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Preparation and imaging of rhenium-188 labeled human serum albumin microsphere in orthotopic hepatoma rats

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HIGHLIGHTS

- The present study relates to a method for preparing ^{188}Re-labeled human serum albumin microspheres (HSAM) by ^{188}Re(I)-tricarbonyl ion($^{188}Re(OH_2)_3(CO)_3)^+$).
- This radioactive particle can be subjected to radioembolization for liver tumor.
- The method of ^{188}Re(I)-tricarbonyl ion labeled HSAM can proceed with high labeling yield.
- We believe this work is important and benefit for the future research and application of cancer radiotherapeutics.
- Furthermore, this method provided a convenient method for radio-labeling of HSAM with ^{188}Re as well as a kit for manufacturing.

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ABSTRACT

Objective: The present study relates to a method for preparing 188Re-labeled human serum albumin microspheres (HSAM) by 188Re(I)-tricarbonyl ion(188Re(OH2)3(CO)3)+). This radioactive particle can be subjected to radioembolization for liver tumor.
Methods: The particle sizes and conformations of HSA microspheres were analyzed by Particle sizes-Malvern mastersizer and Scanning Electron Microscope (SEM). For preparing 188Re(I)-tricarbonyl ion, the 188ReO4- was eluted from a 188W/188Re generator with saline. The radio labeling efficiency was analyzed with high-performance liquid chromatography (HPLC). Amino borane-reduced 188ReO4-was interacted with carbon oxide to form (188Re(OH2)3(CO)3]+). For preparing 188Re-HSA microspheres, the 188Re(I)-tricarbonyl ion was added into a vial with HSA microspheres. The in vitro stability was investigated. The rat was injected with 188Re-HSA microspheres via hepatic artery route. Nano-SPECT/CT Imaging was acquired after injection of 188Re-HSA microspheres.
Results: The shape of HSA microsphere was rough surfaced sphere or oval-shaped. The particle size was distributed between 20 and 35 μm. In the RP-HPLC-UV chromatography, the yield of 188Re(I)-tricarbonyl ion was 75–80%. The labeling efficiency of 188Re-HSA microspheres in this method was more than 85%. After incubation, the 188Re(I)-tricarbonyl ion labeled HSA microspheres were found to be stable in vitro in normal saline and rat plasma. The result of Nano-SPECT/CT Imaging quantification analysis indicated that the percentage of injection dose %ID was maintained at 95% ID-88% ID from 2 to 72 h after injection with 188Re- HSA microspheres.
Conclusions: The method of 188Re(I)-tricarbonyl ion labeled HSA microspheres can proceed with high labeling yield. Furthermore, this method provided a convenient method for radio-labeling of HSA microspheres with 188Re as well as a kit for manufacturing.

1. Introduction

Radioactive microspheres radioembolization offers promise for treatment of liver cancer. The radioactive microspheres are injected directly into the blood vessel feeding tumor through hepatic catheterization. The beta-radionuclide labeled microspheres can emit radiation for a very limited distance and surrounding normal

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tissues remain unaffected. For this purpose, variety of radioisotopes including rhenium-188 (^{188}Re) has been used for labeling appropriate particles. ^{188}Re is a radionuclide with a physical half-life of 16.9 h and has several advantages compared to other beta-emitting radionuclides. It emits beta particles with a maximum energy of 2.12 MeV and a 155 keV gamma ray, which are suitable for therapy and imaging (Wunderlich et al., 2005a). The HSA microspheres showed several advantages, including biodegradable, bio-compatible, non-toxic and non-antigenic. The highly mechanical stability and chemical stability of HSA microsphere are able to resist hydrolysis and radiolysis. So, HSA microsphere can be an ideal radionuclide carrier for liver cancer treatment.

Wang et al. (1998a), (1998b) described method for labeling resin microspheres with ^{188}Re. In this report, large amount of tin salt was used to radioactively labeling; however it may cause unexpected pharmacological effects. In addition, the acidify condition would hydrolyze proteins. Wunderlich et al. disclosed ^{188}Re labeled HSA microspheres (Wunderlich et al., 2005a; Wunderlich et al., 2005b; Wunderlich et al., 2010). In this report, the radiolabeling process was carried out by combination of the reductive reaction of Re(VII) with Sn(II) and a particle surface-related co-precipitation effect of tin hydroxid colloid with reduced, hydrolyzed rhenium. The labeling yield was significantly higher in this process, and the amount of tin salt used in reaction is limited. However, in vitro stability is not stable. The particle-bound radioactivity was decreased to 86% within 48 h at ambient temperature. Hence, ^{188}Re or tin chloride may release from microsphere surface and cause side effects.

In this study, the goal is to achieve the high labeling yield and stability of ^{188}Re HSA microspheres. The ^{188}Re(I)-tricarbonyl ion was introduced as a precursor for HSA microspheres radio-labeling. ^{188}Re was covalently bound to the surface of HSA microspheres. The stability of ^{188}Re-HSA microspheres were investigated in saline and rat plasma. The bioactivity study of ^{188}Re-HSA microspheres was performed by nano-SPECT/CT imaging via intra-arterial (i.a.) injection.

2. Materials and methods

2.1. Preparation of HSA microspheres

A 1.6% solution of human serum albumin (Sigma) was added dropwise to a 1000 mL flat-bottomed glass beaker, containing of refine olive oil (800 mL) during continuous stirring with a stirring bar putting in a magnetic heat plate. The HSA solution was reacted at 60–110 °C, continuous stirring with different speed. After removing all oil form HSA microspheres, the 200 mL acetone was added to wash free oil and dried with 40 °C. Finally the HSA microspheres, was filtered with 20–53 μm sieves.

2.2. Determination of particle size and conformation of HSA microspheres

The diffraction scatting angle of the particle is on proportion to the particle-size when a bunch of micron particles pass through a beam of light by means of Fraunhofer Diffraction method. The size of particles can be measured by detecting various intensities of diffracted beam superimposition of various diffraction angles with the photo-sensor. (Malvern) mastersizer 2000 was used to analyze HSA microspheres. HSA microspheres (10 mg/mL) were injected to measuring cell. The HSA microspheres suspension were formed particle flow by continuous cycle of flow and pass the light beam and measure the particle size. HSA microspheres were analyzed with Scanning Electron Microscope (HITACH S-4800 SEM). Proper amount of specimen is mounted on a specimen stub using electrically conductive double-sided adhesive tape. Specimen stub was putted into the chamber of SEM. Specimen was excited by secondary electron under vacuum environment. And then, the surface image of specimen can be projected on the screen. The voltage and current for measurement is 10 V and 2 A. The working distance width is 15 mm.

2.3. Preparation of ^{188}Re(I)-tricarbonyl ion

The processes for preparing ^{188}Re(I)-tricarbonyl ion were shown as following: CO gas kept flushing into vial containing 8 mg borane ammonia (NH_3BH_3). The vial was sealed and flushed with 1 atm CO gas for 15 min; the pressure of CO gas in the vial was sustained through a balloon flushed with CO gas inserted in the rubber stopper. $^{188}ReO_4^-$ was eluted from the ^{188}W/^{188}Re generator with saline. About 1 mL of sterilely filtered ^{188}Re perrhenate (1–20 mCi) dissolved in 0.9% NaCl and 7 μL 85% Phosphoric acid were injected into the vial with NH_3BH_3. The solution mixture in the vial was incubated in a shaking water bath at 95 °C for 20 min with 80 rpm. After cooling down to room temperature, the solution mixture was analyzed with high-performance liquid chromatography (HPLC). The Waters HPLC system using a RP C-18 column (Vydac 218TP, 10 μ, 250 × 4.6 mm^2) equipped with a radiometric detector. The eluent consisted of methanol and 0.05 M triethylammonium phosphate (TEAP) buffer pH 2.25. The gradient elution started with 100% of 0.05 M TEAP buffer from 0 to 5 min and switched at 6 min to 75% of 0.05 M TEAP buffer and 25% of methanol. At 9 min it switched to 66% of 0.05 M TEAP buffer and 34% of methanol, followed by a linear gradient program from 66% of 0.05 M TEAP buffer to 0% of 0.05 M TEAP buffer and 100% of methanol at 15 min. Then sustain 100% of methanol for 5 min. The flow rate was 1 mL/min.

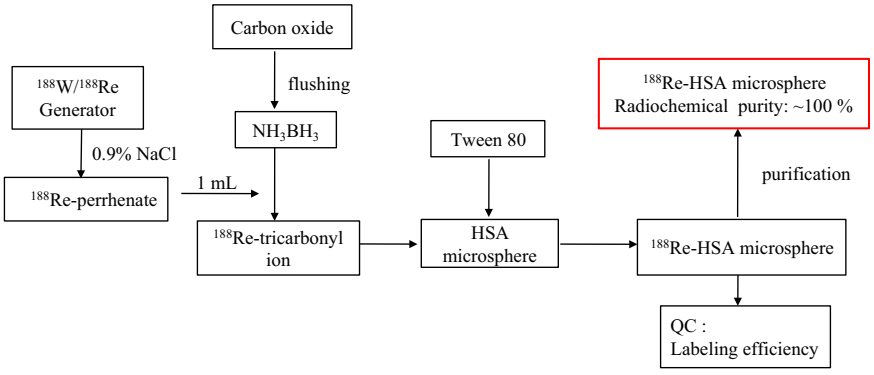

Fig. 1. Flow chat of ^{188}Re-HSA microsphere preparation.