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Intratumoral Injection with [^{188}Re]Rhenium Sulfide Suspension for Treatment of Transplanted Human Liver Carcinoma in Nude Mice

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ABSTRACT. Hepatocellular carcinoma (HCC) is one of the most common malignancies in China. Direct intratumoral injection of nonremovable radioactive material has been widely studied because it could deliver high doses of radiation to target sites and minimize radiation leakage to non-target organs or tissues. Thirty nude mice bearing SMMC 7721 human liver carcinoma were used for the biodistribution study after intratumoral injection of [^{188}Re]rhenium sulfide suspension or sodium [^{188}Re]perrhenate solution. Another 30 tumor-bearing mice were divided into six groups, four groups of which were treated with a 0.1 ml [^{188}Re]rhenium sulfide suspension at doses of 3.7, 7.4, 18.5, 29.6 MBq by a single intratumoral injection. For control studies, to study the tumor inhibiting ratio, the remaining two groups were injected with nonradioactive rhenium sulfide suspension and Hanks' balanced salt solution, respectively. The injections were repeated 6 days later. The retention percentages of radioactivity (%ID) in tumors injected with [^{188}Re]rhenium sulfide suspension were 90.96 ± 6.63%, 86.09 ± 22.58% and 87.62 ± 13.97% at 1, 24 and 48 h, respectively. Tumor inhibition ratios are as high as 89% when the outer space of tumor (0.5–0.6 cm from center) received about 507.6 Gy doses. Intratumoral injection of [^{188}Re]rhenium sulfide suspension results in high tumor retention indicating this approach has strong potential for the treatment of hepatic carcinoma. NUCL MED BIOL 27;4:347–352, 2000. **© 2000 Elsevier Science Inc. All rights reserved.**

KEY WORDS. [^{188}Re]Rhenium sulfide, Suspension, Intratumoral injection, Tumor inhibiting ratio, Hepatoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in China. According to the World Health Organization statistics, of 6,350,000 cancer cases reported each year, 4% are HCCs, 42% of which occur in China (6). Traditional treatment of liver tumors typically has been surgical resection and chemotherapeutic when the tumors are not resectable (1). As an alternative, direct intratumoral injection of nonremovable radioactive material has been widely studied because it can deliver high doses of radiation to target sites and minimize radiation leakage to non-target organs or tissues and the results are encouraging (4–6, 12).

The preparation of rhenium sulfide colloid as a radiation synovectomy agent has been reported by Venkatesan and Wang (7, 8). We have investigated the reaction conditions and developed a very satisfactory method for routine preparation of [^{188}Re]rhenium sulfide suspension (10). In the present studies, we have evaluated use of [^{188}Re]rhenium sulfide suspension as an effective radiopharmaceutical for treatment of transplanted human live carcinoma in nude mice following intratumoral injection.

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MATERIALS AND METHODS

Preparation of [^{188}Re]rhenium sulfide suspension

The rhenium sulfide suspension was prepared by the reaction of sodium thiosulfate with potassium perrhenate in acid solution and the main particle size was 1–10 μm (10).

Animals and tumor cell line

Female athymic nude mice weighing 18–20 g, 7 weeks of age were purchased from the Shanghai Institute of Pharmacology, Academia Sinica. Nude mice were maintained in an aseptic environment with laminar filtered ventilation. The animals were handled under sterile conditions. Human liver carcinoma cell line SMMC 7721 (2) was obtained from the Second Military Medical College in Shanghai and grown in RPMI 1640 medium supplemented with 10% newborn calf serum, and incubated at 37°C in humidified 5% CO_2.

Tumor xenograft

SMMC 7721 cells were harvested by trysinization, washed by Hanks' balanced salt solution, and centrifuged into a cell pellet which was suspended in sterile Hanks' balanced salt solution and 5×10^6 cells were injected SC into the right flank of the nude mice. Animals were used for experiments when the tumor diameter was 0.9–1.2 cm. The animal experiments were performed in accordance with the "Principles of Laboratory Animal Care" (NIH publication No. 86-23, revised 1985).

Biodistribution studies

The tissue biodistribution of [^{188}Re]rhenium sulfide suspension was determined in 15 nude mice. The mice were sacrificed at 1, 24 and 48 h (five mice at each time) after intratumoral injection of approximatedly 0.5 MBq [^{188}Re]rhenium sulfide suspension in a volume of 0.1 ml. Another 15 nude mice served as the controls and were also sacrified after intratumoral injection of approximatedly 1.3 MBq 0.1 ml $Na^{188}ReO_4$ solution. Organ samples and tumor were weighed and counted in a "well-type" gamma counter to calculate the resident activity.

Tumor inhibiting studies

Thirty nude mice bearing SC xenograft tumors were divided into six groups. Four groups were treated with a 0.1 ml [^{188}Re]rhenium sulfide suspension at doses of 3.7, 7.4, 18.5, 29.6 MBq by a single intratumoral injection. Two groups served as the controls and were injected with nonradioactive rhenium sulfide suspension and Hanks' balanced salt solution, respectively. The injections were repeated 6 days later. The tumors were measured every 2 or 3 days with vernier calipers in two dimensions. Tumor volume was calculated as

$$\frac{1}{2}ab^2$$

where a = length and b = breadth (3). Nude mice were killed after 14 days from the first injection and tumors were weighed to calculate the tumor inhibiting ratio. The tumor inhibiting ratio was calculated as

$$\frac{n1 - n2}{n1} \times 100\%$$

where n1 = the average weight of control tumors and n2 = the average weight of treated tumors) (9). The levels of radiation were calculated as described elsewhere (11).

Histological procedures

Six representative sections from tumors of each group were fixed in a 10% formalin solution (The fixed pieces were dehydrated in an ethanol-butanol series) and embedded in paraffin. Transverse sections were hydrated and stained with hematoxylin and eosin. When the sections were completed, microphotos were taken using a microscopic camera (Biostar, American Optical Co., Buffalo, NY USA).

RESULTS

The retention percentages of radioactivity (%ID) in tumors injected with [^{188}Re]rhenium sulfide suspension were 90.96 ± 6.63%, 86.09 ± 22.58% and 87.62 ± 13.97% at 1, 24 and 48 h, respectively. The results of the biodistribution study, expressed as the percent injected dose per gram (%ID/g) of organs, are summarized in Table 1. The radioactivity in the tumor is much higher than that in other organs (Table 2). The highest activity outside the tumors was found in the lung, liver and spleen. For control, the radioactivity retention in the tumor injected with sodium [^{188}Re]perrhenate solution was very low and had no notable difference with other organs (Table 3). The %ID values were only 1.66 ± 0.35%, 0.02 ± 0.01% and 0.01 ± 0.01% at 1, 24 and 48 h, respectively.

Figure 1 shows a very significant difference in group of tumors between treated and control groups of mice. At the time of the first injection, tumor volumes were similar in the six groups. As time progressed, tumor growth in the control groups continued its natural course, while treated tumors grew slowly. By day 14, the differences between the tumor volumes in the treated and control groups were very significant. The average volumes of tumors injected with Hanks' balanced salt solution and nonradioactive rhenium sulfide suspension were 3.66 and 3.65 cm^3. In comparison, the volumes injected with 3.7, 7.4, 18.5 and 29.6 MBq [^{188}Re]rhenium sulfide suspension were 2.52, 1.72, 1.29 and 0.25 cm^3, respectively. These differences are further illustrated in Figures 2 and 3. (Figure 2 shows the final tumor weights of each group. Figure 3 illustrates photos of the tumors and tumor-bearing mice.)

The experimental results of internal irradiation for treatment of transplanted human liver carcinoma in nude mice are summarized in Table 4. The tumor inhibiting ratio was about 20% when the outer areas of tumor (0.5–0.6 cm from tumor center) received about 63.5 Gy of radiation. When the injected dose increased to 59.2 MBq, the ratio increased to about 89%. The tumors were much smaller than those in the control groups. Most of the tumor cells were killed and replaced by scar and collagen tissue which is apparent in Figure 4F. Figures 4A and 4B show normal tumor cells, and Figures 4C, 4D, 4E, 4F show variable degrees of necrosis, fibrosis, capsulation and collagen tissue.

DISCUSSION

Direct intratumoral injection of nonremovable radioactive material is an attractive method for tumor treatment because it can prevent normal tissues from radiation while delivering radiopharmaceutical to target sites. Such an approach can also provide high energy to cure tumors in a short time period. In our experiments, about 90% of total administered radioactivity were retained in tumors after 2 days and the radioactivity in normal organs was very low. Beta emissions with high energy of ^{188}Re can kill most of the tumor cells. In tumor inhibition studies, the tumor inhibiting ratio was as high as 89% when the outer regions of the tumor (0.5–0.6 cm from center) received a dose of about 507.6 Gy. Such a strategy is safe for the normal liver because only 7.8 Gy was received by the tissue at the distance of 0.8 cm from center if it was applied to cure live tumor. For these reasons [^{188}Re]rhenium sulfide suspension appears to be very suitable for liver tumor treatment following intratumoral injection.

The particle size of the [^{188}Re]rhenium sulfide suspension plays an important role in preventing leakage of radioactivity from tumors. Tables 1 and 3 show the levels of sodium [^{188}Re]perrhenate solution which escaped from tumors to the body by the abundant blood of tumors. Tumor tissue is not as tightly a closed-system as the knee is since it is surrounded by abundant blood vessels through which small particles of suspension could spread throughout the body. We have compared the differences of biodistribution and tumor retention by intratumoral injection of [^{188}Re]rhenium sulfide suspension with different sizes (11). The differences of biodistribution and radioactive retention in tumors were notable, although the difference of suspension particles ultrasonicated for 10 and 5 min was not. So 1–10 μm particle size of [^{188}Re]rhenium sulfide suspension is necessary for tumor treatment.

Another consideration is the method of injection. Because the average penetration of rhenium-188 beta particles in tissue is 3.8

TABLE 1. Biodistribution after Intra-tumor Injection of [^{188}Re]Rhenium Sulfide Suspension in Mice (%ID/g, Mean ± SD, n = 5)

Hours post-injection	Blood	Heart	Lung	Liver	Spleen	Kidney	Brain	Muscle	Bone	Gastrointestinal tract	Tumor
1 h	0.02 ± 0.02	0.05 ± 0.06	0.77 ± 0.98	0.13 ± 0.14	0.19 ± 0.10	0.01 ± 0.01	0.07 ± 0.05	0.03 ± 0.02	0.22 ± 0.16	0.16 ± 0.12	84.79 ± 2.53
24 h	0.03 ± 0.03	0.10 ± 0.12	1.21 ± 0.74	0.88 ± 0.69	0.74 ± 0.44	0.16 ± 0.08	0.02 ± 0.04	<0.01	0.10 ± 0.11	0.18 ± 0.05	125.20 ± 63.13
48 h	0.08 ± 0.12	0.32 ± 0.20	1.10 ± 1.81	1.27 ± 1.74	1.16 ± 1.96	0.47 ± 0.08	0.37 ± 0.42	<0.01	0.09 ± 0.15	0.01 ± 0.01	150.92 ± 38.48

TABLE 2. The Ratio of Target (Tumor) to Non-target Organs Injected with [^{188}Re]Rhenium Sulfide Suspension in Mice (n = 5)

Hours post-injection	T/blood	T/heart	T/lung	T/liver	T/spleen	T/kidney	T/brain	T/muscle	T/bone	T/gastrointestinal tract
1 h	4240	1696	110	652	446	8479	1211	2826	385	530
24 h	4173	1252	103	142	169	783	6260	>12520	1252	696
48 h	1887	472	137	119	118	321	5031	>15092	1677	15092

T = Target (tumor).

TABLE 3. Biodistribution after Intra-tumor Injection of [^{188}Re]Sodium Perrhenate Solution in Mice (%ID/g, Mean ± SD, n = 5)

Hours post-injection	Blood	Heart	Lung	Liver	Spleen	Kidney	Brain	Muscle	Bone	Gastrointestinal tract	Tumor
1 h	6.66 ± 1.60	1.56 ± 0.43	5.58 ± 0.18	2.73 ± 0.31	1.96 ± 0.09	2.54 ± 0.25	0.38 ± 0.16	1.23 ± 0.19	2.83 ± 0.07	20.45 ± 26.87	2.88 ± 0.01
24 h	0.05 ± 0.01	<0.01	<0.01	<0.01	0.02 ± 0.02	<0.01	<0.01	<0.01	<0.01	0.21 ± 0.09	0.02 ± 0.01
48 h	0.07 ± 0.01	<0.01	<0.01	<0.01	0.16 ± 0.16	0.05 ± 0.02	0.08 ± 0.08	0.11 ± 0.09	0.31 ± 0.31	0.09 ± 0.07	0.02 ± 0.01

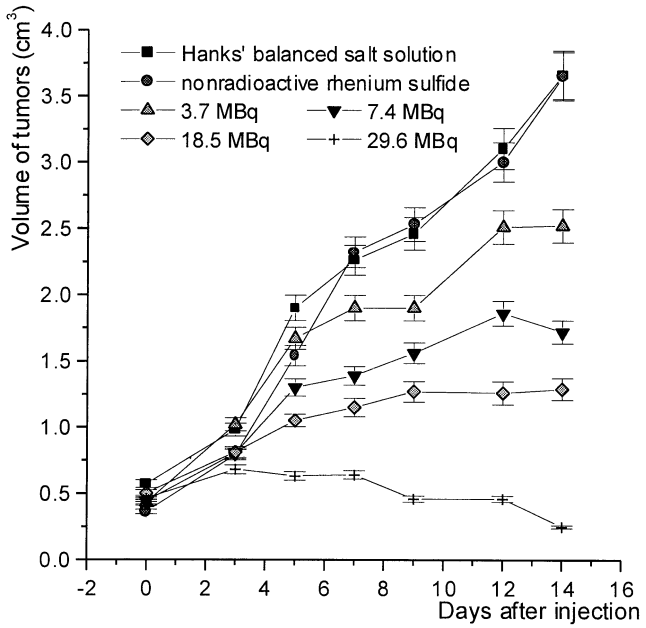

FIG. 1. The curves of tumor growth injected with Hanks' balanced salt solution, nonradioactive rhenium sulfide suspension, 3.7, 7.4, 18.5 and 29.6 MBq [^{188}Re]rhenium sulfide suspension (n = 5).

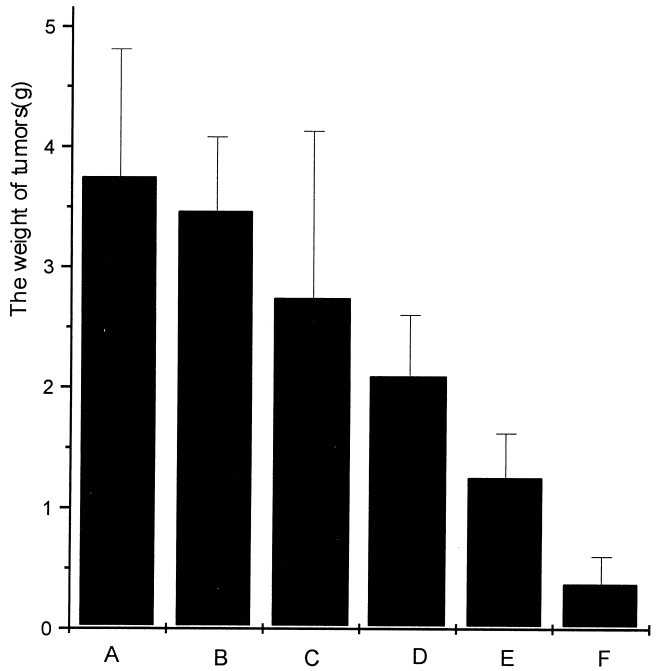

FIG. 2. The final weights of (A) tumors injected with Hanks' balanced salt solution; (B) nonradioactive rhenium sulfide suspension; (C) 3.7 MBq [^{188}Re]rhenium sulfide suspension; (D) 7.4 MBq [^{188}Re]rhenium sulfide suspension; (E) 18.5 MBq [^{188}Re]rhenium sulfide suspension; and (F) 29.6 MBq [^{188}Re]rhenium sulfide suspension (n = 5).

mm (maximum, 11 mm), the positive results depend greatly on the shape of tumor though the tumors' volume and the radiation received. Spherical tumor size decreased significantly when their outer space received about 317.3 Gy. In case of the tumors with irregular shapes, the center cells were killed while the cells in outer space were still growing even under the same dose. For these tumors, muti-injection may be more effective than a single injection.

In conclusion, [^{188}Re]rhenium sulfide suspension is an attractive potential radiopharmaceutical for treatment of hepatic tumors following intratumoral injection.

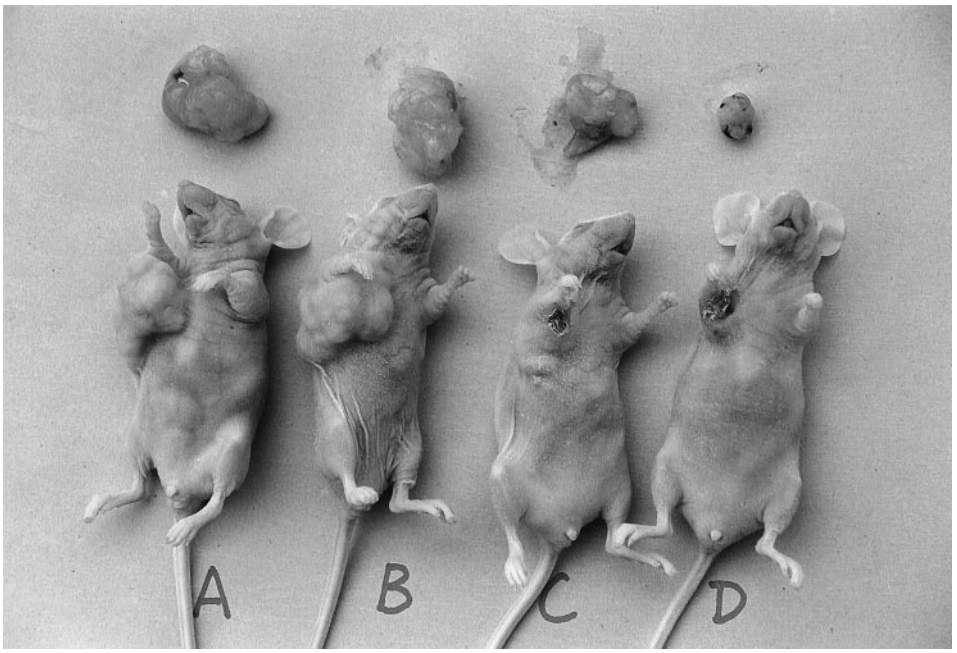

FIG. 3. Photographs of tumors and tumor-bearing nude mice. (A) Injected with Hanks' balanced salt solution; (B) Injected with nonradioactive rhenium sulfide suspension; (C) Injected with 18.5 MBq [^{188}Re]rhenium sulfide suspension; (D) Injected with 29.6 MBq [^{188}Re]rhenium sulfide suspension

TABLE 4. Results of Internal Irradiation for Liver Carcinoma (n = 5)

Injected dose (MBq)	Absorbed dose ratio*[1] (Gy/s)	Absorbed dose*[2] (Gy)	Tumor inhibiting ratio	
			A*[3]	B*[4]
3.7 × 2*[5]	1.08×10^{-3}	63.5	20.8%	26.7%
7.4 × 2	2.16×10^{-3}	126.9	39.3%	43.8%
18.5 × 2	5.4×10^{-3}	317.3	63.6%	66.3%
29.6 × 2	8.64×10^{-3}	507.6	89.0%	89.8%

*[1,2] 0.5 cm from tumor center.
*[3] Using tumors injected with nonradioactive rhenium sulfide suspension as control group.
*[4] Using tumors injected with Hanks' balanced salt solution as control group.
*[5]: ×2 means injected twice.

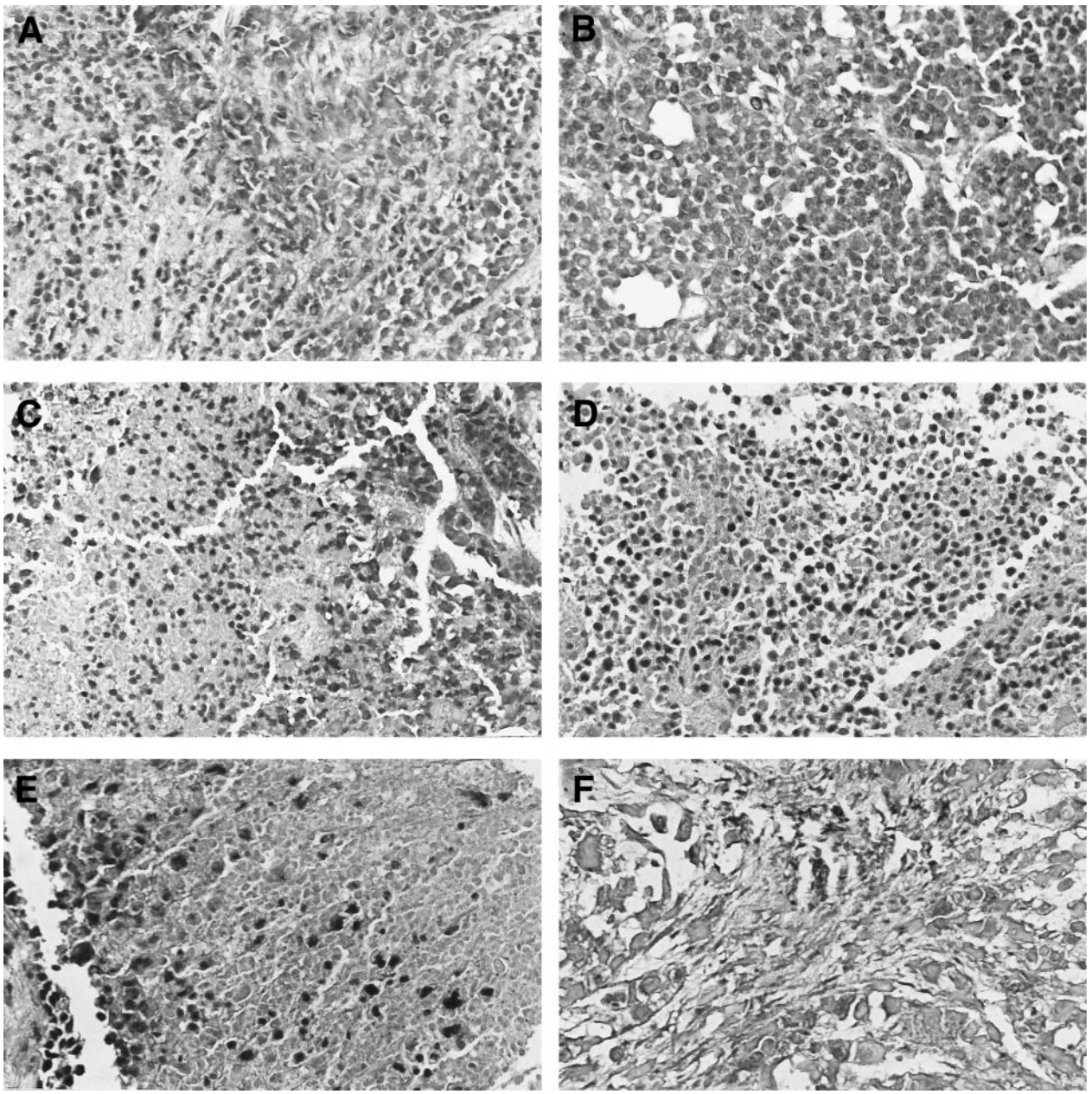

FIG. 4. Microphotos of histologic sections of tumors injected with (A) Hanks' balanced salt solution , (B) nonradioactive rhenium sulfide suspension, (C) 3.7 MBq [^{188}Re]rhenium sulfide suspension, (D) 7.4 MBq [^{188}Re]rhenium sulfide suspension, (E) 18.5 MBq [^{188}Re]rhenium sulfide suspension and (F) 29.6 MBq [^{188}Re]rhenium sulfide suspension. Photos taken by microscopic camera (×280, Biostar, American Optical Co.).

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