

Direct *in vivo* injection of ^{131}I -GMS and its distribution and excretion in rabbit

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Abstract

AIM: To explore the distribution and metabolism of ^{131}I -gelatin microspheres (^{131}I -GMSs) in rabbits after direct injection into rabbits' livers.

METHODS: Twenty-eight healthy New Zealand rabbits were divided into seven groups, with four rabbits per group. Each rabbit's hepatic lobes were directly injected with 41.336 ± 5.106 MBq ^{131}I -GMSs. Each day after ^{131}I -GMSs administration, 4 rabbits were randomly selected, and 250 μL of serum was collected for γ count. Hepatic and thyroid functions were tested on days 1, 4, 8, 16, 24, 32, 48 and 64 after ^{131}I -GMSs administration. Single-photon emission computed tomography (SPECT)

was taken for each group on days 0, 1, 4, 8, 16, 24, 32, 48, 64 after ^{131}I -GMSs administration. A group of rabbits were sacrificed respectively on days 1, 4, 16, 24, 32, 48, 64 after ^{131}I -GMSs administration. Their livers were taken out for histological examination.

RESULTS: After ^{131}I -GMSs administration, the nuclide was collected in the hepatic area with microspheres. The radiation could be detected on day 48 after ^{131}I -GMSs administration, and radiography could be seen in thyroid areas in SPECT on days 4, 8, 16 and 24. One day after ^{131}I -GMSs administration, the liver function was damaged but recovered 4 d later. Eight days after ^{131}I -GMSs administration, the levels of free triiodothyronine and free thyroxine were reduced, which restored to normal levels on day 16. Histological examination showed that the microspheres were degraded to different degrees at 24, 32 and 48 d after ^{131}I -GMSs administration. The surrounding parts of injection points were in fibrous sheathing. No microspheres were detected in histological examination on day 64 after ^{131}I -GMSs administration.

CONCLUSION: Direct *in vivo* injection of ^{131}I -GMSs is safe in rabbits. It may be a promising method for treatment of malignant tumors.

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Key words: ^{131}I ; Label; Gelatin microspheres; Animal; Rabbit; Hepatic; Direct injection

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INTRODUCTION

The treatment of malignant tumors in hepatic, biliary and pancreatic systems has been and will always be a tough and key part of general surgery^[1-3]. Surgical removal has been considered a positive approach for most of malignant tumors in these systems^[4]. But the success rate of surgical removal is very low and the prognosis after operation is unsatisfactory^[5-8]. For example, the number of cases of primary hepatic carcinoma in China was around 384 119 in 2005^[9-12] and the number of deaths was 357 624^[13-15], only 10%-30% are related to surgical removal, and 25% of them survived over 5 years^[16]. The number of cases of pancreatic cancer in America was around 42 470 and the number of death was around 35 240 in 2009^[17]. Surgical procedure has much limitations for treatment of malignant cancers. Many patients with malignant tumors in the three systems at middle to advanced stages are in the urgent need of new and effective non-surgical treatment.

Radionuclide labeled microspheres and seeds are the new progress made in the area of tumor therapy and interventional radiotherapy. It has now become a fast developing sub-field of medicine combining nuclear medicine and oncotherapy. Nuclide microspheres under study over recent years include ⁹⁰Y glass and resin microsphere^[18-20], ¹⁸⁸Re glass microsphere^[21], ³²P glass microsphere^[22,23] and ¹⁶⁶Ho glass microsphere^[24]. The ⁹⁰Y glass microsphere is being increasingly recognized by the medical community as an important strategy for the treatment of primary and secondary neoplasm, which was officially approved in 1999 in America and Canada for treatment of malignant tumors. Now hundreds of publications have described the treatment of ⁹⁰Y glass microsphere^[25]. The therapeutic practices in thousands of patients with liver cancer in about 80 medical centers around the globe show that hepatic arterial injection of ⁹⁰Y glass microsphere is a safe and effective method for liver cancer treatment. Carr reports a group of clinical control study involving 65 cases of primary liver cancer. Forty-two cases are Okuda stage 1 with a median survival of 649 d (244 d for the control group), 23 are Okuda stage 2 with a median survival of 302 d (64 d for the control group)^[26]. Salem reports another clinical study involving 49 cases of liver cancer with a median survival of 85.9 mo. Twenty-eight patients reached the condition for tumor removal after the treatment and had their tumors removed, and 13 patients had tumor necrosis after the treatment. Their survival rate for 1, 3 and 5 years are 98%, 64% and 57%, respectively^[27]. Intratumoral injection of ⁹⁰Y glass microsphere has also been studied. Tian reported that the tumor was reduced by 92% in 27 cases of primary liver cancer and 6 cases of metastatic liver cancer. α -fetoprotein in 13 cases recovered entirely after intratumoral injection^[28]. These studies showed similar clinical therapeutic efficacy of ⁹⁰Y glass microsphere to that of surgical procedure. The ¹²⁵I-seeds used for prostate cancer in Europe and America has

been tried in intratumoral implantation in China to treat advanced pancreatic carcinoma, which has demonstrated effects in alleviating pains and prolonging life^[29-31]. In conclusion, directional radiotherapy using nuclide microsphere or nuclide particle is a potential alternative to treat malignant tumors in hepatic, biliary and pancreatic systems.

China is a country with a large population in the world and has the highest incidence and death rate of primary liver cancer. And the incidence of biliary and pancreatic malignant tumors is increasing. However, most of the patients with middle to advanced stage cancers of the three systems cannot be treated with surgery. Therefore, directional radiotherapy using nuclide microsphere will significantly improve the prognosis of patients with middle to advanced stage cancers. Since ⁹⁰Y glass microspheres have to be activated in an accelerator to get radioactivity, and when they are activated, they have relatively short half-life. We have been studying the nuclide microspheres for local brachytherapy for hepatic, biliary and pancreatic malignant tumors. In the 1990s, we developed the ³²P glass microsphere with relatively long half-life, and treated 40 patients with advanced liver cancer from 1992 to 1994 after the completion of the metabolism tests *in vivo* in tumor-carrying animals^[32]. In recent years, we have developed the gelatin microspheres (GMSs) with a diameter of 50-70 μ m carrying a high concentration of ¹³¹I to treat patients with advanced liver cancer with hepatic arterial transfusion and embolotherapy. Since its half-life is 8.04 d and free ¹³¹I in the body is either collected in thyroid tissue or discharged out of the body quickly, ¹³¹I is safe and has relatively weak influence on other tissues^[33-35]. And the GMS is one of the degradable biomaterials with good biocompatibility, which can also bind ¹³¹I nuclide at a high concentration^[36]. So in this study, we prepared ¹³¹I-GMSs with a diameter of 10-30 μ m for intratumoral implantation, which is expected to be easily applied for the hepatic, biliary, pancreatic and other malignant tumors that cannot be removed. Healthy rabbits are used as models to observe the metabolism of ¹³¹I-GMSs *in vivo* and the tissue reaction in their livers.

MATERIALS AND METHODS

Materials

Lime-processed gelatin (sigma G-9382) with an isoelectric point of 4.8-5.2 was purchased from Sigma Co. Ltd., USA; ¹³¹I-sodium-iodine solution (37 GBq/mL) was purchased from China Nuclear Group Chengdu Gaotong Isotope Co. Ltd., Chengdu, China. All other chemicals were of the highest commercially available purity.

Laboratory animals

This study was approved by the Animal Ethics Committee of Sichuan University. Twenty-eight healthy New Zealand rabbits weighing 1.8-2.5 kg were supplied by the animal experimental center of the Medical School of

Sichuan University and were divided into seven groups (groups 1-7), with four rabbits per group. Half of the rabbits were female and half were male. The rabbits were fed with a particulate (3-5 mm) chow and housed in a layered stainless steel coop. Rabbits had *ad libitum* access to running water. The air humidity and temperature were maintained at 50%-70% and 20-29°C, respectively. Eight days before the operation, four rabbits were randomly selected as control animals to collect serum from their hearts for the measurement of the liver and thyroid function.

Preparation of GMSs

GMSs were produced according to the modified method of Tabata *et al.*³⁷. Briefly, 10 mL of 10% lime-processed gelatin solution was added dropwise while stirring, to 80 mL of liquid paraffin (Kelong Chemical Reagent Co. Ltd., Chengdu, China), which was preheated to 55°C with 0.8 mL span-80 (Shenyu Chemical Reagent Co. Ltd., Chongqing, China). The mixture was then stirred at 550 r/min at 55°C for 15 min to yield a water-in-oil emulsion. The stirring was then continued for 30 min at 4°C. Next, 3 mL of glutaraldehyde (25%, Kermel Chemical Reagent Co. Ltd., Tianjin, China) was added to the mixture after it cooled for 5 min to induce crosslinking and solidification of the microspheres. The resulting microspheres were removed by suction filtration and washed three times with acetone (Changlian Chemical Reagent Industries, Ltd., Chengdu, China) after dehydration by immersion in 30 mL of acetone for 15 min. After air-drying, the GMSs were examined by the Analyzing and Testing Center of Sichuan University and imaged under a scanning electron microscope.

Preparation of ¹³¹I-GMS

The ¹³¹I was labeled by a modification of the chloramine-T method. Briefly, 50 mg of GMSs were placed in test tubes, rehydrated with 190 μL of phosphate-buffered saline (pH 7.0) for 10 min. Next, 3.4 μL of ¹³¹I-sodium-iodine solution (37 GBq/mL) and 200 μL of chloramine-T solution (Bodi Chemical Reagent Co. Ltd., Tianjin, China) (20 mg/mL) were added to each test tube. After the reaction mixture had been incubated for 5-15 min at room temperature, with agitation, 200 μL of sodium pyrosulfite solution (Jiangbei Chemical Reagent Industries, Ltd., Wuhan, China) (15 mg/mL) was added to stop the reaction. The mixture was centrifuged (Eppendorf® 5702R refrigerated centrifuge, Eppendorf Co. Ltd., Hamburg, Germany) at 4400 r/min for 4 min to separate the ¹³¹I-GMSs. Finally, the products were washed seven times with normal saline and sterilized by Co60 irradiation.

Implantation of ¹³¹I-GMS in the liver

Each rabbit was injected intramuscularly with 0.1-0.2 mL/kg SuMianXin® (Veterinary Institute of the Chinese Military Academy of Medicine, Changchun, China) veterinary injection for anesthesia and placed on an animal

operation table. The epigastrium was shaved, disinfected, and covered with a drape. The abdomen was opened with a 5-cm incision along the median line, and the left middle lobe or right middle lobe of the liver was fixed by the left index finger and thumb of the operator. Then, the mixture of 41.336 ± 5.106 MBq of the ¹³¹I-GMSs, as measured in a Capintec® CRC-15R (Capintec, Inc., New Jersey, USA) dose calibrator, and 1 mL of 25% glucose solution (Kelun Pharmaceutical Industries, Ltd., Chengdu, China) drawn by a 1 mL syringe with a 4-G needle was slowly injected into the liver, after checking the needle was not inserted into a capillary. Two or three injections of 0.3-0.5 mL of the mixture were administered. When the injection points were not observed to be bleeding after being pressed for approximately 1 min after withdrawing the needle, the abdomen was closed and the operation was finished. The rabbit was immediately administered intramuscularly with 40 000 U gentamycin sulfate (Tianjin Pharmaceutical Co. Ltd., Tianjin, China).

Radioassay of rabbit serum

Four rabbits were chosen randomly for collecting 1 mL of blood from the ear veins between 0 and 24, then at 28, 32, 48 and 64 d after the administration of the GMSs. Those blood were centrifuged at 4400 r/min, and 250-μL aliquots of serum were used for γ counting in a γ counter (No. 262 Industry, Ltd., Xi'an, China).

Single-photon emission computed tomography, hepatic and thyroid functions examinations

The single-photon emission computed tomography (SPECT) scan (Skylight SPECT Camera, Philips Co. Ltd., Amsterdam, Netherlands) was conducted by the Nuclear Medicine Department of West China Hospital at 4 h, and at 1, 4, 8, 16, 24, 32, 48 and 64 d after administration. The animals in group 1, 2, 3, 4, 5, 6 and 7 were sacrificed at 1, 4, 16, 24, 32, 48 and 64 d after the surgery, respectively. Eight milliliters of blood was collected from each deceased animal. On day 8, four rabbits were randomly selected, and a further 8 mL of blood was collected from the heart of each rabbit on this day. Blood samples were used to assess hepatic and thyroid functions, which was conducted at the Biochemical Laboratory and the Hormonal Laboratory of the Experimental Medicine Department of West China Hospital.

Histological examination

Liver samples were fixed in 10% formalin solution (Kelong Chemical Reagent Co. Ltd., Chengdu, China) for 48 h. Liver samples were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) for histological examination.

Statistical analysis

Results were expressed as mean ± SD and were analyzed by *t* tests using SPSS 11.5 software. The level of significance was regarded at a *P* value of < 0.05.

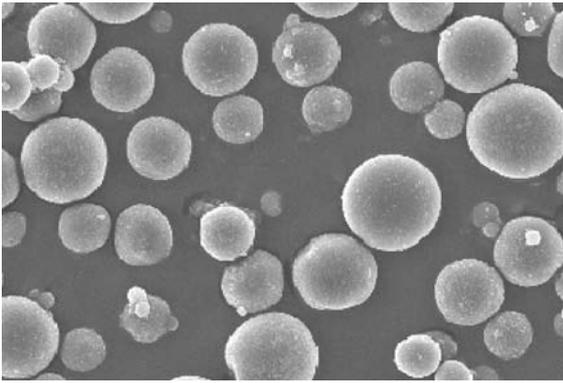


Figure 1 Scanning electron microscopic (20 kV) images of metal-coated gelatin microspheres (original magnification, × 500).

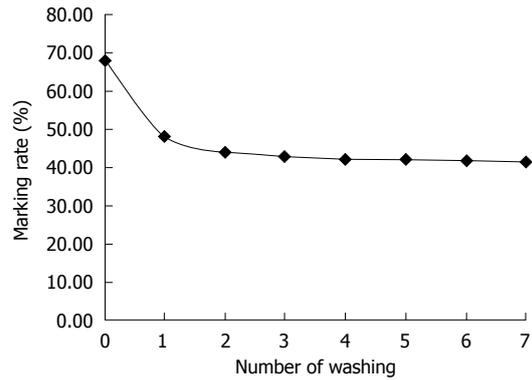


Figure 2 The labeling rate decreases with increasing the number of washes. The slope decreased gradually, nearly reaching a straight line, which demonstrates that the ¹³¹I-labeled gelatin microspheres after washing contained very low amounts of nuclides conjugated by physical adsorption.

Table 1 Effect of washing on labeling rate (n = 6)

Number of washes	Labeling rate (%)
0	68.01 ± 2.09
1	47.74 ± 2.26
2	43.68 ± 2.19
3	42.72 ± 2.23
4	42.22 ± 2.27
5	41.91 ± 2.28
6	41.56 ± 2.27
7	41.00 ± 2.29

Table 2 Ratio of radioactivity between the liver and thyroid (n = 6) after the administration of ¹³¹I-labeled gelatin microspheres

Time	Liver/thyroid
4 h	15.910 ± 0.740
Day 4	27.197 ± 5.467
Day 8	81.467 ± 24.637
Day 16	91.670 ± 23.278
Day 24	93.601 ± 21.337
Day 32	112.608 ± 12.787
Day 48	162.875 ± 7.955

RESULTS

Morphology of the microspheres

The GMSs were uniform in appearance, with a diameter of 10-30 μm, and a good divergence (Figure 1).

Effect of washing on GMS ¹³¹I content

The GMSs labeled with ¹³¹I were washed seven times to inhibit physical adsorption. Then, 50 mg of the GMSs labeled with ¹³¹I showed decreased radioactivity levels with increasing number of washes. The slope for the relative ¹³¹I content decreased gradually until it nearly reached a straight line (Table 1 and Figure 2).

Findings of SPECT imaging

The rabbits showed normal behaviors after the operation. SPECT imaging showed that the radioactive nuclide was concentrated in the liver, in regions surrounding the site of injection at 4 h and at 1, 4, 8, 16, 24, 32 and 48 d after ¹³¹I-GMSs administration. However, SPECT did not reveal any nuclide labeling on day 64. The thyroid also showed low levels of nuclide accumulation on days 4, 8, 16 and 24. Furthermore, there was faint nuclide labeling in the bladder of four rabbits before day 24, although this disappeared by day 32. SPECT imaging showed no accumulation of nuclide in other tissues, including the lung, heart, stomach, intestines and kidney in any rabbits for the entire observation period (Figure 3). The radioactive ratios between the injected parts of liver and thyroids was assessed by region of interest analysis and increased

with time, from 15.91 ± 0.74 at 4 h after ¹³¹I-GMSs administration to 162.875 ± 7.955 at day 48 (Table 2).

Radioactive changes in serum

According to the serum γ counts, the radioactivity level decreased markedly over the first 2 d after ¹³¹I-GMSs administration. The decline in radioactivity continued to decline thereafter, but at a slower rate until day 24. At this time, there was no difference in relative radioactivity level compared with the background level (Figure 4).

Hepatic and thyroid function

The levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) increased rapidly, particularly that of ALT, within 1 d after ¹³¹I-GMSs administration. The levels of these enzymes then decreased gradually, reverting to the normal level by day 4. The values of alkaline phosphatase and γ-glutamyltransferase were relatively stable. Similarly, the total protein, albumin and globulin did not change markedly during the study period (Table 3 and Figure 5).

The levels of free triiodothyronine (FT3) and free thyroxine (FT4) were significantly decreased on day 8 (*P* < 0.05), but returned to normal levels at day 16, and remained at the normal level until day 64 (Table 4 and Figure 6). The level of thyrotropic-stimulating hormone (TSH) remained < 0.005 mU/L throughout the study.

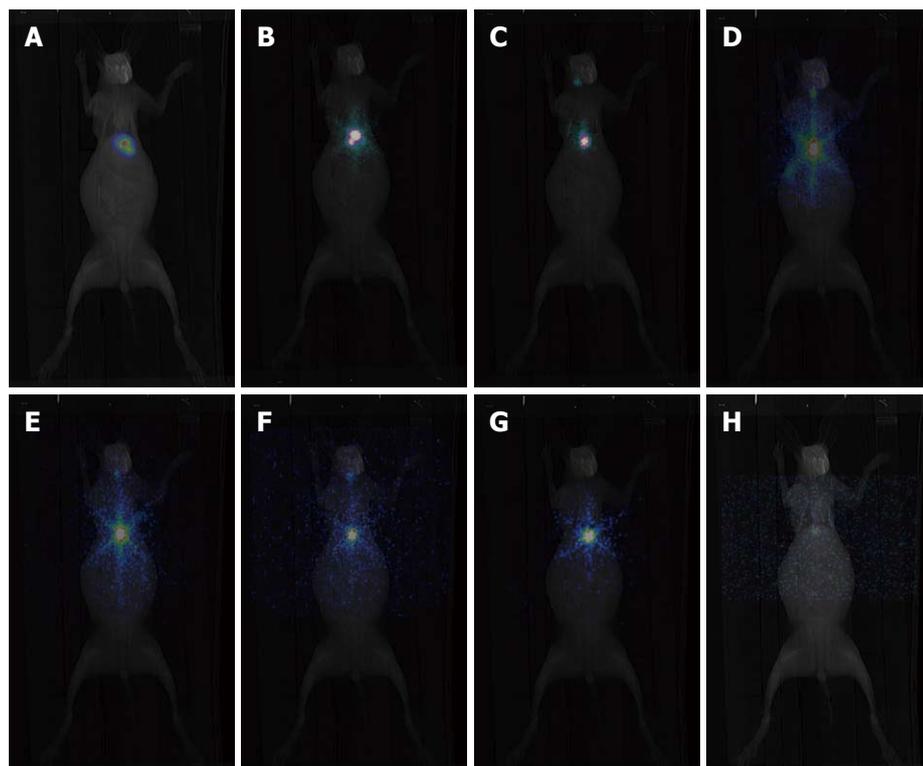


Figure 3 Single-photon emission computed tomography (SPECT) imaging performed at 4 h (A) and 1 (B), 4 (C), 8 (D), 16 (E), 24 (F), 32 (G) and 48 (H) d after the administration of ^{131}I -labeled gelatin microspheres.

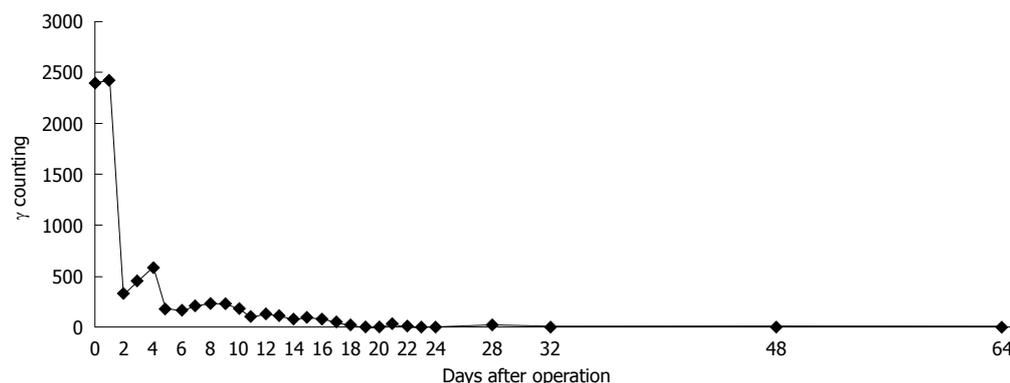


Figure 4 Dynamic changes in serum γ count after the administration of ^{131}I -labeled gelatin microspheres.

Table 3 Effects of the administration of ^{131}I -labeled gelatin microspheres on hepatic function ($n = 4$)

	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	GGT (IU/L)	TP (g/L)	ALB (g/L)	GLB (g/L)
Before administration	67.75 ± 11.87	55.00 ± 11.87	99.75 ± 14.99	6.00 ± 1.83	54.33 ± 3.16	46.05 ± 2.32	8.28 ± 2.79
After administration							
1 d	291.50 ± 12.79	146.00 ± 13.88	115.00 ± 25.92	9.25 ± 3.30	60.23 ± 1.96	45.65 ± 5.17	15.00 ± 5.34
4 d	46.75 ± 4.79	41.25 ± 10.69	91.00 ± 29.59	7.25 ± 1.26	58.43 ± 8.45	47.55 ± 3.53	12.23 ± 5.03
8 d	48.50 ± 14.39	48.00 ± 17.32	79.75 ± 14.77	7.50 ± 0.58	58.10 ± 4.40	44.63 ± 2.33	13.65 ± 4.33
16 d	37.25 ± 9.32	39.75 ± 11.81	76.00 ± 9.83	9.50 ± 3.11	62.13 ± 5.84	44.38 ± 7.41	19.85 ± 6.79
24 d	46.50 ± 16.01	45.25 ± 9.95	96.25 ± 17.08	8.00 ± 2.83	60.28 ± 4.45	47.60 ± 7.38	14.50 ± 3.54
32 d	52.25 ± 14.29	46.25 ± 8.66	96.75 ± 35.58	12.50 ± 3.87	63.55 ± 4.90	46.40 ± 4.06	18.63 ± 6.72
48 d	43.75 ± 10.01	40.25 ± 14.45	97.25 ± 7.93	9.25 ± 1.71	60.78 ± 4.08	46.83 ± 4.06	14.23 ± 4.22
64 d	45.50 ± 4.80	45.75 ± 3.24	91.75 ± 29.62	10.50 ± 3.42	61.92 ± 8.18	50.15 ± 5.30	15.05 ± 3.89

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ -glutamyltransferase; TP: Total protein; ALB: Albumin; GLB: Globulin.

Histological findings

The histological specimens showed that the ^{131}I -GMSs were quite concentrated, with a few inflammatory cells surrounding the injection sites on day 1 (Figure 7A) and

some hepatic cells had died by day 4 (Figure 7B). Fibrous sheaths coating the ^{131}I -GMSs and sequential degradation of the ^{131}I -GMS were observed on days 16, 24 and 32. Most of the hepatic cells around and within the

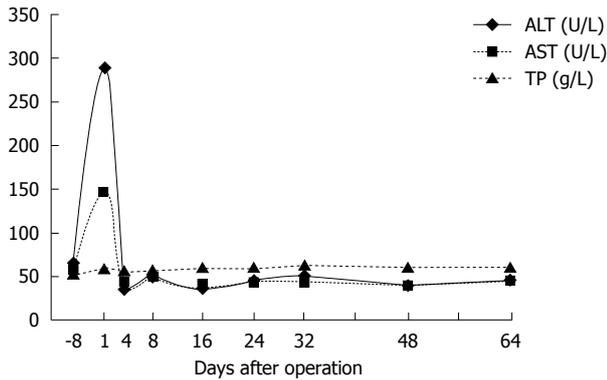


Figure 5 Changes in hepatic function after the administration of ¹³¹I-labeled gelatin microspheres.

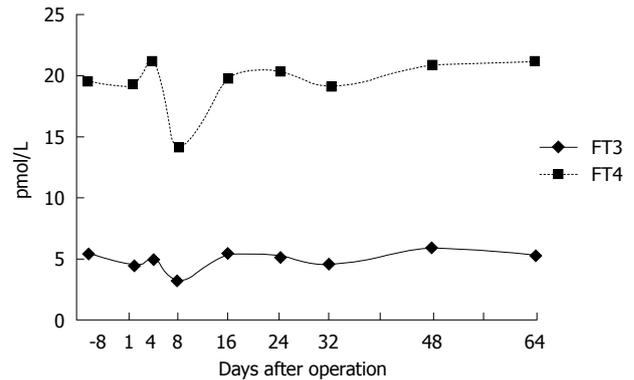


Figure 6 Curves of thyroid functional changes after the administration of ¹³¹I-labeled gelatin microspheres.

Table 4 Effects of the administration of ¹³¹I-labeled gelatin microspheres on thyroid function (*n* = 4)

	FT3 (pmol/L)	FT4 (pmol/L)	TSH (mIU/L)
Before administration	5.55 ± 0.57	19.61 ± 1.54	< 0.005
After administration			
1 d	4.54 ± 1.53	19.31 ± 6.47	< 0.005
4 d	5.09 ± 1.68	21.31 ± 4.68	< 0.005
8 d	3.29 ± 0.83	14.23 ± 2.81	< 0.005
16 d	5.53 ± 1.01	19.95 ± 2.56	< 0.005
24 d	5.31 ± 0.57	20.39 ± 2.38	< 0.005
32 d	4.66 ± 2.17	19.18 ± 2.98	< 0.005
48 d	6.02 ± 2.77	20.94 ± 10.78	< 0.005
64 d	5.34 ± 1.34	21.23 ± 3.42	< 0.005

FT3: Free triiodothyronine; FT4: Free thyroxine; TSH: Thyrotropic-stimulating hormone.

sheaths died, leaving the hepatic cell cords on days 24 and 32. By day 24, the ¹³¹I-GMSs had started to degrade and had an irregular shape (Figure 7C-E). However, the remnant ¹³¹I-GMSs could still be histologically identified on day 48 (Figure 7F). No microspheres were found on day 64.

DISCUSSION

In recent years, many radioactive nuclide microspheres and nuclide particles have been successfully developed to target malignant tumors. However, because of the potential toxicity and side-effects associated with ⁹⁰Y, ³²P and other radioactive nuclides on marrow and other tissues, nonbiodegradable glass is often used as a carrier for most nuclides to avoid causing unwanted damage to normal tissues. However, glass has some limitations, including a protracted bioavailability due to its nonbiodegradable characteristics. Furthermore, its high specific gravity may adversely affect its injection and distribution. In this study, we generated degradable GMSs carrying the ¹³¹I nuclide. Gelatin has a similar specific gravity to blood, and could be conjugated to many other drugs and nuclides *via* physical adsorption or chemical keys. Gelatin also shows good histocompatibility and degrades gradually *in vivo*^[38-40]. Therefore, as a carrier for slow release

of drugs, GMSs have been widely used by the medical community^[41,42]. Meanwhile, ¹³¹I is the most widely used radioactive nuclide in clinical settings, and has shown good anti-tumor effects in many clinical cases^[43]. *In vivo*, dissociative ¹³¹I mainly accumulates in the thyroid and is excreted *via* the kidney^[44-46]. Therefore, ¹³¹I that is released by the degradation of ¹³¹I-GMS into the serum could result in tissue damage, particularly the thyroid gland. Therefore, detailed evaluation of the metabolic characteristics of ¹³¹I-GMS, including its release, distribution and excretion *in vivo*, and the potential damage to the body should be evaluated to comprehensively appraise its safety. The study has provided some insight into these concerns.

The initial labeling rate of ¹³¹I includes chemical binding and physical adsorption. When radionuclide-labeled microspheres are injected into the body, the nuclides conjugated to the microspheres *via* physical adsorption are more likely to dissociate. This has been reported to cause a severe de-iodinated state *in vivo*. In this study, we washed the microspheres seven times after the initial labeling, until the dissociative curve flattened, indicating that most of the ¹³¹I conjugated *via* physical adsorption had been eluted and the ¹³¹I-GMSs mainly exist *via* chemical combination. Although this reduces the labeling index, the *in vivo* de-iodination process is also attenuated, protecting against unwanted de-iodination effects.

Some studies have shown that injecting the microspheres at multiple sites could provide a more even distribution of the microspheres in the tumor tissues^[47]. Therefore, in this study, we injected the microspheres in several sites in the liver. SPECT imaging revealed that the nuclides were principally localized to the injection sites at 4 h and at 1, 4, 8, 16, 24, 32 and 48 d after administration. The extrahepatic labeling with ¹³¹I was mainly found in the thyroid gland between days 4 and 24 after administration, without any accumulation in other tissues, including the lung, heart, kidney, brain, stomach and intestines. Radioactivity of the serum could be detected for 24 d after the operation; after this time, the serum radioactivity level was not different to that of the background level. Because the thyroid gland is the

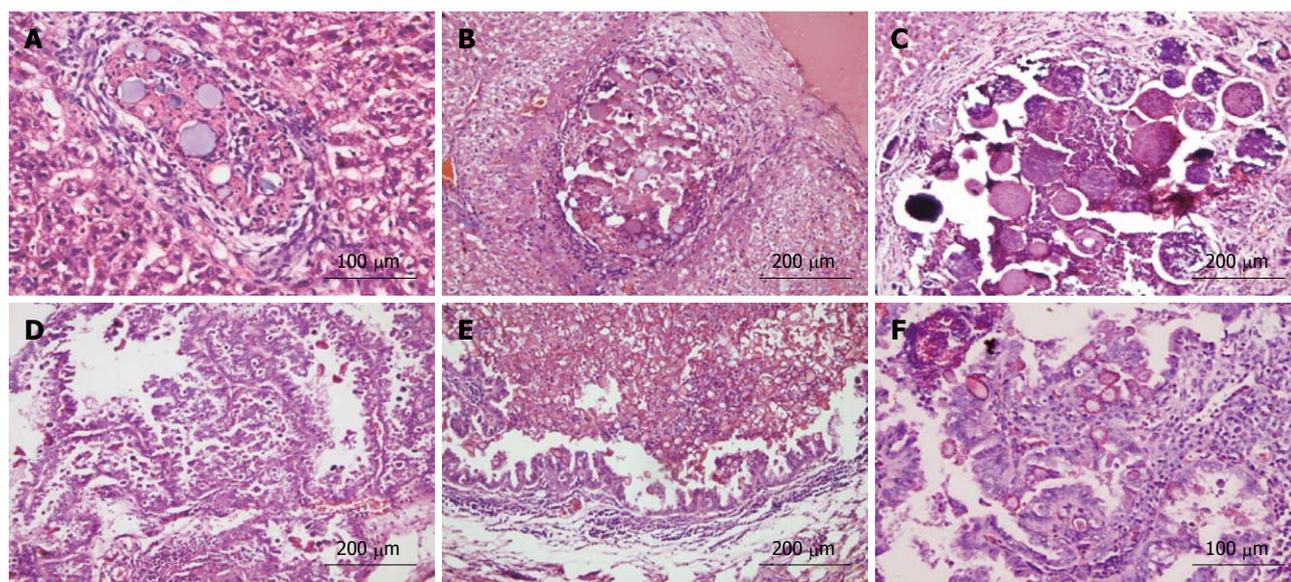


Figure 7 Pathological examination at 1 (A), 4 (B), 16 (C), 24 (D), 32 (E) and 48 (F) d after the administration of ^{131}I -labeled gelatin microspheres (HE stain; original magnification, $\times 200$ in B, C, D and E, and $\times 400$ in A and F).

principle site of iodine absorption and accumulation, it can absorb ^{131}I from the serum into thyroid follicles, which is shown on the SPECT scan. Based on the radioactivity ratio between the site of injection in the liver and the thyroid, it is clear that only a small amount of ^{131}I is absorbed by the thyroid.

SPECT imaging before day 24 revealed low radioactivity levels in the bladder, but not thereafter. This may be related to the full state of the rabbits' bladders when they were scanned, with full bladders showing some radioactivity as a result of excretion *via* the urinary system. Therefore, we believe that the release of ^{131}I from the microspheres mainly occurs within 24 d after administration, but only very small amounts are released.

Assessment of thyroid function revealed that the TSH level was consistently below 0.005 mU/L, while FT3 and FT4 declined on day 8, but returned to normal levels after day 16. This suggests that the thyroid is only subject to transient damage. Because the thyroid has its own repair mechanisms, the damage caused by some radiation doses can be repaired, without causing hypothyroidism^[48]. In this study, a small amount of radioactive nuclides released into the blood was absorbed by the thyroid gland, but did not cause permanent damage or long-lasting hypothyroidism.

The assessment of hepatic function revealed that the ALT and AST increased rapidly compared with the normal level within 1 d after ^{131}I -GMSs administration. However, these parameters returned to the normal level 4 d later. This suggests that the administration of 41.336 MBq of ^{131}I caused notable liver damage in these experimental animals; however, because of the liver's capacity for self-repair and compensation, these impairments were transient and resolved within 4 d. This demonstrates the safety profile of radionuclide microspheres in the treatment of liver cancers.

From the pathological examination, we could conclude that the microspheres were gradually surrounded by fibroblasts to form fibrotic sheaths between days 16 and 24. This seemed to delay the degradation of the GMSs and reduced the rate of radionuclide release. This may explain the absence of thyroid radiolabelling from day 24 after surgery. Ohta reported that, after injecting GMSs into the renal artery of rabbits for 2 wk, the microspheres became wrapped with fibrous tissue and the GMSs in the embolism were completely biodegraded within 1 mo^[49,50]. However, in this experiment, we used the intra-tissue implantation method, which differs from the arterial embolism method. Indeed, over four half-lives of ^{131}I decay (i.e. 32 d), the GMSs had degraded to varying degrees, but there was no sign of disappearance, with a large number of fiber-coated GMSs present, even by day 48. This difference may be due to the different methods of administering the GMSs. Previous studies have shown that gelatin is degraded in the body by degrading enzymes^[51]. In this study, the ^{131}I -GMSs administered into the liver are, on the one hand, treated as a foreign body and induce foreign body reactions, with the activation of inflammatory cells, fibrotic cells and Kupffer cells to encapsulate and phagocytose the GMSs. On the other hand, the radiation will cause cells surrounding the GMSs to die to prevent phagocytosis by macrophages. This may explain why, in this experiment, the GMSs degrade slowly than that in the arterial embolism.

In conclusion, the hepatic administration of ^{131}I -GMSs in rabbits caused marked hepatic damage. Furthermore, there was some ^{131}I accumulation in thyroid tissue, causing slight, but only transient damage to the thyroid tissue. Other tissues showed no radioactive accumulation. Fibrous sheaths formed around the injected GMSs, which likely hampered the degradation of the

GMSs and protracted the release of ¹³¹I. Taken together, we believe that it is safe to inject ¹³¹I-GMSs into tissues *in vivo*, and these are likely to be effective against malignant tumors.

COMMENTS

Background

Malignant tumors in hepatic, biliary and pancreatic systems are very commonly encountered and treated surgically worldwide. In recent years, the incidence of malignant tumors in these three systems was reported to be rising worldwide. However, the rate of surgical removal of the malignant tumors of the three systems is very low and the prognosis after surgery is very unsatisfactory.

Research frontiers

Internal radiotherapy has become an important facet of clinical therapy for malignant tumors. ⁹⁰Y, ¹⁸⁸Re and ¹⁶⁶Ho microspheres have already been shown to be safe and are frequently used to therapy malignant tumors. However, their vehicle material is usually glass, which cannot degrade in the body. Unfortunately, once the nuclides have decayed completely, the vitreous carriers become foreign bodies that persist in the patient's body, and trigger foreign body reactions.

Innovations and breakthroughs

Nuclide microspheres and nuclide particles represent a new generation of tumor therapies and interventional radiotherapies. The authors used gelatin microspheres (GMSs) that can be easily produced to carry radionuclides, and can degrade *in vivo*. This study provides a foundation to show how nuclides attached to GMSs are distributed and metabolized *in vivo*. Healthy rabbits were used as a model to observe the metabolism of ¹³¹I-GMSs. Overall, ¹³¹I-GMSs were found safe for administration in rabbits.

Applications

By understanding the distribution and metabolism of ¹³¹I-GMSs administered in the livers of rabbits, this study supports the use of ¹³¹I-GMSs in directional internal radiotherapy for the treatment of hepatic malignant tumors. This approach could be applied to pancreatic, hepatic, biliary and other material malignant tumors that cannot be removed by surgery. This approach could also be considered to treat osteoarthritis.

Terminology

¹³¹I-GMSs are protein microspheres containing the radionuclide ¹³¹I. The half life of ¹³¹I is 8.04 d and free ¹³¹I either in the body or accumulates in the thyroid is quickly excreted via the urinary system. Compared with glass microspheres, the gelatin microsphere is a biodegradable material with good biocompatibility. In recent years, the authors have developed 50-70 μm nuclide protein microspheres that can be labeled with high concentrations of ¹³¹I. These microspheres can be applied for hepatic arterial transfusion and embolotherapy for patients with advanced liver cancer.

Peer review

This is an interesting experimental study. The technique used in this study can be an effective method for treatment of malignant tumors in the patients.

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