducible moieties, 2(4)-nitroimidazoles as tumor hypoxia-imaging agents.[6,7] In order to extend the previous studies, two new hydroxyiminoamide ligands containing nitrobenzoimidazole and nitrotriazole: 1-(3-1,2,4-nitrotriazole-1-yl)-propanhydroxyimino-amide (NTPA) and 1-(6-nitrobenzoimidazole-1-yl)-propanhydroxyiminoamide (NBIPA) were synthesized (Scheme 1) and labeled with ^{99m}Tc, and the biodistribution of ^{99m}Tc complexes in mice bearing S180 tumor and the accumulation in aerobic versus hypoxic cells were evaluated.

3-Nitro-1*H*-1,2,4-triazole (1.5 g, 13.15 mmol) was dissolved in triethylamine (40 mL) and ethanol (20 mL). Acrylonitrile (15 mL) was added dropwise to a stirred solution, and the mixture was refluxed under an atmosphere of N_2 for 6 h. After cooling, the light yellow solid was filtered off and washed with 1 M NaOH solution, then water to neutrality. 3-(3-Nitro-1,2,4-triazol-1-yl)-propionitrile (**I**)[8] was obtained as a needle-like light yellow crystalline solid by recrystallization from methanol.

The previous product **I** (1.0 g, 6.0 mmol) was refluxed for 8.5 h with a methanolic solution of hydroxylamine (12 mmol) at 80 °C. Standing at 4 °C overnight gave the yellow crystalline powder, which was recrystallized from methanol to give 0.77 g of NTPA (**II**)[8] as yellow crystals.

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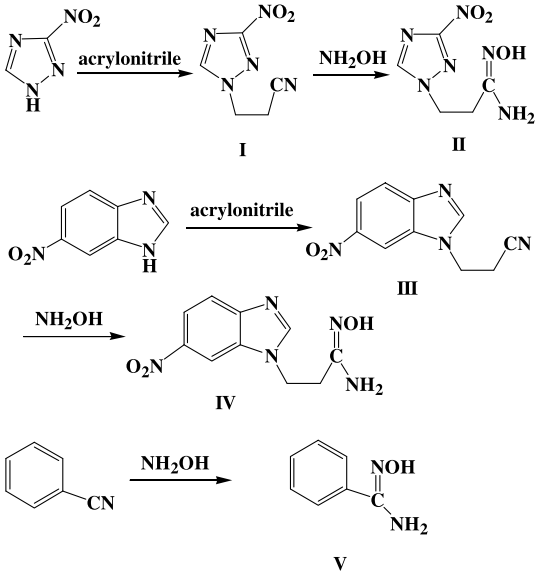

Scheme 1. Synthesis of 1-(3-1,2,4-nitrotriazole-1-yl)-propanhydroxyiminoamide, 1-(6-nitrobenzoimidazole-1-yl)-propanhydroxyiminoamide, and benzenehydroxyiminoamide.

3-(6-Nitrobenzoimidazol-1-yl)-propionitrile (**III**) was synthesized as previously described.[9] Compound **III** (1.22 g, 5.6 mmol) was refluxed for 11 h with a methanolic solution of hydroxylamine (0.026 mol). Standing at 4 °C overnight gave the green crystalline powder, which was recrystallized from water to give 0.57 g NBIPA (**IV**)[8] as a needle-like light green crystalline solid.

According to our previous studies' procedure,[6,7] the ^{99m}Tc complexes were prepared. NaCl solution (0.9%) for ^{99m}Tc-NTPA or methanol for ^{99m}Tc-NBIPA was used as the developer for TLC, and Polyamide strips (purchased from Siqing Biochemical Material Factory, Taizhou, Zejiang, China) were used as the fixed phase to determine the yield. Both ^{99m}Tc-NTPA and ^{99m}Tc-NBIPA migrated to the middle of the strip (R_f = 0.3–0.6 for ^{99m}Tc-NTPA and R_f = 0.3–0.5 for ^{99m}Tc-NBIPA), while the insoluble radiochemical impurities and $^{99m}TcO_4^-$ remained near the origin (R_f = 0–0.1). The labeling yield was 94–96%. And the complexes were observed to be stable at room temperature for a period of 6 h. In addition, the paper electrophoresis showed that these complexes were neutral under physiological conditions.

For comparison, a hydroxyiminoamide ligand without nitrobenzoimidazole or nitrotriazole, benzenehydroxyiminoamide (Bham, **V**) was also prepared,[10] and its ^{99m}Tc-complex was synthesized according to the published method.[11]

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 containing a Teflon-coated magnetic spinbar 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- to 45-min equilibration period, the radiolabeled compound (0.3 mL) was added to each vial at a final activity of approximately 0.25 MBq/mL and a drug concentration of approximately 0.7 μg/mL. The 0.9-mL samples were removed at various time intervals. It has been shown that S180 cells in this stirred suspension system maintain >90% viability for 4–6 h under aerobic and hypoxic conditions. From each sample, the 0.2-mL sample was centrifuged at 1500 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. C_{in}/C_{out} was calculated as (residue counts − supernatant counts)/supernatant counts. At each time-point, three samples were determined. The effect of hypoxic or aerobic conditions on the accumulation of three complexes in S180 cells as a function of time is illustrated in Figure 1.

From Figure 1, it is shown that the accumulation of ^{99m}Tc-NTPA ands ^{99m}Tc-NBIPA steadily increased with time in hypoxic cells, but fluctuated with time and had no fixed trend in aerobic cells, while ^{99m}Tc-Bham had no obvious difference between hypoxic cells and aerobic cells. The hypoxic/aerobic differences at 4 h were 1.85-, 1.60-, and 1.15-fold for ^{99m}Tc-NTPA, ^{99m}Tc-NBIPA, and ^{99m}Tc-Bham, respectively. It was concluded that the accumulation of ^{99m}Tc-NTPA and ^{99m}Tc-NBIPA in hypoxic cells was only attributed to the bioactive group, nitrotriazole or nitrobenzoimidazole.

Biodistribution studies were performed in Kunming male mice (weighing 20–25 g) bearing S180 tumor, which grew to a leg diameter of 10–15 mm. The ^{99m}Tc

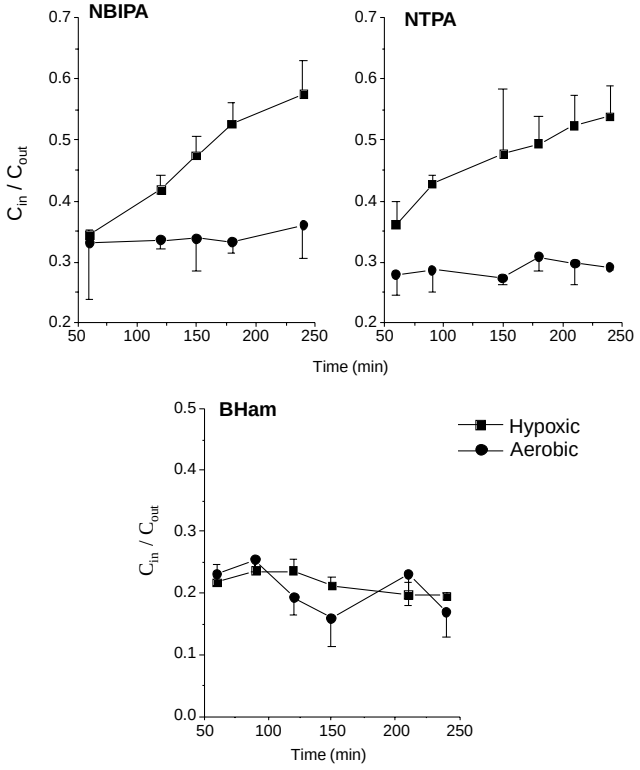

Figure 1. The accumulation of three complexes in hypoxic and aerobic S180 cells as a function of time.

Table 1. Biodistribution of ^{99m}Tc complexes in mice bearing S180 (%ID/g)

Tissue	^{99m}Tc-NTPA		^{99m}Tc-NBIPA		
	2 h	4 h	2 h	4 h	8 h
Blood	0.60 ± 0.15	0.35 ± 0.08	0.40 ± 0.11	0.24 ± 0.06	0.20 ± 0.04
Heart	0.18 ± 0.03	0.10 ± 0.02	0.13 ± 0.05	0.10 ± 0.02	0.09 ± 0.02
Lung	0.30 ± 0.07	0.19 ± 0.01	0.26 ± 0.08	0.23 ± 0.05	0.21 ± 0.04
Liver	0.78 ± 0.14	0.37 ± 0.08	1.14 ± 0.50	0.76 ± 0.25	0.69 ± 0.18
Spleen	0.16 ± 0.03	0.10 ± 0.03	0.24 ± 0.08	0.18 ± 0.05	0.18 ± 0.04
Kidney	4.81 ± 1.29	3.40 ± 1.34	1.23 ± 0.39	1.05 ± 0.15	0.80 ± 0.11
Stomach	1.74 ± 0.52	1.32 ± 0.38	0.40 ± 0.23	0.47 ± 0.29	0.20 ± 0.06
Muscle	0.14 ± 0.04	0.08 ± 0.04	0.25 ± 0.05	0.08 ± 0.02	0.07 ± 0.04
Brain	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.01	0.01 ± 0.01
Tumor	0.46 ± 0.12	0.34 ± 0.17	0.27 ± 0.09	0.19 ± 0.05	0.20 ± 0.04
Intestine	0.67 ± 0.26	0.28 ± 0.06	0.55 ± 0.11	0.62 ± 0.42	0.18 ± 0.04
T/B	0.78 ± 0.17	0.94 ± 0.25	0.81 ± 0.27	0.82 ± 0.18	1.07 ± 0.42
T/M	3.37 ± 0.48	4.67 ± 0.71	1.25 ± 0.12	2.39 ± 0.68	3.22 ± 1.03

complex (100 μL, 1×10^6Bq) was injected into the mice via the tail vein and the mice were sacrificed by cervical dislocation at various time intervals after injection. The organs or tissues were moved, weighed, and counted. Such injection solution (0.1 mL) was taken as standard for calculating the percent injected dose per gram of tissue, that is, %ID/g. Tumor-to-tissue ratios were calculated from %ID/g of the tumor and relevant organs. The final results were expressed as means ± SD. All biodistribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation.

From Table 1, it can be seen that the tumor uptake decreased slowly, from 0.46%ID/g at 2 h to 0.34%ID/g at 4 h for ^{99m}Tc- NTPA and 0.27%ID/g at 2 h to 0.20%ID/g at 8 h for ^{99m}Tc- NBIPA, while the uptake in the normal tissues was lower and their clearance was faster. At 4 h postinjection, the tumor-to-blood ratios (T/B) were 0.94, 0.82 for ^{99m}Tc-NTPA and ^{99m}Tc-NBIPA, respectively, and the tumor-to-muscle ratios (T/M) were 4.67 for ^{99m}Tc-NTPA and 2.39 for ^{99m}Tc-NBIPA. The ratios went up with time, this increasing trend was kept at 8 h for ^{99m}Tc-NBIPA, the tumor-to-blood ratio was 1.07 and tumor-to-muscle ratio was 3.22. Compared with some radiolabeled 2-nitroimidazoles at 2 h after injection, ^{99m}Tc-BMS181321 0.31 ± 0.05(T/B), 2.63 ± 0.57(T/M)[12]; ^{99m}Tc-BRU59-21 0.86 ± 0.21(T/B), 3.84 ± 1.54(T/M),[13] it was showed that ^{99m}Tc-NTPA was a better one for tumor hypoxia marker but not the best one.

Therefore, the ^{99m}Tc labeled nitrobenzoimidazole and nitrotriazole showed selective accumulation in tumors in vivo and hypoxic cells in vitro, and ^{99m}Tc-NTPA had a slightly better selective hypoxic localization than ^{99m}Tc-NBIPA. These results suggested that the ^{99m}Tc labeled nitrobenzoimidazole and nitrotriazole might be the novel tumor hypoxia markers.

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8. All the new compounds gave satisfactory analytical data. For compound **I**: mp 119–120 °C, yield: 1.54 g (70%), ^{1}H NMR δ (DMSO-d_6): 8.94 (1H), 4.65 (2H), 3.21 (2H). For compound **II**: mp 157.1–159.2 °C, yield: 64.3%, ^{1}H NMR δ (DMSO-d_6): 8.97 (1H), 8.78 (1H), 5.55 (1H), 4.51 (2H) and 2.60 (2H). The analytical data (%) calculated for $C_5H_8N_5O_3$ were: C, 30.00; N, 41.99; H, 4.03. Found: C, 29.89; N, 42.59; H, 4.13. For compound **IV**: mp 196.0–197.4 °C, yield: 41%, ^{1}H NMR δ (DMSO-d_6): 8.85 (1H), 8.71 (1H), 8.51 (1H), 8.11 (1H), 7.82 (1H), 5.58 (2H), 4.58 (2H) and 2.53 (2H). The analytical data (%) calculated for $C_{10}H_{11}N_5O_3$ were: C, 48.19; N, 28.10; H, 4.45. Found: C, 48.42; N, 28.03; H, 4.24.
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