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Biodistribution characteristics of ^{188}Re-TSC after TAE in rabbits bearing VX_2 liver tumor

ZHANG Bin[1,2,*] WU Yiwei[2] JIN Yonghai[3] LIU Yizhi[3] LUO Xianwen[2] FAN Wo[1]

[1] *School of Radiation Medicine and Public Health, Soochow University, Suzhou 215123, China*

[2] *Department of Nuclear Medicine, the First Affiliated Hospital of Soochow University, Suzhou 215006, China*

[3] *Department of Interventional Radiology, the First Affiliated Hospital of Soochow University, Suzhou 215006, China*

Abstract ^{188}Re-tin sulfur colloid (TSC) was prepared to compare its biodistribution characteristics with ^{188}Re-macroaggregates album (MAA) after transhepatic arterial embolization (TAE) in rabbits bearing VX_2 liver tumor. Labeling efficiencies of the ^{188}Re-TSC and ^{188}Re-MAA were 99.94%±0.04% and 99.95%±0.03%, respectively, and they were stable for 72 h in human serum. Sintigraphy and biodistribution in 31 rabbits bearing VX_2 liver tumor after transcatherter hepatic arterial injection of the radiopharmaceuticals were performed, and relevant activity accumulated mostly in the tumor. The percentage of the injected dose per gram of wet tissue (%ID/g) of ^{188}Re-TSC and ^{188}Re-MAA were calculated. Tumor uptake of ^{188}Re-TSC at 1 h and 24 h were 24.32%±11.93% and 21.88%±18.29%, and the radioactive ratios of tumor/liver were 70.89±19.58 and 17.42±13.96, respectively. Tumor uptake of ^{188}Re-MAA at 1 h and 24 h were 38.78%±30.23% and 15.98%±26.64%, and the radioactive ratio of tumor/liver of ^{188}Re-MAA were 39.71±25.06 and 8.13±4.61, respectively. ^{188}Re-TSC is a potential radiopharmaceutical for the therapy of tumors.

Key words ^{188}Re, Tin sulfur colloid (TSC), Transhepatic arterial embolization (TAE), Liver tumor

1 Introduction

Liver cancer is a common malignancy causing about one million deaths annually. Because of late diagnosis, over 85% of the cases are inoperable primary or metastasic liver cancer. In this case, the most important method is intervention therapy[1], by which hepatic tumor-feeding arteries are embolized under angiographic guidance. To enhance the treatment effect, adjunctive therapies using radioisotopes or chemotherapeutic agents are used.

Several β-emitters are possible candidates for intervention therapy[2], such as ^{131}I, ^{90}Y, ^{186}Re and ^{188}Re, among which ^{188}Re has outstanding physical properties for clinical use. With a desirable half-life of 16.9 h, ^{188}Re emits higher energy electrons (maximum 2.12 MeV, and averaged at 0.78 MeV) and 155 keV γ-ray, and enables adequate imaging and monitoring of therapeutic efficacy. It can be produced from a ^{188}W/^{188}Re generator, which offers practical clinical availability and economic advantage for routine applications[1].

It has been reported that ^{188}Re-4-hexadecyl-TDD(HDD)/lipiodol shows excellent targeting of liver cancer after transhepatic arterial embolization (TAE) in Phase I clinical trial[3] and another prospective multicenter clinical trial of ^{188}Re-HDD/lipiodol therapy for hepatocellular carcinoma (HCC)[4]. But ^{188}Re-HDD/lipiodol is more a *chemical* embolization agent than *physical* embolization agent, and low radiochemical yield of the ^{188}Re-HDD/lipiodol labeling procedure is a drawback to implementing high-activity treatment routinely[5]. ^{188}Re-human serum albumin (HSA) microspheres and ^{188}Re-sulfur colloid (SC) have also been reported[6,7]. Transarterial radionuclide therapy (TART) appears to be a safe, effective, and promising therapeutic option in patients with inoperable primary or metastasic liver cancer.

* Corresponding author. *E-mail address:* zbnuclmd@126.com

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In this work, ^{188}Re-tin sulfur colloid (TSC) was synthesized, and biodistribution characteristics of ^{188}Re-TSC and ^{188}Re-macroaggregates album (MAA) after transhepatic arterial embolization in rabbits bearing VX_2 liver tumor were studied.

2 Experimental

2.1 Preparation of ^{188}Re-TSC

The direct method was adopted to label TSC with ^{188}Re-perrhenate eluted from a ^{188}W/^{188}Re generator (Beijing Atom Hightech Co., Ltd, China). Stannous chloride and sodium thiosulphate (Fluka, Germany) were added into a vial of sulfur colloid drug kit (SC kit), which was used to synthesize ^{99m}Tc-SC, containing 2.0 mg $NaS_2O_3{\cdot}5H_2O$, 2.3 mg $Na_2EDTA{\cdot}2H_2O$, and 18.1 mg gelatin. Then, ^{188}Re-perrhenate (1.0 mL, 185 MBq) was added to the vial. After adjusting the pH with 1.5 mL 0.148 N HCl, the mixture was boiled for 30 min in water bath. The labeled ^{188}Re-TSC was neutralized to pH = 6.7–7.0 by adding 1.5 mL of sodium phosphate buffer (38.78 mg NaH_2PO_4 and 11.10 mg NaOH). The stannous chloride and sodium thiosulphate were added into the SC kit of 0.125–1.25 mL or 0.25–1.0 mL. Radiolabeling efficiency was determined by Xinhua 1# paper chromatography (PC) using acetone as the mobile phase. Free ^{188}Re migrated with the solvent front while ^{188}Re-TSC complex remained at the origin. The strip was analyzed with a NaI(Tl) scintillation counter or an AR-2000 imaging scanner (Bioscan Inc, USA) to plot activity against the strip length. Particle size of the ^{188}Re-TSC were measured (n=32) by laser scatterance/ diffraction method on a Mastersizer 2000 (Malvern, UK).

2.2 Preparation of ^{188}Re-MAA

Macroaggregated albumin (MAA) was labeled with ^{188}Re-perrhenate after adding 0.5 mL stannous chloride (20mg/mL) into commercial MAA kit, which was used to prepare ^{99m}Tc-MAA. The time-variation labeling efficiency was measured to evaluate the stability of ^{188}Re-MAA. Labeling efficiency was determined by PC using acetone as the mobile phase. Labeling efficiency was checked by same method with ^{188}Re-TSC.

The *in vitro* stabilities of ^{188}Re-TSC and ^{188}Re-MAA were analyzed in sodium chloride or human serum (v:v=1:10), and the radiochemical purity was determined by PC analysis from 30 min to 72 h.

2.3 The animal model

To establish a model of VX_2 carcinoma, a piece of VX_2 tumor tissue was implanted into the left lobe of the rabbit liver *via* percutaneous puncture under the guide of CT. Thirty-two New Zealand white rabbits were established models of VX_2 liver tumor. The CT and DSA imaging manifestation of the VX_2 liver tumor was analyzed to observe the tumor size. One rabbit bearing VX_2 liver tumor was selected to observe the biological features of tumor by histopathology and microscope photographs.

2.4 Animal experiments

2.4.1 TAE and Planar scintigraphy

Three weeks after tumor implantation, the rabbits were injected in the hepatic arterial with ^{188}Re-TSC (n=12), ^{188}Re-MAA(n=15), or $Na^{188}ReO_4^-$ solution (n=4). Briefly, under general anesthesia, the femoral artery was exposed and the left hepatic artery was catheterized. After injecting the radiopharmaceuticals, the catheter was removed and the femoral artery was ligated.

Planar scans with IRIX3 SPECT (PHILIPS, the Netherland) were obtained at 1 and 24 h after administration. Planar scintigraphy was performed, with the energy window being centered at 155 keV and opened by ±10%. Images were acquired with 4.0×10^5 counts on a 256×256 matrix equipped with a parallel-hole collimator for low-energy and high-sensitivity imaging. Regions of interest (ROI) were drawn around the tumor and liver images, and in the whole-body image.

2.4.2 Biodistribution

The biodistribution characteristics of the ^{188}Re-TSC, ^{188}Re-MAA, and ^{188}Re-perrhenate in rabbits were evaluated at 1 and 24 h after administration. Organs were excised, washed with saline, weighed, and counted on a gamma counter. Organs of interest included tumor, blood, brain, heart, spleen, lungs, liver, kidneys, muscle and intestine. The organ uptakes were

calculated as a percentage of the injected dose per gram of wet tissue (%ID/g). Tumor retention was calculated and compared between ^{188}Re-TSC and ^{188}Re-MAA. The experiments complied with the ethics approval and laws of China.

2.5 Statistical analysis

The results are expressed as mean ±SD. SPSS13.0 software was used for statistical analysis, taking $p<0.05$ as the level of significance. The results were analyzed using the one-way ANOVA test or student's *t*-test.

3 Results and discussion

3.1 Labeling efficiencies and particle size

Radiolabeling efficiency of the ^{188}Re-TSC increased with the volume of stannous chloride (20mg/mL), approaching over 95% (Fig.1). No significant difference among 0.5, 1.0, and 1.25 mL of stannous chloride volume ($p>0.05$). Radiolabeling efficiency of the ^{188}Re-TSC decreased with increasing volume of sodium thiosulphate (Fig.2), and the radiolabeling efficiency of 0 and 0.25 mL of sodium thiosulphate volume did not differ significantly ($p>0.05$). When 0.5 mL stannous chloride and 0.25 mL sodium thiosulphate were added into sulfur colloid kit, labeling efficiency of the ^{188}Re-TSC was 99.94%±0.04%. When 0.5 mL stannous chloride was added into MAA kit, labeling efficiency of the ^{188}Re-MAA was 99.95%±0.03%. Particle size of the ^{188}Re-TSC was 10.83±6.60 μm (D10), 44.91±14.46 μm (D50), and 235.29±126.61 μm (D90). Because the particle size affects the uptake and retention of ^{188}Re-TSC in particular organs, we changed the radiolabeling procedure of ^{188}Re-tin colloid and ^{188}Re-SC by a new method to label TSC with ^{188}Re[8,9]. It was stable with high labeling efficiency. The mean particle size of ^{188}Re-TSC was 64 μm (Fig.3), being larger than particle size of ^{188}Re-tin-colloid and ^{188}Re-SC reported in Ref.[7]. The particle size of sulfur colloid labeled with ^{99m}Tc is generally less than 1 μm, and the MAA is 10–60 μm. The diameter of ^{188}Re-HSA microspheres was about 25 μm, which could be "physical" embolization agents to embolize the capillary vascular plexus. When ^{188}Re-SC was used to treat melanoma-bearing animals, there was a significant increase in survival time with increasing amount of the larger-particle-size colloid[10].

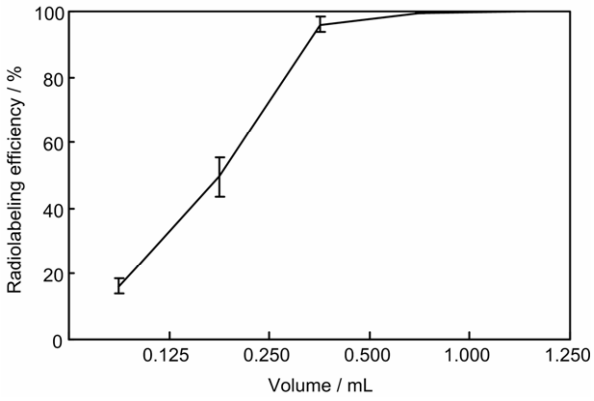

Fig.1 Labeling efficiency of the ^{188}Re-TSC vs. volume of $SnCl_2 \cdot 2H_2O$ (20mg/mL).

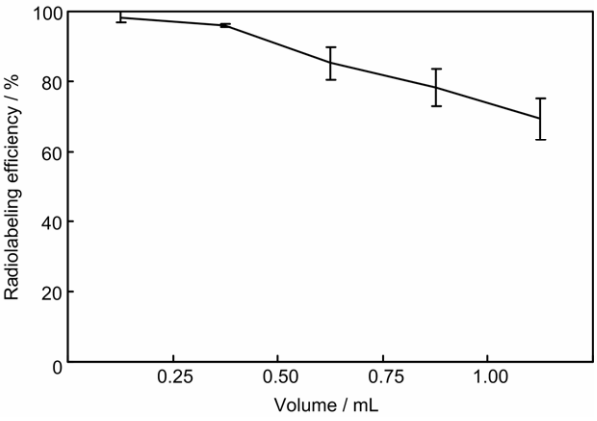

Fig.2 Labeling efficiency of the ^{188}Re-TSC vs. volume of sodium thiosulphate (8 mg/mL).

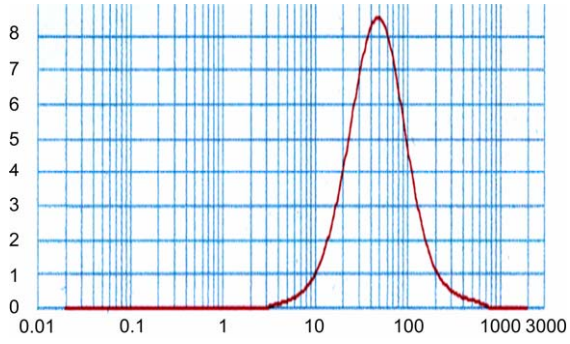

Fig.3 Particle size distribution of the ^{188}Re-TSC.

3.2 Stability

^{188}Re-TSC could be stored for 72 h at room temperature without observable decrease of radiochemical purity. We found that the ^{188}Re-TSC and ^{188}Re-MAA were stable either in human serum or in saline solution at room temperature for 72 h (Table.1). Radiochemical purity of ^{188}Re-TSC stored for 72h in Human serum was higher than that of ^{188}Re-MAA.

Table 1 Radiochemical purity in medium at different time point (%)

Samples	Medium	0.5 h	4 h	24 h	48 h	72 h
^{188}Re-TSC	non	99.94±0.04	99.87±0.05	99.86±0.05	99.63±0.20	99.61±0.17
	Saline solution	96.50±0.12	96.77±0.54	94.77±1.33	94.28±1.15	93.23±0.64
	Human serum	99.49±0.21	99.06±0.11	99.23±0.88	98.93±0.72	95.77±0.54
^{188}Re-MAA	non	99.95±0.03	99.80±0.18	98.87±0.97	97.08±1.10	93.27±3.95
	Saline solution	99.92±0.08	99.60±0.22	96.28±0.83	95.58±0.79	92.53±1.20
	Human serum	99.90±0.07	99.52±0.22	96.43±0.95	95.44±0.91	92.13±1.21

3.3 Planar imaging

Thirty-two rabbits were implanted with VX_2 tumor in the liver by paunching, and the tumors demonstrated by hypodensity or isodensity on plain CT scans meanwhile peripheral rim enhancement on contrast enhanced arterial phase images. In 17 of the 27 rabbits, TAE was performed successfully. The radioactive ratio of tumor/liver in region of interesting (ROI) of SPECT images was 2.15±0.80 and the tumor can be seen clearly in planar imaging (Fig.4). The tumor in other 10 rabbits couldn't be differentiated from the liver on planar images of SPECT.

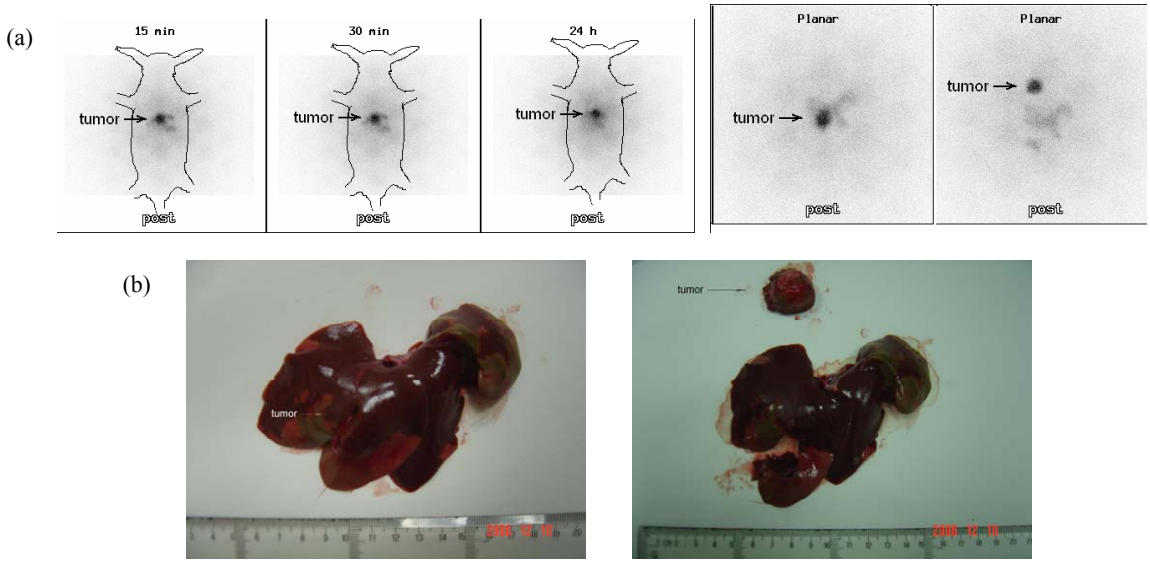

Fig.4 Serial planar images of ^{188}Re-TSC in rabbits after TAE and the scintigraphy images of liver and tumor *in vivo* and *in vitro*, respectively (a) and the liver and tumor *in vitro* (b).

3.4 Biodistribution

Biodistribution of the ^{188}Re-TSC, ^{188}Re-MAA, and $^{188}ReO_4^-$ in rabbit after TAE are shown in Table 2. At 1 h and 24 h after injection, the %ID/g ratio of tumor/liver were respectively 70.89 ± 19.58 and 17.42 ± 13.96 with ^{188}Re-TSC, and 39.71 ± 25.06 and 8.13 ± 4.61 with ^{188}Re-MAA. Retention ratio of the ^{188}Re-TSC for 24 h to 1 h in tumor was 89.95%, being higher than 41.22% of the ^{188}Re-MAA. At 24 h after injection, the tissue accumulation is in the sequence of tumor (21.88%), liver (1.41%), kidney (1.07%), spleen (1.00%), and lung (0.11%). Because direct administration of radioactivity to the lesion is involved, the radioembolization in TAE depends considerably on skill of an operator. In this study, the radioactive ratio of tumor/liver appears so different, because the rabbit TAE model has several technical difficulties uncommon in humans, such as severe vasospasm, spillover of the agent from the selected artery, and intratumoral arteriovenous shunts. Moreover, minute selection of the tumor-feeding artery was nearly impossible because of the small diameter of the artery. In human TAE, however, well trained interventional

radiologists can easily select the feeding artery. Therefore, the radioactivity should be delivered more efficiently in humans[1]. Administration of the radiopharmaceutical as close as possible to the tumor feeding arteries might avoid further deterioration of the liver function and show enhanced antitumoral activity.

Table 2 Biodistribution data of ^{188}Re-TSC, ^{188}Re-MAA, and $^{188}ReO_4^-$ in rabbits after TAE

Organ	$^{188}ReO_4^-$	^{188}Re-TSC		^{188}Re-MAA	
	1h(n=4)	1h(n=3)	24h(n=5)	1h(n=5)	24h(n=4)
Blood	0.390±0.136	0.130±0.009	0.104±0.144	0.130±0.072	0.030±0.006
Lung	0.332±0.066	0.135±0.038	0.107±0.084	0.393±0.690	0.123±0.117
Brain	0.013±0.005	0.006±0.001	0.018±0.013	0.019±0.021	0.017±0.014
Heart	0.216±0.041	0.063±0.008	0.040±0.021	0.087±0.088	0.039±0.025
Muscle	0.100±0.039	0.030±0.026	0.082±0.062	0.036±0.041	0.065±0.077
Intestine	0.102±0.050	0.054±0.035	0.051±0.042	0.043±0.028	0.054±0.051
Kidney	0.439±0.091	0.307±0.081	1.067±1.169	0.664±0.410	1.469±0.933
Spleen	0.153±0.015	0.266±0.308	0.999±1.139	1.379±1.869	0.121±0.093
Liver	0.335±0.189	0.391±0.266	1.413±0.598	1.719±2.110	1.536±1.824
Tumor	0.743±0.499	24.318±11.931	21.875±18.290	38.781±30.233	15.984±26.641
T/Liver	2.23±0.95	70.89±19.58	17.42±13.96	39.71±25.06	8.13±4.61
T/Blood	2.14±1.92	183.07±82.35	726.15±505.10	347.15±164.37	473.59±79.20
T/Kidney	1.73±1.31	80.27±41.27	59.27±16.58	86.99±71.85	11.64±9.12
T/Lung	2.06±0.97	205.66±135.64	299.92±74.03	401.93±231.10	88.76±39.69
T/Spleen	4.67±2.89	169.95±147.30	102.26±61.54	570.04±405.50	265.39±239.90
T/Muscle	8.49±6.08	2764.9±351.53	685.29±442.08	1729.91±849.02	746.61±461.70

The injected dose was corrected by time.

4 Conclusions

The results indicate that this method of synthesizing ^{188}Re-TSC is stable and high labeling efficiency can be obtained. The tumor uptake of ^{188}Re-TSC and ^{188}Re-MAA were high and the tumor retention of ^{188}Re-TSC significantly improved compared with that of ^{188}Re-MAA. ^{188}Re-TSC is a potential radiopharmaceutical for the therapy of tumors.

Considering that many therapeutic details have not been studied, it is necessary to investigate further whether TAE with ^{188}Re-TSC improves the survival of tumor-bearing animals and is a safe, effective, and promising therapeutic option in patients with inoperable primary or metastasic liver cancer.

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* Corresponding author. *E-mail address:* zbnuclmd@126.com

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