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# DA-7911, $^{188}$Rhenium-tin Colloid, as a New Therapeutic Agent of Rheumatoid Arthritis

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Radiation synovectomy is one of the most useful methods for treating patients with refractory synovitis because of its convenience, long-term effects, repeatability and the avoidance of surgery. In this study, we investigated the toxicity, stability and biodistribution of a rhenium-188 ($^{188}$Re)-tin colloid to evaluate its suitability as a synovectomy agent. Twenty four hours after injecting the $^{188}$Re-tin colloids (74 KBq/0.1 mL) into the tail vein of ICR mice, most of the $^{188}$Re-tin colloidal particles was found in the lungs. In addition, there were no particle size changes at either room temperature or at 37°C after injecting the $^{188}$Re-tin colloids in human plasma and synovial fluid. In vitro stability tests showed that the $^{188}$Re-tin colloid remained in a colloidal form without a critical size variation over a 2-day period. We investigated the leakage of $^{188}$Re-tin colloids from the intraarticular injection site with gamma counting in New Zealand white rabbits. The $^{188}$Re-tin colloids (55.5 MBq/0.15 mL) were injected at the cavum articular and the mean retention percentage of the $^{188}$Re-tin colloid was 98.7% for 1 day at the injection site, which suggests that there was neither change in the particle size nor leakage at the injection sites. In the biodistribution study with the SD rats, the liver showed the highest radioactivity (0.0427% ID/organ) except for the injected knees (99.49%). In the SD rats, mild toxicities including the skin or a synovium inflammation were observed as a result of a radioactivity of 15 mCi/kg at the intraarticular injection site. However, there was no systemic toxicity. In the Ovalbumin (OVA)-induced arthritic rabbits, the $^{188}$Re-tin colloid improved the macroscopic, the histological score and reduced the knee joint diameter when compared to the arthritic control. In conclusion, a $^{188}$Re-tin-colloid is considered as a strong candidate for radiation synovectomy with a superior efficacy and safety.

**Key words:** $^{188}$Re-tin colloids, Radiation synovectomy, Rheumatoid arthritis

## INTRODUCTION

Radiosynovectomy is a type of radiotherapy that has been used for more than 40 years to relieve pain and inflammation from rheumatoid arthritis, particularly steroid refractory chronic synovitis (Ures *et al.*, 2002). Radiation synovectomy was developed as an alternative to surgical synovectomy for treating rheumatoid arthritis (Wang *et al.*, 1995). The procedure consists of injecting a β-emitting radionuclide into the joint capsule (Davis and Chinol, 1989), where it remains in contact with the synovial membrane or the synovium. The intraarticular-administered radiopharmaceutical is then phagocyted by the lining cells, which are on the synovial surface. While the radionuclide decays, the absorbed dose is given to the synovium.

Rhenium-188 ($^{188}$Re) has optimal characteristics for synovectomy owing to its deep tissue penetration (β-ray; 2.1 MeV, maximum 11 mm, average 3.8 mm) and relatively short physical half-life (16.9 hours), which makes it suitable for treating of the knee (Johnson *et al.*, 1995; Wang *et al.*, 2001). $^{188}$Re decays into the stable $^{188}$Os, with a γ-ray emission of 155 KeV, which is suitable for image acquisition. This fact allows target uptake to be evaluated, as well

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as allowing the absorbed radiation dose to be estimated. In addition, $^{188}$Re is readily available on a routine bases from the tungsten-188/rhenium-188 generator system, which has a useful shelf life of several months (Knapp *et al.*, 1998).

$^{188}$Re should be attached to a particle that is sufficiently small to be phagocytosed, but not too small because it might leak from the joint before being phagocytosed. In addition the radiolabeled particles should be distributed homogeneously in the joint without initiating an inflammatory response. The appropriate size range can be achieved by conjugation it with a tin-colloid (Ures *et al.*, 2002).

In this study, we investigated the toxicity, stability and biodistribution in order to evaluate the suitability of the rhenium-188 ($^{188}$Re)-tin colloid to be used as a synovectomy agent.

## MATERIALS AND METHODS

### Preparation of $^{188}$Re-tin colloid

The $^{188}$Re-tin colloid was prepared according to the methods reported by Jeong *et al.* (Jeong *et al.*, 2000). Briefly, 0.5 mL aliquots of nitrogen-purged 0.1 N HCl containing 0.5-30 mg $SnCl_2$-$H_2O$ (Sigma-Aldrich) were dispensed in o vials under nitrogen environment. The vials were capped and sealed with aluminum and then stored in a refrigerator until needed. $^{188}$Re-tin colloid in the vials remained stable for 3 months. Radiolabeling was performed by adding a 0.5 mL aliquot of $^{188}$Re-perrhenate (~30 MBq) freshly eluted with saline from an alumina-based $^{188}$W/$^{188}$Re-generator (Oak Ridge National Laboratory) into each vial and the mixture was incubated it at room temperature or 100 °C. The labeling efficiency was estimated by chromatography (ITLC-SG/normal saline) and radioactivity was monitored using a TLC scanner (Imaging Scanner System 200, Bioscan). The radiolabeled $^{188}$Re-tin colloid was neutralized by adding an adequate quantity of a 0.2 M sodium phosphate buffer (pH 8.0) solution.

### Stability test

Each radiolabeled colloid was stored at room temperature and the stability was evaluated for 24 h according to the previously reported methods (Jeong *et al.*, 2000). To evaluate the stability in body fluid, each labeled colloid was incubated with human plasma and human synovial fluid for 72 h in a $CO_2$-incubator, which preserves 5% $CO_2$ at 37° C. The human plasma and synovial fluid were obtained from normal volunteers or rheumatoid arthritis patients, respectively. The stability was evaluated by chromatography (ITLC-SG/normal saline) and a TLC scanner was used to monitor the radioactivity. The soluble free $^{188}$Re was moved to the solvent-front and the colloidal $^{188}$Re remained at the origin in this condition.

### Determination of particle size

The particle size of each colloid preparation was determined using filters with pore sizes of 10, 5, 1 and 0.22 μm according to the methods reported by Jeong *et al* (2000) (Nucleopore Co. or Millipore Co.). Briefly, a 1 mL aliquot (~35 MBq) of each radiolabeled colloid was passed through serially connected filters that were stacked beginning with a 10 μm filter. After washing the filters by passing additional 4 mL of normal saline, the radioactivity remaining in the pooled eluent and each filter was measured by a gamma scintillation counter (Packard Co.). The mean values were obtained from two separate experiments to determine the particle size.

### Ovalbumin (OVA)-induced arthritis in the rabbits

Arthritis was induced by injecting ovalbumin (OVA) into the joint of OVA-immunized rabbits according to the method reported by Pettipher *et al* (1989). Briefly, the New Zealand white (NZW) rabbit weighing 3-3.5 kg were immunized by an intra-dermal injection of 1 mg OVA in 1 mL of Freunds complete adjuvant to initialize the antigen-induced arthritis model. The animals were again immunized three weeks later in the same way. Three weeks after the second immunization, arthritis was induced in one knee joint by an intraarticular injection of 5 mg OVA in 1 mL of sterile saline. The contralateral knee joint was injected with 1 mL sterile saline to serve as the within-animal control. The rabbit knees were injected with the 55.5 MBq/head $^{188}$Re colloids 2 weeks after injecting the OVA. Joint swelling was estimated as the difference in the joint diameter between the pre-arthritic and post-arthritic joint diameter. The animals were sacrificed six weeks after inducing arthritis. The knee joint diameter at three weeks and six weeks were measured, and the macroscopic (Table I) and histological score (Table II) were measured at six weeks.

**Table I.** Criteria for macroscopical scoring of the arthritic knee joints

| Criteria | Score | Appearance |
|---|---|---|
| Synovium | 1 | Slight hyperplasia and vascularization |
| | 2 | Score 1 plus occasional petechiae |
| | 3 | Hyperplasia with petechiae and some discoloration |
| | 4 | Hyperplasia with many petechiae and discoloration |
| Cartilage and Bone | 1 | Cartilage erosion |
| | 2 | Cartilage erosion with limited bone erosion of femoral condyles |
| | 3 | Extensive erosion of femoral condyles |
| | 4 | Erosion also affecting the intercondylar fossa and patella |

**Table II.** Criteria for histological scoring of the arthritis knee joints

| Score | Appearance |
|---|---|
| 1 | Normal synovium with a few plasma cells and lymphocytes |
| 2 | Moderate synovial hyperplasia with plasma cells and lymphocytes |
| 3 | Increased hyperplasia and cellular infiltrate with vascul tis |
| 4 | Cellular infiltration of whole synovium, pannus formation with abnormal chondrocyte distribution and cartilage erosion |
| 5 | Dense cellular infiltrate with erosion of cartilage and bone |

### Acute toxicity and biodistribution of $^{188}$Re-tin colloids in rodents

The $^{188}$Re-tin colloids (0.15, 0.45, 1.5, and 4.5 mCi/head) were injected either into the tail vein of the ICR mice or into the joint of the SD rats and the general signs were examined for 14 days. Fourteen days after injecting the $^{188}$Re-tin colloids, the animals were sacrificed and the hematological and blood biochemical changes were measured, and necropsy was performed. The tissues were collected at 24 or 72 h after $^{188}$Re-tin colloids injection and radioactivity was measured by gamma scintillation counter (Packard Co.) to test the tissues distribution of the$^{188}$Re-tin colloids in rats.

### Statistical analysis

The results are expressed as means ± SE. The statistical differences between the groups were established using the Students *t*-test or an analysis of the variance (ANOVA) and considered as significant when the P value was less than 0.05.

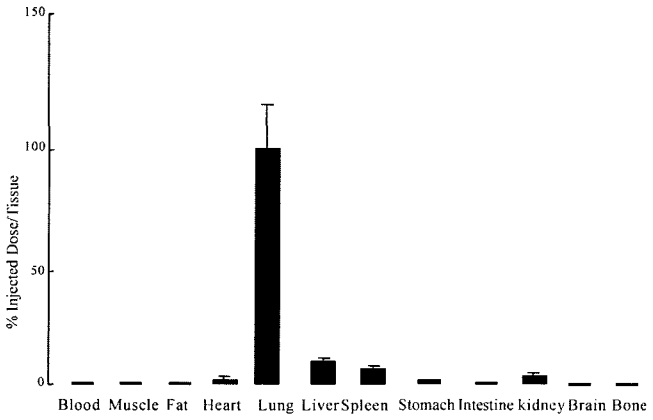


**Fig. 1.** Biodistribution of the $^{188}$Re-tin colloid at 1 h after the intravenous injection into the tail of the ICR mice. Data are expressed as mean ± SE (n=4).

## RESULTS

### The stability of $^{188}$Re-tin colloids

At 24 h after injecting the $^{188}$Re-tin colloids (74 KBq/ 0.1 mL) in the tail vein of the ICR mice, most of the $^{188}$Re-tin colloidal particles were found in the lungs (Fig. 1). This suggests that the $^{188}$Re-tin colloidal particle sizes are so large that they cannot across the capillary vessel. The $^{188}$Re-tin colloids (55.5 MBq/0.15 mL) were injected into the New Zealand white rabbits at the cavum articular, and 98.7% of $^{188}$Re-tin colloids were detected at the injection site (Table III). After injecting the $^{188}$Re-tin colloids in the human plasma and synovial fluid, there was no particle sizes change at room temperature or at 37°C (Table IV). This suggests that $^{188}$Re-tin colloids are stable at both room temperature and 37°C either before or after the injection into the cavum articular.

### Effects of $^{188}$Re-tin colloids in OVA-induced arthritic rabbits

A single intraarticular injection (1.5 mCi/head) of the $^{188}$Re-tin colloid significantly inhibited the lesion of the macroscopic score when compared to the arthritic control. The histology of the arthritic rabbits and the knee joint diameter were examined. The histological score was also reduced after the $^{188}$Re-tin colloid treated (Fig. 2). The knee joint diameter was higher in the arthritic rabbit compared to the control. However, in the $^{188}$Re-tin colloid groups, the increases in the knee joint diameter was lower than that observed in the arthritic control (Table V).

**Table IV.** Particle size change of the $^{188}$Re-Tin colloids at either room temperature or 37°C (parenthesis) in human plasma and synovial fluid

| Time \ Particle Size | <0.45 μm | 0.45-1.2 μm | 1.2- 5 μm | >5 μm |
|---|---|---|---|---|
| 0 | 1.63% | 5.23% | 1.54% | 91.6% |
| 2 h | 2.36 (1.91)% | 4.10 (4.26)% | 4.24 (7.7)% | 89.3 (86.1)% |
| 4 h | 2.03 (1.93)% | 4.55 (4.20)% | 0.77 (1.54)% | 91.6 (92.3)% |
| 6 h | 1.81 (2.11)% | 4.65 (3.97)% | 0.39 (0.39)% | 93.2 (93.5)% |
| 8 h | 2.13 (1.55)% | 4.33 (4.20)% | 2.31 (8.86)% | 91.2 (85.4)% |
| 24 h | 4.31 (2.68)% | 3.97 (5.42)% | 3.20 (4.24)% | 88.5 (87.7)% |

**Table III.** Biodistribution of the intraarticularly injected the $^{188}$Re-Tin colloid in rabbits

| Tissue concentration (% injection dose/organ/24 h) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Blood | Muscle | Heart | Lung | Spleen | Stomach | Intestine | Liver | Kidney | Cavum articular |
| 0.171 | 0.206 | 0.003 | 0.0004 | 0.0000 | 0.044 | 0.126 | 0.179 | 0.194 | 98.7 |

Data are expressed as mean (n=5).

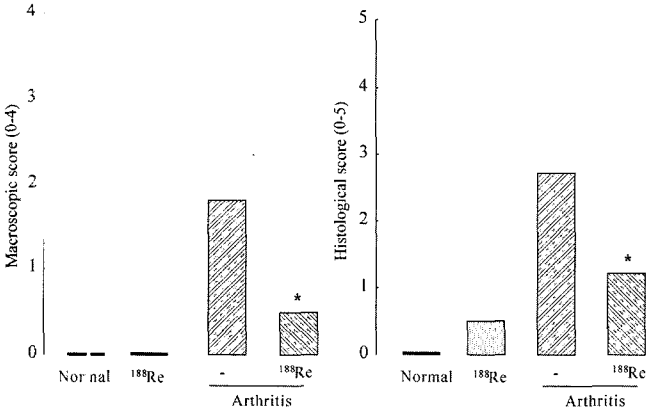


**Fig. 2.** The effects of $^{188}$Re-tin colloid on the macroscopic or histological score in the OVA-induced arthritic rabbits. $^{188}$Re-tin colloid significantly inhibited the increase in the macroscopic or histological score when compared to the arthritis control. The data is expressed as a mean ± SE. * $P<0.05$ vs. arthritis control (n=17).

**Table V.** Effect of the $^{188}$Re-Tin colloid on the knee joint diameter (mm) of arthritic rabbits

| | Knee joint diameter (change %) | | |
|---|---|---|---|
| | 0 | 3 weeks | 6 weeks |
| Normal (N=4) | 18.8 ± 0.2 | - | - |
| Normal-$^{188}$Re (N=6) | 18.9 ± 0.4 | 18.9 ± 0.4 (100) | 18.8 ± 0.4 (99.5) |
| Arthritis control (N=6) | 24.5 ± 0.7 | 23.6 ± 1.1 (96.7) | 23.6 ± 1.2 (96.7) |
| Arthritis-$^{188}$Re (N=6) | 25.0 ± 0.7 | 22.4 ± 1.1 (89.6) | 22.5 ± 1.0 (90.6) |

Data are expressed as mean ± SE

**Table VI.** The white blood cell (WBC) population after or before the $^{188}$Re-Tin colloid injection into the ICR mice

| | Normal | 0.15 mCi /head | 0.45 mCi /head | 1.5 mCi /head | 4.5 mCi /head |
|---|---|---|---|---|---|
| WBC ($10^3$/μl) | 7.2 ± 1.4 | 6.3 ± 0.8 | 4.3 ± 0.9 | 3.6 ± 0.2 | – |

Data are expressed as mean ± SE

### Acute toxicity and Pharmacokinetics of $^{188}$Re-tin colloids

In the mouse toxicity test, the $^{188}$Re-tin colloid was intravenously injected from at doses ranging from 1.5 to 4.5 mCi/head and the white blood cell (WBC) population was measured. In the normal ICR mice, the WBC population was 7.2 ± 1.4×$10^3$ per μL. As the $^{188}$Re-tin colloid concentration was increased, the WBC population decreased with $IC_{50}$ of 0.9 mCi/kg (Table VI). In the rat toxicity test, the clinical sign, body weight, mortality, hematology, blood biochemical changes, and necropsy were examined but there were no significant systemic changes (Data not shown). We investigated the leakage of the colloid from the intraarticular injection site using gamma counting in normal rats. In the biodistribution study, the liver showed the highest radioactivity (0.0427% ID/organ) except the injected knees (99.49% ID/organ) (Fig. 3).

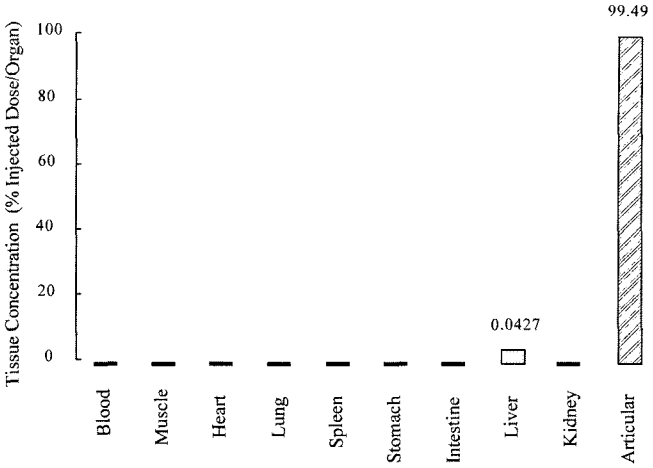


**Fig. 3.** Biodistribution of the $^{188}$Re-tin colloid at 24 h after the intraarticular injection into the knee of the SD rats. The liver produced the highest radioactivity (0.0427% ID/organ) except for the injected knees (99.49% ID/organ). The data is expressed as a mean (n=4-5).

## DISCUSSION

Rheumatoid arthritis (RA) is a chronic, systemic disease, which is characterized by the progressive destruction of the affected joints. Synovial hyperplasia, massive cellular infiltration, erosion of the cartilage and bone, and abnormal immune response are the main characteristics of RA. This disease affects approximately 1% of the population and results in a reduced life expectancy. Although much has been learnt about the mediators that drive the pathology in RA in recent years, the underlying causes of the disease are still unclear (Feldmann *et al.*, 1996; Jorgensen and Gay, 1998).

Radiation synovectomy has been used as an alternative treatment for rheumatoid arthritis for more than 40 years (Ures *et al.*, 2002). However, it has not been widely adopted because of radiation leakage from the treated joints, its limited availability and high costs (Zuckerman *et al.*, 1987). One of the most important characteristics of a radiopharmaceutical for synovectomy is to deliver a radiation dose in the target tissue. Good stability and low cost are also very important features. The $^{188}$Re-tin colloid fulfills both objectives, which makes it an attractive radiotherapeutical agent for treating rheumatoid arthritis in the knee. When measured at 24 hours after administration, nearly 98% of the total radiation dose remained inside the knee joint. The main advantage of $^{188}$Re is its convenience of preparation, since it can be produced by a generator system. The long physical half-life of the $^{188}$W patent (69 days) gives the generator system a long shelf life and results in a lower $^{188}$Re production cost. The $^{188}$W/$^{188}$Re generator, with a

shelf life of six months, has a suitable performance for this clinical application (Ures *et al.*, 2002).

For radiation synovectomy, the particle size of the radiocolloid should be between 2 to 30 μm. If the particle size is ≤1 μm, leakage from the synovial space can occur and the particles will not be phagocytosed by the synovial tissue. If the particle is ≥30 μm, the particle cannot be phagocytosed by the synovial tissue (Jeong *et al.*, 2000). $^{188}$Re is an ideal radiopharmaceutical agent because the beta ray (2.1 MeV) that is emitted from $^{188}$Re are appropriate for synovial cell ablation and the gamma ray (155 KeV) are ideal for dosimetry. The ideal particle size (2-5 μm) was achieved by a conjugation with a tin-colloid. According to the results, the $^{188}$Re tin colloid radiolabeled at 100°C had an adequate particle size for the radiation synovectomy. One practical problem of the $^{188}$Re-sulfur colloid was the residual radioactivity in the syringe after use, which makes it difficult to predict the injected dose, and leads to a waste of radioactivity (Kim *et al.*, 1998). The hydrophobic property of the $^{188}$Re-sulfur colloid is believed to be the cause of this problem. In order to prevent this problem, a stabilizing agent (e.g. gelatin) needs to be added to the preparation. However, the $^{188}$Re-tin colloid does not require any stabilizing agents (Jeong *et al.*, 2000).

If the size of the colloid is larger than the diameter of the capillary, the particles will mainly accumulate in the lung after the intravenous injection due to a capillary blockade, while smaller size colloids mainly accumulate in the liver due to phagocytosis of the reticuloendothelial system (Jeong *et al.*, 2000). The results of the biodistribution in the rats were consistent with this assumption. The $^{188}$Re-tin colloid was well retained in the synovial space for 24 h in the rats. The $^{188}$Re-tin colloid was retained in the knee throughout the study after the intra-articular injection. Since the mean retention percentage of radioactivity in the injection site, as determined by a gamma counter, was 99.49% at 24 h, the estimated doses in other organs were very low.

In conclusion, the $^{188}$Re-tin-colloid is a strong candidate for radiation synovectomy owing to its superior efficacy and safety.

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