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Kit formulation for preparation and biological evaluation of a novel ^{99m}Tc-oxo complex with metronidazole xanthate for imaging tumor hypoxia

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ABSTRACT

Introduction: Achieving an ideal ^{99m}Tc labeled nitroimidazole hypoxia marker is still considered to be of great interest. Metronidazole xanthate (MNXT) ligand was synthesized and radiolabeled with ^{99m}Tc-glucoheptonate (GH) to form the ^{99m}TcO-MNXT complex, for the potential use as a novel probe for imaging tumor hypoxia.
Methods: For labeling, ^{99m}TcO-MNXT was prepared by ligand-exchange reaction with ^{99m}Tc-GH. The radiochemical purity of the ^{99m}TcO-MNXT complex was measured by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). The distribution coefficient and stability of the complex was investigated. The structure of the ^{99m}TcO-MNXT complex was verified by preparation and characterization of the corresponding stable rhenium complex. The cellular uptake of the ^{99m}TcO-MNXT complex was determined in murine sarcoma S180 cell lines under hypoxic and aerobic conditions. The biodistribution and single photon emission computed tomography (SPECT) image studies of the ^{99m}TcO-MNXT complex were performed in mice bearing S 180 tumor.
Results: The radiochemical purity of the ^{99m}TcO-MNXT complex was over 90%. It had good in vitro stability and its distribution coefficient indicated that it was a hydrophilic complex. When ^{99m}Tc and Re complexes were coinjected in HPLC, both radioactivity (for ^{99m}Tc complex) and UV detectors (for Re complex) showed nearly identical HPLC profiles, suggesting their structures are similar. The tumor cell experiment and the biodistribution in mice bearing S 180 tumor showed that the ^{99m}TcO-MNXT complex had a good hypoxic selectivity and accumulated in the tumor with high uptake and good retention. Single photon emission computed tomography (SPECT) image studies showed that the tumor detection was observable.
Conclusions: ^{99m}TcO-MNXT is prepared from a kit without the need for purification and shows high tumor uptake, tumor/blood and tumor/muscle ratios, suggesting that it would be a promising candidate for imaging tumor hypoxia.

1. Introduction

For several years, it has been known that hypoxia plays an important role in the resistance of cancers to treatment by radiotherapy [1]. The identification of tumor tissue hypoxia may be related to individual treatment planning and monitoring as well as predicting prognosis. Thus, it is very important to develop methods to detect tumor hypoxia. A number of invasive methods, including polarographic needle electrodes, have been developed for the measurement of hypoxia, but none of these are 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. Noninvasive imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) with hypoxia targeted radiopharmaceuticals are considered to be reasonable alternatives. Up to now, much effort has been made to develop radiolabeled hypoxia imaging agents. Among them, nitroimidazole derivates are thought to undergo bioreduction reaction in the hypoxic cell and thus can be retained in hypoxic tissue [2,3]. Thus, different kinds of PET and SPECT tracers containing nitroimidazole group have been evaluated as hypoxia imaging agents [4–24]. At present, PET tracer [^{18}F]Fluoromisonidazole ([^{18}F]FMISO) is one of the most clinically studied hypoxia tracers. However, the short half life, high cost and limited availability of the [^{18}F] isotope are some limitations. Due to the favorable characteristics of ^{99m}Tc (ideal half-life, optimal γ-energy, inexpensive cost and in-house availability) and larger number of SPECT scanners in the world, there has been great interest in developing ^{99m}Tc-labeled nitroimidazole derivatives for targeting

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tumor hypoxia. As compared to 2-nitroimidazole, metronidazole, a cheaper commercial 5-nitroimidazole derivative, can also be efficiently reduced and retained in hypoxia cells, thus making it suitable as a starting material for the preparation of ^{99m}Tc radiopharmaceuticals for hypoxia imaging [25–31]. As nonfunctionalized metronidazole is usually weak ligand for chelating with ^{99m}Tc, functionalization with an external chelating group or the insertion of some functional groups is crucial to obtain strong metal-binding compounds. Metronidazole has a pendant –CH_2OH group, thus making it suitable to react with carbon disulfide in NaOH solutions to form the corresponding xanthate product. Xanthate can be used to form stable ^{99m}Tc complex on the basis of efficient binding of the group to four sulfur atoms. In the development of ^{99m}Tc radiopharmaceuticals, most mononuclear ^{99m}Tc(V) compounds at tracer level are characterized by the presence of $[^{99m}TcO]^{3+}$ core, such as ^{99m}Tc(V)-glucoheptonate (GH), ^{99m}Tc(V)-L,L-ethylene dicysteine diester (ECD), ^{99m}Tc(V)-D,L-hexamethylpropyleneamineoxime (HMPAO), ^{99m}Tc(V)-mercaptoacetyltriglycinate (MAG_3), ^{99m}Tc(V)-L,L-ethylene dicysteine (EC), ^{99m}Tc(V)-dimercaptosuccinic acid (DMSA) and so on [32]. Bearing in mind that ^{99m}Tc(V)-glucoheptonate (GH) is a well defined complex containing the $[^{99m}TcO]^{3+}$ core and a weak ^{99m}Tc chelating complex, it is suitable to be a good labeling precursor for preparing the novel ^{99m}Tc-oxo complexes. This background encouraged us to synthesize the ^{99m}TcO-MNXT complex by ligand-exchange reaction with ^{99m}Tc-GH to find a good tracer for imaging tumor hypoxia. In this study, the synthesis and biological evaluations of the ^{99m}TcO-MNXT complex as a potential agent to target tumor hypoxia are reported.

2. Experimental

2.1. Materials

Metronidazole was purchased from Alaf Aesa, China. Glucoheptonate (GH) kit containing 0.1 mg of stannous chloride dihydrate, 5.0 mg of GH was obtained from Beijing Shihong Pharmaceutical Center, Beijing Normal University, China. All other chemicals were of reagent grade and were used without further purification. ^{99}Mo/^{99m}Tc generator was obtained from the China Institute of Atomic Energy (CIAE). IR spectrum was obtained with an AVATAR 360 FT-IR spectrometer using KBr pellets. NMR spectrum was recorded on a 400 MHz Bruker Avance spectrophotometer. Elemental analyses were performed on a Vario EL elemental analyzer model. HRMS was recorded on a Bruker Solarix mass spectrometer. HPLC analysis was carried out with a reversed-phase column (Kromasil 100-5C, 250 4.6 mm), Shimadzu SCL-10A VP series.

2.2. Synthesis

The synthetic procedures and the spectral data for MNXT are as follows. Metronidazole (0.342 g), carbon disulfide (0.456 g) and NaOH (0.120 g) were dissolved in 25.0 mL water. The mixture was stirred at 3 °C for 2.0 h and continued to react overnight at room temperature. Most of the solvent was removed, and the precipitate was collected by filtration. The crude product was recrystallized from $CH_3OH/CH_3CH_2OCH_2CH_3$ to give 0.266 g of MNXT (brown solid). Yield: 49.5%. MNXT was characterized by IR, ^{1}HNMR, ^{13}C-NMR and elemental analysis. IR (KBr)/cm^{-1}: νC-O: 3519.0, νNO_2: 1529.4, 1364.5, νC = S: 1073.7. ^{1}H-NMR (D_2O): δ7.92 (s, 1H, CH), δ4.39-4.36 (t, 2H, CH_2), δ3.80-3.78 (t, 2H, CH_2), δ2.37 (s, 3H, CH_3); ^{13}C-NMR (D_2O): δ206.52(CS_2); δ162.73(C); δ152.59(C); δ132.74(CH); δ60.09(CH_2); δ48.07(CH_2); δ13.31(CH_3). Elemental analysis calculated (%) for $C_7H_8N_3NaO_3S_2$: C, 31.22; N, 15.60; H, 2.99. Found: C, 31.47; N, 15.57; H, 3.13.

2.3. Radiolabeling and quality control

For preparing ^{99m}TcO-MNXT, $[^{99m}TcO_4]^-$ (1.50×10^7 Bq) was added to a GH kit containing 0.1 mg of stannous chloride dihydrate, 5.0 mg of GH. The mixture was kept at room temperature for 15 min. Successively, 1.0 mg of MNXT dissolved in 1.0 mL water 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 (Kromasil 100-5C, 250 × 4.6 mm), Shimadzu SCL-10A VP series, working at a flow rate of 1.0 mL/min. Water (A) and acetonitrile (B) were used as the mobile phase, and the following gradient elution technique was adopted for the preparation: For ^{99m}TcO-MNXT, 0–20 min 50%–90% B, 30 min 90% B, 40 min 100% B.

2.4. Preparation of ReO-MNXT

To verify the proposed structure of ^{99m}TcO-MNXT, corresponding ReO analogue was also synthesized. First, 100 mg of GH was dissolved in 1.0 mL water in a 10 mL of vial and 3.0 mg of $KReO_4$ was added into the solution immediately. Then, 100 μL of $SnCl_2 \cdot 2H_2O$/HCl solution (80 mg $SnCl_2 \cdot 2H_2O$/1 mL 1 mol/L HCl) was added to the above solution and 1.0 mol/L NaOH was added to adjust the pH to about 5. The solution was incubated at room temperature for 1.0 h and the color of the solution turned to light blue. Successively, 100 μL of the above solution was transfered into another 10 mL of vial. Then, 100 μL of MNXT (10.0 mg/mL) solution was added and the pH of the solution was adjusted to about 8 quickly. The mixture solution was heated at 100 °C for 0.5 h, and then the solution was purified by HPLC to obtain the ReO-MNXT solution. The solvent was removed and a brown residue was obtained. In order to obtain enough solid powder for characterization, the above steps should be repeated several times to get enough ReO-MNXT. ReO-MNXT powder was characterized by ^{1}H-NMR, IR and HRMS-MALDI. ^{1}H-NMR (D_2O): δ7.89 (s, 2H, CH), δ4.28–4.25 (t, 4H, CH_2), δ3.79-3.76 (t, 4H, CH_2), δ2.19 (s, 6H, CH_3); IR (KBr)/cm^{-1}: νC-O:3431.1, νNO_2:1508.9, 1336.9, νC = S:1026.2, νReO: 976.0; HRMS-MALDI: found m/z: 694.95223, calcd for $C_{14}H_{16}N_6O_7S_4Re^+$: 694.95208.

For the purpose of characterization by comparison, the ReO-MNXT solution was mixed with 0.5 mL of ^{99m}TcO-MNXT solution (3.70×10^6 Bq). Then the solution was co-injected into HPLC to get the corresponding HPLC pattern.

2.5. In vitro stability study

^{99m}TcO-MNXT was kept in the labeling milieu at room temperature (25 °C) and the radiochemical purity was assayed by TLC for up to 6.0 h after labeling. To test the stability of the complex in serum, 0.5 mL of ^{99m}TcO-MNXT was incubated in 1.0 mL human serum albumin (HSA) (1 mg/mL) at 37.0 °C for 4.0 h and then the radiochemical purity of the complex was analyzed by TLC. The stability of the complex in mouse liver homogenate was determined according to the reported methods [33]. In brief, 200 uCi of ^{99m}TcO-MNXT was incubated in the solution of 0.2 mL mouse liver homogenate at 37.0 °C for 4.0 h. Proteins were precipitated by adding 0.4 mL acetonitrile, after centrifugation at 5000*g* for 15 min at −4 °C. The radiochemical purity of the complex was checked by TLC.

2.6. Determination of the distribution coefficient (logD)

The distribution coefficient (log*D*) between 1-octanol and phosphate buffer (0.025 mol/L, pH 7.4) of the complex was measured in order to evaluate their lipophilicity. In a centrifuge tube, containing 2.0 mL of each phase, 0.1 mL of ^{99m}TcO-MNXT was added, and the mixture was shacked on a Vortex mixer for 1 min and then centrifuged at 5000*g* for 5 min. Three samples (0.1 mL each) from each layer were counted in a well gamma γ-counter. The distribution coefficient, *D*, was calculated as the mean value of counts per minute in octanol divided by that of the buffer. Usually the final distribution coefficient value was expressed as log*D*. The log*D* value was reported as an average of three measurements plus the standard deviation.