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Synthesis and application of ^{188}Re-MN-16ET/Lipiodol in a hepatocellular carcinoma animal model

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Abstract

Introduction: Hepatocellular carcinoma is the most common form of primary hepatic carcinoma. A new N_2S_2 tetradentate ligand, *N*-[2-(triphenylmethyl)thioethyl]-3-aza-19-ethyloxycarbonyl-3-[2-(triphenylmethyl)thioethyl]octadecanoate (H_3MN-16ET), was introduced and labeled with ^{188}Re to create ^{188}Re-MN-16ET in the Lipiodol phase. The potential of ^{188}Re-MN-16ET/Lipiodol for hepatoma therapy was evaluated in a hepatocellular carcinoma animal model of Sprague–Dawley rats implanted with the N1S1 cell line.

Methods: Synthesis of H_3MN-16ET was described, and characterization was identified by infrared, nuclear magnetic resonance and mass spectra. We compared the effects of transchelating agents (glucoheptonate or tartaric acid) and a reducing agent (stannous chloride) on the complexing of ^{188}Re-perrhenate and H_3MN-16ET. Twenty-four rats implanted with hepatoma were injected with 3.7 MBq/0.1 ml of ^{188}Re-MN-16ET/Lipiodol or ^{188}Re-MN-16ET via transcatheter arterial embolization. Biodistribution experiments and single-photon emission computed tomography imaging were performed to investigate tumor accumulation.

Results: H_3MN-16ET was proved to easily conjugate with the Re isotope and showed good solubility in Lipiodol. The radiochemical purity of ^{188}Re-MN-16ET/Lipiodol with 10 mg tartaric acid and stannous chloride was shown to be more than 90%. The major distribution sites of ^{188}Re-MN-16ET in Sprague–Dawley rats were hepatoma and the liver. However, the radioactivity at the tumor site postadministered with ^{188}Re-MN-16ET was quickly decreased from 9.15±0.23 (at 1 h) to 2.71%±0.18% of injected dose/g (at 48 h). The biodistribution and micro-single-photon emission computed tomography/computed tomography image data showed that ^{188}Re-MN-16ET/Lipiodol was selectively retained at the tumor site, with 11.55±1.44, 13.16±1.46 and 10.67%±0.95% of injected dose/g at 1, 24 and 48 h postinjection, respectively. The radioactivity in normal liver tissue was high but significantly lower than that of the tumors.

Conclusion: H_3MN-16ET is a suitable tetradentate ligand for ^{188}Re labeling. From the animal data, we suggest that ^{188}Re-MN-16ET/Lipiodol has the potential to be a therapeutic radiopharmaceutical for hepatoma treatment.

Keywords: ^{188}Re; Hepatocellular carcinoma; H_3MN-16ET; Lipiodol; Transarterial embolization

1. Introduction

Primary hepatocellular carcinoma (HCC) is the most common form of hepatic carcinoma, particularly in Asia and sub-Saharan Africa [1]. The incidence of hepatoma has increased dramatically in Europe and the United States in recent years due to the widespread occurrence of hepatitis C [2,3]. Although surgery is usually considered the treatment of choice, more than 85% of liver cancer cases are inoperable because of late diagnosis [4]. Other therapeutic modalities such as chemotherapy, radiotherapy and transhepatic arterial embolization (TAE) have shown some efficacy but unsatisfactory results. Alternative therapeutic methods such as using Lipiodol as a carrier for chemotherapeutic agents or radioisotopes are currently under investigation [5,6].

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Lipiodol, an iodinated and esterified lipid of poppy seed oil, was initially developed as a radiocontrast agent. Due to its high-viscosity character, Lipiodol has been found to be retained selectively in liver tumors after infusion via the hepatic artery [7]. It is currently used as a common embolizing agent for TAE in clinics. Iodine-131 (^{131}I) Lipiodol has been investigated as a radioembolizing material and is presently commercially available. However, the low β energy and high-energy gamma emission of ^{131}I decrease the competitiveness of ^{131}I-Lipiodol. Although yttrium-90 (^{90}Y)-labeled Lipiodol has also been developed for the treatment of hepatoma, the possibility of accumulation in the skeletal system limits the clinical value of ^{90}Y-Lipiodol [8]. Among several β emitters (e.g., ^{166}Ho, ^{90}Y, ^{186}Re and ^{188}Re), ^{188}Re has become the most probable candidate for labeling Lipiodol because of its convenient, economical and energy characteristics. Since ^{188}Re is obtained as an aqueous solution and Lipiodol is available as an oily solution, many chelating agents were developed to link Lipiodol and chelate with ^{188}Re. In 1996, Wang et al. used *N*,*N*,*N*′,*N*′-tetrakis(2-benzymidazolylmethyl)-1,2-ethanediamine as a chelating agent and successfully labeled Lipiodol [9]. However, the preparation process consumes a lot of time and is not suitable for the half-life of the ^{188}Re isotope (16.9 h). N_2S_2 or diamine-dithiol (DD) compound is known to make a stable chelate with rhenium or technetium. We used ethyl cysteinate dimer (ECD) as a chelating agent for ^{188}Re and Lipiodol in a previous study [10]; however, the tumor uptake was unsatisfactory at 48 h postinjection with ^{188}Re-ECD/Lipiodol. Lee et al. [11] have developed DD and its long-chain alkyl derivatives to be labeled with ^{188}Re, and this synthesis has shown enhanced uptake in liver cancer. Recently, ^{188}Re-Lipiodol conjugated with 4-hexadecyl-2,2,9, 9-tetramethyl-4,7-diaza-1,10-decanedithiol (HDD) was used in the treatment of inoperable HCC for International Atomic Energy Agency (IAEA)-conducted clinical trials [12,13]. It has been proven to be a reasonable choice for treating primary HCC. However, the research group reported that acetylated HDD instead of HDD provided better kit stability for hepatoma therapy [14].

In this study, the new monoamine-monoamide-dithiol (N_2S_2) tetradentate ligand, *N*-[2- (triphenylmethyl) thioethyl]-3-aza-19-ethyloxycarbonyl-3-[2-(triphenylmethyl)thioethyl]octadecanoate (H_3MN-16ET), is introduced. H_3MN-16ET was complexed with ^{188}Re to produce ^{188}Re-MN-16ET. ^{188}Re-MN-16ET was further dissolved in Lipiodol to obtain the radiopharmaceutical ^{188}Re-MN-16ET/Lipiodol. We studied the biodistribution in Sprague–Dawley (SD) rats with hepatic tumors and evaluated its potential for treatment of hepatoma. The single-photon emission computed tomography (SPECT) images and biodistribution data demonstrated that ^{188}Re-MN-16ET/Lipiodol has a high and stable tumor accumulation in an N1S1 hepatoma rat model and shows the potential to be a therapeutic radiopharmaceutical for hepatoma treatment.

2. Materials and methods

2.1. Materials

All laboratory chemicals were of reagent grade and obtained from commercial sources. 2-Thiothylamine hydrochloride and 16-bromohexadecanoic acid were purchased from Sigma-Aldrich. Carrier-free ^{188}Re was eluted with normal saline from ^{188}W/^{188}Re generator manufactured by Institute of Nuclear Energy Research (Taiwan). $ReO(PPh_3)_2Cl_3$ was prepared as described previously [15]. Infrared spectra were run on a Bio-Rad FTS-40 spectrometer. The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 2000 spectrometer. Gas chromatography was scanned on a Shimadzu GCMS-QP 1100EX model.

2.2. Synthesis of H_3MN-16ET and Re-MN-16ET

2.2.1. 2-[(Triphenylmethyl)thio]ethylamine (**1**)

To a boiling solution of 2-thioethylamine hydrochloride (10.0 g, 88.4 mmol), triphenylmethanol (22.0 g, 85.0 mmol) and triethylamine (14.0 ml, 99.7 mmol) in trichloromethane (100 ml) were added and refluxed for 4 h. Then the solution was concentrated under reduced pressure, and the residue was extracted with methanol (100 ml). The extract was concentrated and further triturated with saturated sodium bicarbonate solution. The resultant white solid was isolated by filtration, washed thoroughly with water and dried in a vacuum system to give **1** (27.9 g, 99%).

Infrared (IR) (neat) ν 3381 (NH_2) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.42 (m, 3 H, Ph), 7.30 (m, 12 H, Ph), 2.58 (t, J=6.6 Hz, 2 H, CH_2N), 2.32 (t, J=6.6 Hz, 2 H, CH_2S), 1.45 (br, 2 H, NH_2). ^{13}C NMR ($CDCl_3$) δ 144.80, 192.52, 127.81 and 126.60 (Ph), 66.51 (CPh), 40.94 (CH_2N), 36.09 (CH_2S). MS *m*/*z* 319 (M^+), 243 (M^+-$C_6H_5^{+1}$).

2.2.2. N-[2-(Triphenylmethyl)thioethyl] bromoacetamide (**2**)

An ice-cooled solution of **1** (2.62 g, 8.2 mmol) and triethylamine (1.38 ml, 9.8 mmol) in trichloromethane (80 ml) were slowly added to a solution of bromoacetyl bromide (0.78 ml, 9.8 mmol) in trichloromethane (10 ml). The mixture was stirred for 30 min at room temperature and washed successively with 1.0 N hydrochloric acid (120 ml), water (100 ml) saturated with sodium bicarbonate and water (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated by a vacuum system to afford **2** (2.81 g, 86.6%).

IR (neat) ν 3413 and 3306 (NH), 1662 (CO) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 7.41 (m, 3 H, Ph), 7.24 (m, 12 H, Ph), 6.48 (br, 1 H, NH), 3.97 (s, 2 H, CH_2Cl), 3.12 (q, J=6.3 Hz, 2 H, CH_2N), 2.43 (t, J=6.3 Hz, 2 H, CH_2S). ^{13}C NMR ($CDCl_3$) δ 165.63 (CO), 144.47, 129.48, 127.97 and 126.81 (Ph), 66.52 (CPh), 42.54 (CH_2Cl), 38.35 (CH_2N), 31.67 (CH_2S). MS *m*/*z* 397 and 395 (M^+), 243 ((CPh_3)$^+$).

2.2.3. N-[2-(triphenylmethyl)thioethyl] [2-(triphenylmethyl) thioethylamino]acetamide (**3**)

A mixture of **1** (2.2 g, 6.9 mmol), **2** (2.7 g, 6.9 mmol) and triethylamine (1.5 ml, 10.4 mmol) in dichloromethane (60