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Synthesis and radiolabeling of ^{64}Cu-labeled 2-nitroimidazole derivative ^{64}Cu-BMS2P2 for hypoxia imaging

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ABSTRACT

The objective of this study was to develop a positron emission tomography (PET) probe with hypoxia targeting specificity and a relatively long half-life. The synthesis, ^{64}Cu-labeling in vitro and in vivo study of the novel 2-nitroimidazole complex ^{64}Cu-BMS2P2 is presented in this study. The hypoxia targeting capacity of ^{64}Cu-BMS2P2 in vitro was evaluated and compared with the ^{64}Cu-BMS181321, and confirmed by PET imaging in vivo and immunohistochemistry for carbonic anhydrase 9 (CA9) in a tumor mouse model. These results suggest that ^{64}Cu-BMS2P2 is a promising candidate for PET hypoxia imaging and worthy of further investigations in dynamic hypoxia imaging.

Malignant tumor is a major public health problem in the world. About 1.65 million new cancer cases are estimated to occur in the United States in 2015,[1] and radiation therapy has been a vital part of comprehensive treatment for human malignancies, as nearly 70% of patients need radiotherapy to fight the disease. However, tumor hypoxia plays an important role in radiation therapy resistance and increases the risk of metastasis and poor prognosis for the patients.[2,3] Thus how to accurately assess tumor hypoxia before and during radiation therapy treatment is essential concerning that a higher radiation doses to hypoxic tumor tissue can improve clinical survival.[4]

Recently, a number of Positron Emission Tomography (PET) imaging agents for hypoxia have already been developed and studied to delineate the hypoxia volume.[5–8] Up to now two major classes of hypoxia imaging agents are synthesized as follows: the nitroimidazole complexes and non-nitroimidazoles complexes. The former complexes are known for their ability to be selectively retained in hypoxic tissues and the mostly used for clinically studied is ^{18}F-FMISO, and ^{64}Cu-ATSM is one of non-nitroimidazoles complexes studied widely for hypoxia imaging. (The structural formulas of ^{18}F-FMISO and ^{64}Cu-ATSM are shown in Figure 1.)

As a nitroimidazole agent, ^{18}F-FMISO is specific to hypoxic tissues,[9] and it could provide radiation oncologists hypoxia imaging information to paint a higher radiotherapy doses in the refractory area of hypoxia for improving cancer local control and prognosis. However, it has a relative short half-life (110 min) which goes against its 4 h waiting time before a good image acquisition due to its slow washout from normoxic tissues,[10] and it is also unsuitable for a long-time dynamic hypoxia monitoring before and during radiotherapy treatment for the 110 min short half-life as hypoxia is not changeless during radiotherapy. Furthermore, ^{64}Cu-ATSM, a long half-life (12.7 h) non-nitroimidazole hypoxia imaging agent, has been developed for dynamic hypoxia imaging, but it has been proven to be unspecific to hypoxic tissues yet.[9] Thus, radiolabeled PET probes with the double features of a relatively long half-life and hypoxia-specificity such as ^{64}Cu-radiolabeled nitroimidazole complexes, may result in an ideal hypoxia imaging agent.

The relative long half-life of ^{64}Cu could provide several image acquisitions to give radiation oncologists more information for assessment of hypoxia before and during radiation therapy as fluctuation of hypoxia during treatment.[11] In addition, the nitroimidazole group especially 2-nitroimidazole is more hypoxia-specific than non-nitroimidazole ligands[9] or 4-nitroimidazole as we previously reported.[12]

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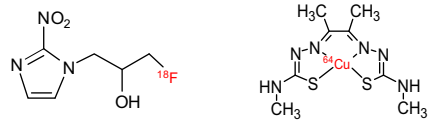


Figure 1. Structural formulas of ^{18}F-FMISO and ^{64}Cu-ATSM.

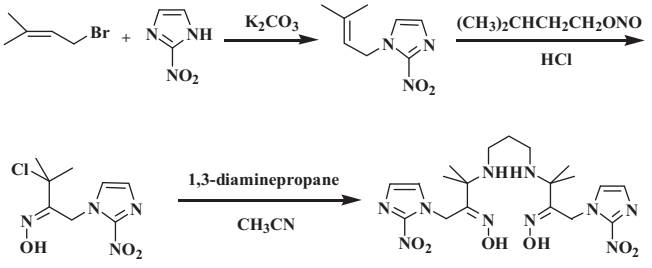
To date, there are limited examples of hypoxia radiotracers using nitroimidazole ligand to complex ^{64}Cu. Indeed, only Bonnitcha[13] report the use of ^{64}Cu to radiolabel nitroimidazole complex. However, the hypoxia imaging in vivo has not been reported in the study.

Herein, the novel bisnitroimidazole complex BMS2P2 was synthesized and radiolabeled with ^{64}Cu and evaluated both in vitro and in vivo as a hypoxia probe. Moreover, its performance was also compared with mononitroimidazole complex ^{64}Cu-BMS181321 (the structure of BMS181321 is similar to BMS2P2 except the lack of one nitroimidazole. The structural formula and ^{64}Cu-radiolabeling of BMS181321 is shown in Supplementary data Scheme S1).

Compound BMS2P2 (Scheme 1) was synthesized following the previous method with some modifications.[12] The crude product was purified by flash chromatography (silica gel, CH_2Cl_2/CH_3-OH = 6:1) and recrystallized from acetonitrile to provide BMS2P2 as a yellow solid. The product BMS2P2 was characterized by the ^{1}H NMR and Mass Spectrum (The data were supported in Supplementary data Figs. S1 and S2).

In order to optimize radiolabeling conditions and characterize the radio-labeled compound, the "cold" counterpart Cu-BMS2P2 was synthesized by complexing the BMS2P2 with $CuCl_2$ according to reported procedures with some modifications.[14,15] The optimum reaction condition for Cu-BMS2P2 was determined as follows: 50 μL precursor BMS2P2 (2 mg/mL), 200 μL of sodium acetate buffer (pH 5.5), 50 μL of $CuCl_2$ (0.05 mol/L), the mixture was reacted at room temperature for 10 min. The target product Cu-BMS2P2 was identified by MALDI-TOF mass spectrum (Bruker Daltonics), The MALDI-TOF mass spectrum (Supplementary data Fig. S3) showed that a mass spectrum peak at 556, which was accorded with the expected molecular weight of Cu-BMS2P2 (MW = 556). BMS2P2 was then radiolabeled with $^{64}CuCl_2$ (Atom-Hitech Beijing, China) following the same procedure described above (Scheme 2). Briefly, 37 MBq of $^{64}CuCl_2$ in 0.1 N HCl was diluted in 200 μL of 0.1 N sodium acetate buffer (pH 5.5) and added to the 2 μL of BMS2P2 (2 mg/mL), the reaction mixture was incubated at room temperature for 10 min.

The radiochemical yield was determined by radio–HPLC (Radio–HPLC was carried out at a flow rate of 1.0 mL/min. NH_4OAc buffer (pH 4.6, 0.1 M) (A) and CH_3CN (B) mixtures were used as the mobile phase (0–10 min, B: 10–45%; 10–20 min, B: 45–60%; 20–25 min, B: 60–10%)). The radioactive peaks on radio–HPLC corresponding to products ^{64}Cu-BMS2P2 and starting material $^{64}CuCl_2$ were well separated (Fig. 2). The retention time of the product was at 10.95 min (A), and $^{64}CuCl_2$ was at 3.75 min (B). The



Scheme 1. The synthetic process of BMS2P2.

Radio–HPLC showed the initial radiolabeling yield and radiochemical purity of product ^{64}Cu-BMS2P2 were >99% and >99%, respectively. The initial radiolabeling yield of ^{64}Cu-BMS2P2 was higher than ^{99m}Tc-PnAO complex ^{99m}Tc-BMS181321 (radiolabeling yield: 92.1%[16]) as Cu^{2+} could be well chelated by PnAO cycle[17] under a relatively simple condition.

In vitro stability analysis of the radiolabeled complex in physiological saline and sodium acetate buffer was performed to determine the stability of ^{64}Cu-BMS2P2 by radio–HPLC (Supplementary data Fig. S4). ^{64}Cu-BMS2P2 can stay >90% radiochemical purity in physiological saline for 60 h, which is more stable than ^{99m}Tc-PnAO complex ^{99m}Tc-BMS181321 (60 h vs 26 h[16]).

To investigate the selectivity of ^{64}Cu-BMS2P2 for hypoxia, a cell uptake experiment in vitro was performed using the protocol reported before,[12,18] under hypoxia environment (2% air plus 98% carbon dioxide) or normally environment (95% air plus 5% carbon dioxide), respectively, and using ^{64}Cu-BMS181321 as a control (radiolabeling yield and radiochemical purity of ^{64}Cu-BMS181321were >99% and >99%, respectively). Briefly, cancer cells in suspension were cultured in DMEM containing 10% fetal bovine serum, then they were spun down from suspension and resuspended in fresh growth medium at a desired cell concentration of 2×10^6/mL. The cells (in 20 mL) were equilibrated for 35 min in the hypoxia environment or normally environment. After equilibration, the ^{64}Cu-labeling probe was added to the suspension at a final radioactivity of 0.30 MBq/mL in each glass vial. Then the samples were removed from the vials to 1 mL centrifugal tubes without disturbing the oxygenation status of cells in the vials and centrifuged at 3000 rpm for 3 min to remove the supernatant liquid, the cell suspensions were collected and measured in a gamma counter (1470 Wizard, PerkinElmer Finland). The cell uptake experiment was conducted at a different time of 5 min, 1 h, 2 h, 3 h, 4 h with five parallel samples, expressed as mean ± standard deviation (n = 5) for each time point. The cellular uptakes (% of applied radioactivity) of the ^{64}Cu-complexes versus time were shown in Fig. 3. At 5 min, the cellular uptake of ^{64}Cu-BMS2P2 was considered no significant hypoxic/normally differential (Student's T-test, $p > 0.05$), the hypoxic/normally uptake ratios were 1.93 (42.16/21.87), 2.28 (53.92/23.67), 2.59 (57.33/22.12) and 2.26 (56.86/21.67) times at 1, 2, 3, 4 hours with statistics differential (Student's T-test, $p < 0.05$). Compared with ^{64}Cu-BMS181321, ^{64}Cu-BMS2P2 had better cellular uptakes (Student's T-test, $p < 0.05$) 1 hour later in hypoxic environment, which was consistent with our previous study in ^{99m}Tc-BMS2P2 and ^{99m}Tc-BMS181321 as introducing a second nitroimidazole may increase hypoxic accumulation.[12,19]

To demonstrate the new radiolabeled compound for hypoxia imaging in vivo, ^{64}Cu-BMS2P2 (15.6 MBq/mouse, filtered through a 0.22 μm filter before injection) was injected through tail vein into A549 tumor bearing mice (five nude mice, tumor xenograft with different diameter) followed by PET imaging. Fig. 4a and b shows the typical PET images of mouse A and mouse B 4 hours after administration of ^{64}Cu-BMS2P2, Fig. 4c shows PET image of mouse B 8 hours later as a multiple-time points monitoring imaging. The ^{64}Cu-BMS2P2 could even acquire a longer-time imaging by the long half-time of ^{64}Cu in mouse C (Supplementary data Fig. S5a), then 15.6 MBq $^{64}CuCl_2$ without radiolabeling any ligand was injected i.v. into another mouse D bearing A549 tumor xenograft as a control (Supplementary data Fig. S5b). Compared with contralateral organs, ^{64}Cu-BMS2P2 uptake in tumor was relatively high (mouse A, B, and C). Moreover, the uptake in the tumor could be clearly visualized over time (mouse B, C) In contrast, there was a much lower signal in the tumor of mouse injected with $^{64}CuCl_2$, and the radioactivity distribution on the mouse was diffused, which is also different from mouse A, B, and C. For mouse B, a wide range of necrosis was found due to anoxia in the tumor center, so