Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **42**, 233-243 (1999)

Preparation of [^{188}Re]Rhenium Sulfide Suspension and its Biodistribution Following Intra-Tumor Injection in Mice

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Key words: [^{188}Re]Rhenium sulfide, Suspension, Biodistribution, Intra-tumor injection, Tumor-bearing mouse

Summary

[^{188}Re]Rhenium sulfide suspension was prepared by the reaction of $Na_2S_2O_3$ and $KReO_4$ containing $Na^{188}ReO_4$ in acid solution and assessed for its applicability in tumor treatment by the techniques of radioisotope

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Received 23 March 1998
Revised 4 September 1998
Accepted 8 September 1998

interventional therapy. The reaction conditions such as the concentration ratio of $Na_2S_2O_3$ to $KReO_4$, heating time, ultrasonic time were optimized. The radiochemical yield of [^{188}Re]Rhenium sulfide suspension is more than 96% and in vitro stability studies demonstrated that more than 99% of the ^{188}Re remained in sulfide form over a 72hrs period. The main particle size after various ultrasonic times is 1-5 μm. [^{188}Re]Rhenium sulfide suspension was injected into tumors and the tumor-bearing mice were killed after some time to determine the retention of ^{188}Re in tumor and the leakage to different organs by γ counter. The mean retention percentages of ^{188}Re in tumors were 83.08 ± 13.71%, 83.92 ± 9.79%, 80.23 ± 3.82% and 78.75 ± 3.02% at 1h, 24, 48 and 72hrs respectively. After 72hrs the highest activity outside the tumors was in the liver, spleen and the kidneys. Our preliminary results indicated that [^{188}Re]Rhenium sulfide suspension may be an effective radiopharmaceutical for tumor treatment by the techniques of radioisotope interventional therapy.

Introduction

Radiotherapy agents are being used to help millions of patients to treat serious diseases, typically cancers (1). An ideal radiotherapy agent should deliver high radiation doses to target sites and low radiation leakage to non-target organs or tissues. Radiolabeled monoclonal antibodies have been studied for several years. However, the results are not satisfactory for the low absolute uptake by the tumor, typically 0.001-0.01% of the injected

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dose/gram (2). Rowlinson et al. (1991) developed a new method of intra-tumor injection to deliver a high dose to an accessible tumor (3). Their results were encouraging.

^{188}Re is an excellent candidate for radiotherapy. It can be easily obtained from a ^{188}W/^{188}Re generator. Beta emissions with energies of 2.12MeV and 1.96MeV are suitable for therapy and the Gamma emission of 155keV allows for imaging and calculating dosimetry. Venkatesan et al. (1990) developed the preparation of [^{186}Re]Rhenium heptasulfide for radiation synovectomy (4). Wang et al. (1995) improved the method to prepare the [^{188}Re]Rhenium sulphur colloid in a quick and easy manner with a good yield (about 90%) (5). However, the free $^{188}ReO_4^-$ could not be separated and the suboptimal particle size distribution (30% of the particles were less than 2 μm) led to approximately 13% leakage of radioisotope from the knee. In this study, we improved Venkatesan's method by investigating the reaction conditions with ^{188}Re and got a very high radiochemical yield. The suspension could be made stable for 72hrs by adding stabilizer(gelatin or PVP) as a protective colloid. The applicability of [^{188}Re]Rhenium sulfide suspension was also assessed by analyzing the biodistribution following intra-tumor injection.

Materials and Methods

^{188}Re production

^{188}Re was obtained from an alumina-based ^{188}W/^{188}Re generator (6), loaded with the ^{188}W solution supplied by the Oak Ridge National

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Laboratory (Tenn., USA). Carrier-free ^{188}Re sodium perrhenate was extracted from the generator eluted with 0.9% NaCl. The nuclear purity of ^{188}Re was more than 99% as analyzed by high purity germanium (HPGe) detector (GEM-15190, EG & ORTEC, USA) and the radiochemical purity of $Na^{188}ReO_4$ was more than 95% by paper chromatography developed with 0.9% NaCl.

Production of [^{188}Re]Rhenium sulfide suspension

The [^{188}Re]Rhenium sulfide suspension was prepared by the reaction of sodium thiosulfate and potassium perrhenate in acid solution. The yield of [^{188}Re]Rhenium sulfide suspension was determined under various conditions of the concentration (mol/L) ratio of $Na_2S_2O_3$ to $KReO_4$ and time of heating. 2ml of the mixture of the $Na_2S_2O_3$ and $KReO_4$ solution (the concentration (mol/L) ratio of $Na_2S_2O_3$ to $KReO_4$ was 35, 70, 91 or 110, respectively) was mixed with 1ml of eluted ^{188}Re and 1ml H_2O. After adding 1ml of 5M HCl the mixture was incubated in a boiling water bath for 7, 12, 15, 20, 25, 30 or 35min, respectively and cooled by tap water for 3min. The solution was centrifuged at the rate of 4000rpm for 10min.. Under these conditions, all of the precipitate deposited on the bottom of the tube. Then the supernant was removed and the residue was washed with water. The procedure was repeated again. In order to obtain a stable suspension and differentiate the different particle sizes, the precipitate was ultrasonically dispersed in 5ml stabilizer (gelatin 28.8mg/ml or PVP 120mg/ml) for 5, 10, 15 or 20min.

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The particle size distributions were determined by using a microscope (2XA-II, Shanghai optical instruments factory, China). The total radioactivity(n) at the beginning of the experiment and the total radioactivity(n') of twice supernants were counted in a "well-type" sodium iodide scintillation crystal. The radiochemical yield of [^{188}Re]Rhenium sulfide suspension = 1 — n'/n.

In vitro stability

Two kinds of experiments proceeded to determine the stability in vitro. In method 1, the [^{188}Re]Rhenium sulfide suspension was allowed to stand at room temperature (15 ℃) for 72 hours and the radiochemical purity was checked at intervals of 0h, 24h, 48h and 72h by paper chromatography (Xinhua#1 chromatography paper) with 0.9% NaCl as the developing solvent; In method 2, aliquots(1ml) of [^{188}Re]Rhenium sulfide suspension were dispensed into tubes containing 1ml of normal saline, phosphate buffered saline(pH7) and calf serum(1:1 v/v with phosphate buffer pH 7). The tubes were stopped, allowed to stand at 37 ℃ for 72 hours and the radiochemical purity was checked at intervals of 0h, 24h, 48h and 72h by paper chromatography. The labeled suspension remains at the origin, while $^{188}ReO_4^-$ migrates with an Rf of approximately 0.7.

Animal model

A Sarcoma-180 (S_{180}) mouse(from Shanghai Institute of Pharmaceutical Research, Academia Sinica) was killed and the tumor was cut into about 2 ~

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$3mm^3$ (7) bits in normal saline. Tumors were initiated by a subcutaneous injection of a bit of tumor in the belly beside one of the front legs of Kunming mice (from Shanghai medical college) weighed 18-20g. Animals were used for experiment 7-10 days later when the tumor diameter was about 1cm. The animal experiments were performed in accordance with the United Kingdom Biological Council's *Guidelines on the Use of Living Animals in Scientific Investigations* (2nd edn.).

Biodistribution

To determine the biodistribution and the retention in tumors of [^{188}Re]Rhenium sulfide suspension, the mice were killed at 1h, 24h, 48h and 72h after intra-tumor injection (0.1ml of [^{188}Re]Rhenium sulfide suspension was injected into tumors. The radiochemical concentration of the suspension was 0.34mCi/ml and the specific activity 2.13mCi/mg). All of different organ samples and tumor were weighed and counted in a "well-type" gamma counter to calculate resident activity.

The effect of varying concentration (mol/L) ratio of $Na_2S_2O_3$ to $KReO_4$ on the yield of [^{188}Re]Rhenium sulfide suspension were examined, using a heating time of 7-8min. Fig.1 shows that the best concentration ratio is 70:1. Fig.2 illustrates the effect of heating time on suspension yield. After 30min of heating, more than 96% of the ^{188}Re was in the sulfide suspension fraction (at the concentration ratio 70:1). The suspension particle size becomes smaller with the longer ultrasonic time (Fig.3). 10min of ultrasonic time was chosen to do the following experiments.

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Results

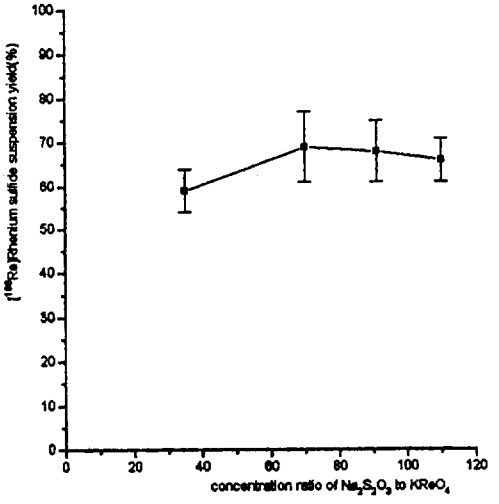

Fig.1 [^{188}Re]Rhenium sulfide suspension yield(%), as a function of the concentration(mol/L) ratio of $Na_2S_2O_3$ to $KReO_4$ (n=4).

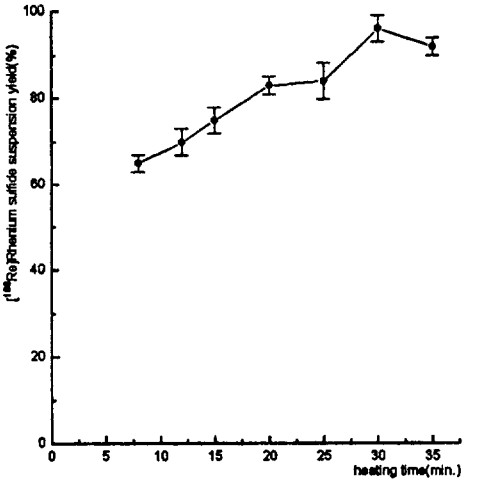

Fig.2 [^{188}Re]Rhenium sulfide suspension yield(%), as a function of heating time (n=3).

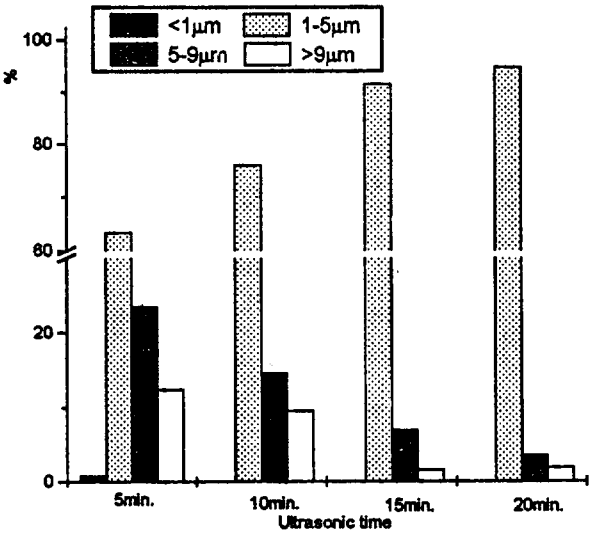

Figure 3 The resultant suspension particle sizes, as a function of ultrasonic time

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In vitro stability

When the suspension was prepared under these optimized conditions, the chemical purity was found to be more than 99% in both methods over a 3-day period.

Animal studies

The retention percentages of radioactivity (%ID) in tumors were 83.08 ± 13.71%, 83.92 ± 9.79%, 80.23 ± 3.82% and 78.75 ± 3.02% at 1h, 24, 48 and 72hrs 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 highest activity outside the tumors was found in the liver, spleen and kidneys after 72hrs (Table 2).

Table 1 Biodistribution of intra-tumor injection of [^{188}Re]Rhenium sulfide suspension in mice(%ID/g, mean ± SD, n = 4)

Hours post-injection	tumor	blood	heart	lung	liver	spleen	kidney	brain	muscle	bone
1 h	206.14 ± 69.83	0.02 ± 0.01	0.02 ± 0.02	0.41 ± 0.32	0.41 ± 0.31	0.23 ± 0.19	0.02 ± 0.01	<0.01	0.02 ± 0.03	<0.01
24 h	436.10 ± 59.82	0.06 ± 0.02	0.11 ± 0.04	0.18 ± 0.10	1.21 ± 0.77	0.92 ± 0.95	0.18 ± 0.04	0.02 ± 0.02	0.04 ± 0.07	0.03 ± 0.06
48 h	196.47 ± 36.60	0.10 ± 0.08	0.18 ± 0.11	0.12 ± 0.16	0.83 ± 0.61	0.67 ± 0.39	0.28 ± 0.15	<0.01	<0.01	<0.01
72 h	128.46 ± 8.97	<0.01	<0.01	<0.01	1.29 ± 0.96	0.92 ± 0.70	0.15 ± 0.09	<0.01	<0.01	<0.01

Table 2 The ratio of target (tumor) to non-target organs (n = 4)

Hours post-injection	T/blood	T/heart	T/lung	T/liver	T/spleen	T/kidney	T/brain	T/muscle	T/bone
1 h	10307	10307	503	503	896	10307	>20614	10307	>20614
24 h	7268	3965	2423	360	474	2423	21805	10903	10537
48 h	1965	1092	1637	237	293	702	>19647	>19647	>19647
72 h	>12846	>12846	>12846	100	140	856	>12846	>12846	>12846

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Discussion

Venkateson et al. reported that the reaction of thiosulfate with perrhenate yielded not only heptasulfide but also thioperrhenates which might decompose further to heptasulfide depending on pH (4) . In the initial stage of our experiments, we didn't separate the supernant from the suspension in order to obtain the suspension directly as Wang et al. did (5). But the highest yield of [^{188}Re]Rhenium sulfide suspension we obtained was about 70%. When we measured the radiochemical yield of [^{188}Re]Rhenium sulfide suspension by paper chromatography with 0.9% NaCl, we often found that there existed a component whose Rf value was 0.53 which was different from sulfide suspension (Rf = 0) and perrhenate (Rf = 0.7). This component could be turned to heptasulfide by longer reaction time or lower pH value and considered as thioperrhenate. Under the conditions we chose, there was only [^{188}Re]Rhenium sulfide suspension without any byproducts.

An ideal radiotherapeutic agent should deliver a high dose of radiation to selected malignant sites in target organs or tissues, while minimizing the radiation doses to surrounding healthy cells (1). In our study, about 80% of total radioactivity was retained in the tumor after 72hrs. It is much higher than that in all normal organs (Table 2). When the suspension was injected directly into tumors, about 83% of total radioactivity was retained in these tumors one hour after the direct injection of the suspension because these were surrounded by abundant blood vessels through which small particles of suspension could spread throughout the body. Interestingly, the retentions of

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the radioactivity in tumors remained nearly unchanged for three days. This phenomenon is encouraging for the application of [^{188}Re]Rhenium sulfide suspension to tumor therapy and a further study will be carried out.

Conclusions

The optimized conditions for the preparation of [^{188}Re]Rhenium sulfide suspension are 70:1 of the concentration (mol/L) ratio of $Na_2S_2O_3$ to $KReO_4$ and 30min of heating time. The radiochemical yield of [^{188}Re]Rhenium sulfide suspension was more than 96% and in vitro stability studies demonstrated that more than 99% of the ^{188}Re remained in sulfide form over a 72-hour period. The preliminary animal experiments show that about 80% of the total radioactivity was retained in the tumor after 72hrs. So [^{188}Re]Rhenium sulfide suspension may broaden the application of tumor treatment by the techniques of radioisotope interventional therapy. The particle size plays an important role in preventing the activity leakage from tumors. Further trials to reveal the relationship between the particle size and the retention of activity in the tumor is necessary.

Acknowledgements---We wish to thank Professor Li Wenxin, Professor He Weiyu, Professor Huang Zhongzhi, Mr. Hu Weiqing and Mr. Zhou Wei for their assistance in these studies. Thank for the part financial support by IAEA.

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