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A comparison of Re-188-MN-16ET-lipiodol and transcatheter arterial chemoembolization in the treatment of hepatoma: An animal study

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ABSTRACT

Introduction: In patients with unresectable HCC, transcatheter arterial chemoembolization (TACE) is a widely used treatment. Recently, as an alternative treatment modality for HCC, transcatheter arterial embolization with radioisotopes has been investigated. In this study, we compared the therapeutic efficacy of an intra-hepatic arterial injection of Re-188-MN-16ET-lipiodol and the TACE method in rats with liver tumors.
Methods: Twelve male rats bearing hepatic tumors were divided into three groups to evaluate the efficacy of treatment (four in each group). Group 1 received an intra-hepatic arterial injection of 0.2 mCi of Re-188-MN-16ET-lipiodol; group 2 received epirubicin (0.5 mg/kg) and 0.1 ml of lipiodol emulsion; group 3 received 0.1 ml of normal saline and served as the control group. Tumor size was measured by liver sonography before injection, at two weeks, four weeks and eight weeks after injection. Survival time was calculated from the day of treatment to 56 days after treatment by the life-table method. The response to treatment and the survival time in each group were evaluated and compared.
Results: All rats treated with Re-188 MN-16ET-lipiodol showed good response to the therapy. Their tumor size decreased and all rats survived over eight weeks. All rats treated with epirubicin plus lipiodol survived over 8 weeks; however, two rats (50%) showed increased tumor size in the 8th week. As for the control group (rats treated with normal saline), all rats survived less than 37 days with continuous tumor growth.
Conclusion: Results showed that Re-188-MN-16ET-lipiodol can be a potential therapeutic pharmaceutical for the treatment of liver tumors.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies in the world, especially in Asia and Africa, with an estimated incidence of more than one million new cases per year [1]. Despite improved diagnosis, as a result of better imaging and screening programs in certain parts of the world, the number of patients eligible for curative surgery remains small. In patients with unresectable HCC, transcatheter arterial chemoembolization (TACE) is a widely used treatment because it induces a marked antitumoral effect in HCC [2,3]. To enhance the effect of TACE, some adjunctive therapies using anticancer agents or radioisotopes have been investigated [4–7].

TACE with an emulsion of iodized oil and various anticancer drugs has been most widely used for the treatment of HCC [4,5,8,2]. Among these anticancer drugs, doxorubicin, cisplatin and epirubicin are commonly used. Although TACE showed good results in many cases, it has limited efficacy in the treatment of tumor cells within capsular or extracapsular invasion, which results in high local recurrence rates [9,10]. Recently, as an alternative treatment modality for HCC, TACE with therapeutic radioisotopes has been investigated [6,11–14]. Among these radiopharmaceuticals, yttrium-90 (Y-90) microspheres have been shown to be a good agent for the treatment of HCC in many reports [11,12]. Y-90 is a pure beta particle emitter with a physical half-life of 64 hr and a mean energy per disintegration of 0.973 MeV. The beta particles have a mean tissue penetrance of 2.5 mm, with a maximum of about 10 mm. The physical characteristics make Y-90 a good isotope for localized internal radiation therapy [11,12]. However, Y-90 is not suitable for imaging. Rhenium-188 (Re-188) has similar beta energy characteristics to Y-90, with many advantages such as generator production, a shorter half life than Y-90 and the emission of 155 Kev gamma rays for tumor imaging. In addition, Re-188 may not have the same bone accretion as Y-90 due to differences

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in aqueous chemistry. Theoretically, these characteristics make Re-188 a better radioisotope than Y-90 for TACE.

Various Re-188-based radiopharmaceuticals were developed to treat inoperable liver malignancy [13–18]. Among them, Re-188 HDD lipiodol was the first to be introduced in clinical studies with encouraging results [13,16]. However, Re-188 HDD lipiodol was reported to have low labeling efficiency, which may limit the synthesis of high therapeutic activities [19]. In a recent study, we successfully labeled Re-188 with a new N2S2 tetradentate ligand, N-[2-(triphenylmethyl)thioethyl]-3-aza-19-ethyloxycarbonyl-3-[2-(triphenylmethyl)thioethyl]octadecanoate (MN-16ET) for the treatment of liver malignancy [14]. The synthesis of Re-188 MN-16ET was simple and the labeling efficiency was high. In that study, Re-188-MN-16ET-lipiodol was selectively retained at the tumor site via an intra-hepatic arterial injection in a rat bearing a liver tumor; hence we concluded that Re-188-MN-16ET-lipiodol has the potential to be a therapeutic radiopharmaceutical for hepatoma treatment.

In this study, we evaluated the therapeutic efficacy of an intra-hepatic arterial injection of Re-188-MN-16ET-lipiodol and the TACE method in rats with liver tumors.

2. Materials and methods

2.1. Animals and tumor cell line

Male rats (Sprague-Dawley rats) weighing 200–250 g were fed on a standard chow diet and given water *ad libitum*. An N1-S1 hepatoma cell line (ATCC, Maryland, USA) was used for tumor implantation. The tumor cells were routinely cultured in Dulbecco's Modified Eagle Medium (Gibco, Paisley, UK) mixed with 5% fetal bovine serum, 1% L-glutamine, and 20% horse serum. After the cells grew exponentially for a week, a concentration of approximately 4×10^6 cells per ml was established. The cell viability was more than 90%, as determined by trypan blue exclusion. This study was approved by the Institutional Animal Care and Use Committee of Taichung Veterans General Hospital.

2.2. Inoculation

A sub-xyphoid laparotomy, 1.5-2 cm in length, was performed to expose the left and right lobes of the rat liver. With a 27-gauge needle, a tumor cell suspension containing 4×10^7 cells in a volume of 0.1 ml was injected slowly into one of the hepatic lobes under the liver capsule, raising a visible pale wheal. The puncture site was gently compressed for 15 s with cotton gauze to prevent bleeding. Then, the wound was closed in layers. Two weeks after inoculation, laparotomy was performed to check tumor growth. Tumors in sizes from 15 mm to 20 mm were chosen for study.

2.3. Re-188 production

The radionuclide W-188 for the W-188/Re-188 generator was purchased from Oak Ridge National Laboratory, TN, USA. The W-188/Re-188 generator was manufactured by the Institute of Nuclear Energy Research. The eluate from the W-188/Re-188 generator was clear and colorless. The pH value of the eluate was in the range of 4~7.0. The radionuclide purity of Re-188 eluted from the generator was >99.9% as assessed by high-performance gamma-ray spectrometry (DSPEC jr2.0, ORTEC Co., Oak Ridge, TN, USA). The chemical purity of the aluminum content was <10 ppm as analyzed by an inductively coupled plasma optical emission spectrometer (ICP-OES) (Ultima 2, Jobin Yvon S.A., Longjumeau Cedex, France). The radiochemical purity of Re-188 perrhenate was >97% as assayed by a thin layer chromatography (TLC) system with silica-gel as the stationary phase and two kinds of developing solution (ethyl acetate and normal saline) [20,21].

2.4. Preparation of Re-188-MN-16ET/lipiodol

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 Re-188 was eluted with normal saline from a 188 W/188Re generator system manufactured by the Institute of Nuclear Energy Research in Taiwan. The detailed procedure for the synthesis of Re-188-MN-16ET/lipiodol was described in our previous study [14]. The radiochemical purity (RCP) of Re-188-MN-16ET was determined using radioisotope high-performance liquid chromatography (radio-HPLC) and radioisotope thin layer chromatography (radio-TLC). Radio-HPLC (Waters 600E, Waters Co., Milford, MA, USA) with a radiometric radiochromatography detector (Series A-100) was used to assess the RCP of Re-188-MN-16ET on a SiO_2 column eluted with the mobile phase (ethylacetate:methanol = 4:1) at a flow rate of 0.8 mL/min with a UV detector (Waters 486, Waters Co., Milford, MA, USA) set at 350 nm. The HPLC column used in this study was "LiChrosorb® Si 60 (5 μm) Hibar® RT 250-4" which was purchased from Meck Millipore. LiChrosorb® Si 60 is a reliable irregular shaped silica sorbents with a pore diameter of 60A for normal phase chromatography. This sorbent is packed into a Hibar® 250-4 HPLC column. In the radio-TLC system, instant thin layer chromatography-silica gel (ITLC-SG) was used as the stationary phase and developed with the same mobile phase as the radio-HPLC system. The chromatograms were analyzed by a radio-thin layer chromatography imaging scanner (AR2000, Bioscan, Inc., Washington, DC, USA).

Lipiodol was added to the reaction vial and the solution was vigorously shaken to extract the labeled Re-188-MN-16ET into the lipiodol phase. The vial was then centrifuged at 3000 rpm at room temperature for 10 min to separate the water and the lipiodol phase. The lipiodol phase containing lipophilic Re-188-MN-16ET was carefully collected to yield Re-188-MN-16ET/Lipiodol. The Re-188-MN-16ET extraction ratio by the lipiodol phase was >40%. The RCP of Re-188-MN-16ET/Lipiodol was determined by using the same method as mentioned above. The RCP of Re-188-MN-16ET/Lipiodol after the extraction procedure was analyzed to be >95% and remained stable for 24 hours.

2.5. Transcatheter arterial embolization

Under anesthesia with intraperitoneal injection of ketamine, a midline laparotomy was performed. The hepatic artery and the gastroduodenal branch were identified and isolated. A temporary sling was placed around the hepatic artery proximal to the gastroduodenal branch to prevent back flow. After ligation of the distal end of the gastroduodenal branch, this artery was cannulated with thin polyethylene tubing #10 (Clay Adams/Becton Dickinson and Company, NJ, USA). The tubing was secured in the vessel with a fine silk tie and connected to a syringe. Following injection of embolic materials in a volume of 0.1 ml, the cannula was flushed with 0.2 ml of saline, removed and the proximal end of the gastroduodenal branch was ligated. The sling around the hepatic artery was removed and hepatic arterial circulation was restored.

2.6. Tumor response and survival time

Twelve male rats bearing hepatic tumors were divided into three groups to evaluate the efficacy of treatment (four in each group). Group 1 received an intra-hepatic arterial injection of 7.4 MBq (0.2 mCi) of Re-188-MN-16ET-lipiodol; group 2 received epirubicin (0.5 mg/kg) and 0.1 ml of lipiodol emulsion; group 3 received 0.1 ml of normal saline and served as the control group. Tumor size was measured by liver sonography (LOGIQ Book, XPGeneral Electric Co., Fairfield, CN, USA) before injection, at two weeks, four weeks and eight weeks after injection. The maximum length and width of the