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Original

Preliminary studies with ^{188}Rhenium-tin colloid for radiation synovectomy: preparation, size determination, *in-vivo* distribution, effects and dosimetry studies

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Abstract

BACKGROUND: Generator-produced beta-emitting radionuclides such as ^{188}Re are gaining in importance for radiosynoviorthesis because of their availability on a regular basis.
MATERIAL AND METHODS: We prepared a ^{188}Re-tin colloid in a reaction carried out either at 100°C or at room temperature (RT). The size of the colloid particles was measured with a laser light-scattering method, and their biodistribution, dosimetric aspects and therapeutic effects were studied in an antigen-induced arthritis (AIA) model in rabbits. ^{188}Re-tin colloid solution was injected intra-articularly into the knee joints of rabbits with AIA and imaging studies were performed. Blood samples were collected post injection for estimation of the blood residence time. We also injected 2 intact rabbits in the same manner with ^{188}Re perrhenate solution in order to observe its effects and distribution in the body. All the treated rabbit knees were subjected to histopathology.
RESULTS: The colloid particle size distribution was different after preparation at the different reaction temperatures, with a more suitable mean of 4.53 μm in the RT preparation. The dose delivered to the synovial surface was between 3.51 and 4.21 Gy and that to the bone surface was between 0.70 and 0.84 Gy. Histopathologic examination revealed the development of fibrous connective tissue in the AIA knees 4 weeks after treatment, but not in the control group.
CONCLUSIONS: The ^{188}Re-tin colloid preparation used in this study was suitable for radiation synovectomy application. It requires modifications in the preparation protocol so as to increase the labeling efficiency in correlation with an appropriate particle size.
Key words: radiosynovectomy, antigen-induced arthritis, rheumatoid arthritis, dosimetry, radiocolloid, radiopharmaceutical

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Introduction

Radiation synovectomy is a technique whereby a beta-emitting radiopharmaceutical is delivered into the affected synovial compartment in order to treat rheumatoid arthritis. Beta-emitting radiocolloids are widely used for this purpose. The ideal radionuclide would possess beta emission with a sufficient energy for a maximum tissue penetration of 5 to 10 mm, gamma emission suitable for gamma camera imaging, a short half-life, and ready availability. A ^{198}Au colloid was first used for radiocolloid synovectomy and this isotope has been continuously investigated [1–3]. The main drawbacks of a ^{198}Au colloid are the 411 keV gamma emission which creates an unnecessary radiation hazard, the small particle size, which results in excessive loss from the joint space by lymphatic drainage, and high radiation doses to the proximal lymph nodes. The high costs have also impeded its routine application. Other radiopharmaceuticals containing isotopes such as ^{32}P and ^{90}Y, have been used as alternatives to ^{198}Au, and show less leakage from the joint [4–8]. The generator-produced ^{188}Re

recently became practically available [9, 10]. Among the ^{188}Re--labeled colloids, ^{188}Re-sulfur colloid has been most extensively investigated [11, 12] and the use of ^{186}Re-sulfur colloid has also been reported [13]. ^{188}Re-hydroxyapatite has been studied for radiation synovectomy as a biodegradable colloid [14] and a ^{188}Re microsphere has been introduced for radiation synovectomy [15]. Jeong et al. studied [16] the preparation and use of a ^{188}Re-tin colloid and reported that it is more suitable than a ^{188}Re-sulfur colloid in stability, labeling efficiency and residual radioactivity. Our experiments were designed to study the circumstances of preparation of ^{188}Re-tin colloid and to evaluate its suitability for radiosynoviorthesis in conjunction with dosimetric aspects.

Material and methods

Radiopharmaceutical preparation

^{188}Re-tin colloid: Aliquots of 0.5 ml of nitrogen-purged 0.1 N HCl containing 5 mg $SnCl_2$ $2H_2O$ (Merck) were dispensed into vials under a nitrogen atmosphere. The vials were capped under nitrogen with a rubber septum and an aluminum seal, and stored in a refrigerator until use. Radiolabeling was performed by adding to each vial a 0.5 ml aliquot of ^{188}Re-perrhenate (~80 MBq) freshly eluted with saline from an alumina-based ^{188}W/^{188}Re generator (Oak Ridge National Laboratory, USA). Incubation was performed for 120 minutes, either at room temperature or at 100°C.

The labeling efficiency was checked by thin-layer chromatography (TLC-SG/acetone, Kieselgel, Merck, Germany). The radioactivity was monitored by cutting the gels into 20 parts, each 1 cm in length, numbering the parts from the starting point and measuring their activities in a well-type gamma counter with an automatic sample changer (NK-350, Gamma, Hungary). Free sodium perrhenate and the examined preparation were dropped in parallel onto TLC plates and after 1 h the plates were processed. Activity vs. distance curves were plotted and the labeling efficiency was calculated. The radiolabeled ^{188}Re-tin colloid was neutralized by the addition of 0.2 M sodium phosphate buffer (pH = 8) solution.

Determination of particle size

The particle diameter spectrum of each colloid preparation was obtained by using a dynamic laser light-scattering device (Dynapro, Protein Solutions, VA, USA). A laser Doppler effect was used to determine the third-degree Brownian motion of the particles, and dedicated software (Dynamics v. 6.0, ProteinSolutions, VA, USA), based on the Stokes-Einstein equation, was applied to determine the average diameter of the particles and the distribution histogram. A spherical molecule standard model was used for the assessment. A 50-μl aliquot of each labeled colloid solution was transferred to the cell of the micro sampler and measured ten times at RT with a laser light of 830 nm wavelength.

Animal experiments

Following the AIA model descriptions by Steinberg et al. and Dumonde et al. [17, 18], 9 male New Zealand White rabbits (3–4 kg) were injected intradermally over 5 sites on the back with 10 mg of ovalbumin solution (Sigma, Germany) emulsified in 1 ml of Freund's complete adjuvant (Sigma, Germany) as first sensitization. The animals were resensitized 3 weeks later. A dose of 0.1 mL of the above solution was administered aseptically intra-articularly into each right knee joint 2 weeks after the second immunization. The intra-articular injection was performed by entering the joint at the middle of the patellar tendon with a 16 G sterile needle, drawing back approximately 0.1 mL of synovial fluid and injecting the challenger solution. The knees were kept immobile for 1 minute and then moved three times. For each procedure, anesthesia was attained with 10 mg/kg of ketamine (SBH-Ketamin, SBH, Hungary) and 5 mg/kg of xylazine (Primazin, Alfasan, The Netherlands), given intramuscularly. Three weeks after the intra-articular challenge, 5 rabbits were injected intra-articularly under the same conditions as during the challenge with a net activity of 49 MBq in 0.25 mL of freshly prepared ^{188}Re-tin colloid solution produced at RT. Four weeks later, the rabbits were euthanased with pentobarbital (Nembutal, Phylaxia-Sanofi, Hungary) and the knees were removed, and subjected to histopathology. Four arthritic rabbits kept as untreated controls, were sacrificed at the same time and also subjected to histopathology. Additionally, 2 healthy non-treated rabbits were subjected to the same intra-articular injection procedure, receiving around 50 MBq of freshly eluted ^{188}Re perrhenate in 0.1 mL of physiological saline. They were treated in the same manner as the treated rabbits during the experiments, with the exception of the blood sampling.

An 18G-cannula (Medicor, Hungary) was implanted into the jugular vein of the treated rabbits, and 2-mL blood samples were taken at 1, 3, 24 and 48 h after the application of the radiopharmaceutical.

All animal studies were carried out by prior permission of the local Animal Experiment Ethical Committee.

Imaging studies

Three, 24, 48 and 72 h after the application of the radiopharmaceutical, images were acquired with a gamma camera (Nucline X-Ring, Mediso, Hungary), with a high-energy general-purpose collimator at a 512 × 512 × 16 matrix size. Region of interest (ROI) data in counts per minute (cpm) were collected by an image processing software (InterView©, Mediso, Hungary). Knee to knee uptake ratios were calculated for all rabbits receiving any radioactive substance in the knee joint.

Dosimetric calculations

Radiation doses throughout treated joints were estimated as a function of time by using the absorbed dose factors. Calculations of doses and absorbed dose factor determinations were conducted according to the work of Johnson et al. [19, 20]. For a location in the joint model and at any time *t* after injection, the radiation absorbed dose from the injected radioactivity is calculated as the product of the cumulated activity $A_{cumulated}(t)$ (MBqs) in the source region and the absorbed dose factor F_i (cGy × cm^2 × MBq^{-1} × s^{-1}) divided by the estimated total synovial surface area S_{syn} of the joint (250 cm^2 — as described in [20] for human synovium) so that

$$D_i(t) = A_{cumulated}(t) \times F_i / S_{syn}$$

where $A_{cumulated}(t) = A_o \times \tau_{knee}$, A_o is the administered activity (MBq), and τ_{knee} is the knee residence time (s). F_i for ^{188}Re and the synovium surface is 0.05 cGy × cm^2 × MBq^{-1} × s^{-1}; for the knee bone surface it is 0.01 cGy × cm^2 × MBq^{-1} × s^{-1}.

Mean residence times were calculated on the basis of the ROI activity data for knee joints. Blood residence times were calculated by measuring the activities (in the well-type gamma counter) and the masses of the samples. Medical Internal Radiation Dosimetry (MIRD) methods were used to determine mean residence times. Using the values of residence times, extrapolation to adult human total body and specific organ absorbed dose values was obtained by the MIRDOSE 3.1 software developed by Michael Stabin et al. at Oak Ridge Institute for Scientific Education and Oak Ridge National Laboratory, US.

Histopathological examinations

The knee joints were dissected, placed into 10% m/v buffered formalin solution for 3 days, and joints were embedded in paraffin. Series of 20-μm thick longitudinal sections were made and stained with hematoxylin-eosin, and synovial membranes, synovial spaces, cartilage, and bones were evaluated for the presence and quality of inflammation and/or fibrous tissue proliferation by an expert histopathologist (R. G.).

Results

Radiolabeling

The labeling efficiency was determined on the basis of chromatograms. Mean values of three measurements are shown for the RT chromatogram in Figure 1. The labeling efficiency was 97.3% for the preparation at 100°C and 63% for that at RT. The solution was clear and transparent after labeling at 100°C and opaque, milky, very slowly sedimenting (during two hours, practically no sedimentation was seen), non-turbid for the reaction carried out at RT; the situation remained similar throughout all procedures.

Size distribution

The particle size distribution was first assessed by the evaluation of ITLC (paper) chromatograms, where the different peaks represent different particle sizes formed in the reaction. For the reaction performed at 100°C, the distribution led to a non-uniform spectrum with a larger number of smaller particle peaks. The chromatogram of the RT product is shown in Figure 1. The size distribution was also measured with the laser scattering method. These measurements confirmed that the size distribution was more uniform for the preparation produced at RT than for that produced at 100°C. In the product of the 100°C reaction, 15% of the particles measured < 1 μm and 85% of the particles measured > 3 μm. A more uniform size pattern, with the 1% of the particles measuring < 1 μm versus 99% measuring > 3 μm, was seen after the RT reaction. The dedicated software of the device gave an assessed mean value of 4.535 μm for the colloid produced at RT.

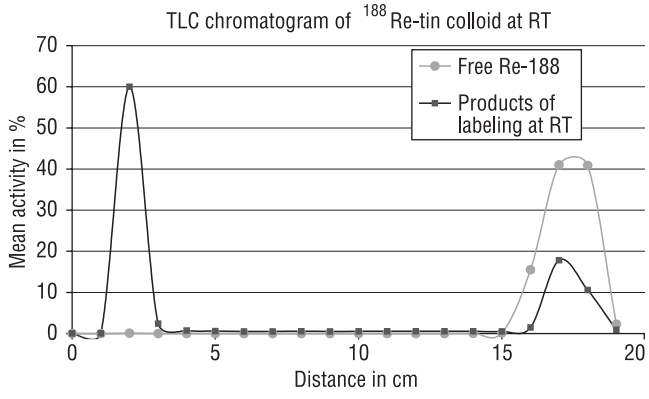

Figure 1. TLC chromatogram of ^{188}Re-tin colloid produced by reaction at room temperature.

Animal experiments

Retention of ^{188}Re-tin colloid in the synovial space of the AIA rabbits was observed up to 72 h. No leakage to the inguinal lymph node (the predilection site of the accumulated outflow of radioactivity) or other lymph nodes could be observed (Fig. 2). Figure 2 presents static scintigraphic images of the knee regions of a rabbit at 3, 24, 48, and 72 h after the injection into the knee. The knee to knee radiopharmaceutical uptake ratios were stable in time; the averages and standard deviations are listed in Table 1. In whole-body scans, no other activity accumulation was seen anywhere in the body (e.g. in the predilection sites such as the thyroid gland and the gastric mucosa). The scans of healthy rabbits receiving only intra-articular ^{188}Re perrhenate solution revealed uptake in the predilection sites — stomach mucosa and thyroid gland were visualized in the images, most prominently 3 h after injection (Fig. 3). Later healthy rabbit scans demonstrated a very fast excretion of perrhenate via the kidneys and bladder: 48 and 72 hours post injection, only background activity could be observed in healthy rabbits receiving perrhenate eluate.

Histopathology

We detected a severe inflammation of the synovial membrane and knee cartilage surfaces, characterized by the infiltration of heterophilic leukocytes, macrophages and a smaller number of lymphocytes, together with thickening of the synovial membrane in the control animals. In intact rabbits receiving intra-articular ^{188}Re perrhenate solution, a mild heterophilic granulocytosis was present. Histopathological examination 4 weeks post injection clearly showed the development of fibrous connective tissue in all of the AIA knees. This was not seen in any animal in the other groups. No adverse effect was observed on the bone surface at the delivered doses.

Dose calculation

Table 2 provides data on the residence times in the knee and blood. The dose delivered to the presumed human synovial surface was between 3.51 and 4.21 Gy, and that to the bone surface was between 0.70 and 0.84 Gy. Tables for different organ doses are shown in Table 3, which also contains an extrapolation to human adult absorbed doses obtained by the MIRDOSE 3.1 software.

Discussion

The main purpose of this preliminary study was to prepare a ^{188}Re-tin colloid and to evaluate it for suitability together with dosimetric analysis, for the treatment of rheumatoid arthritis.

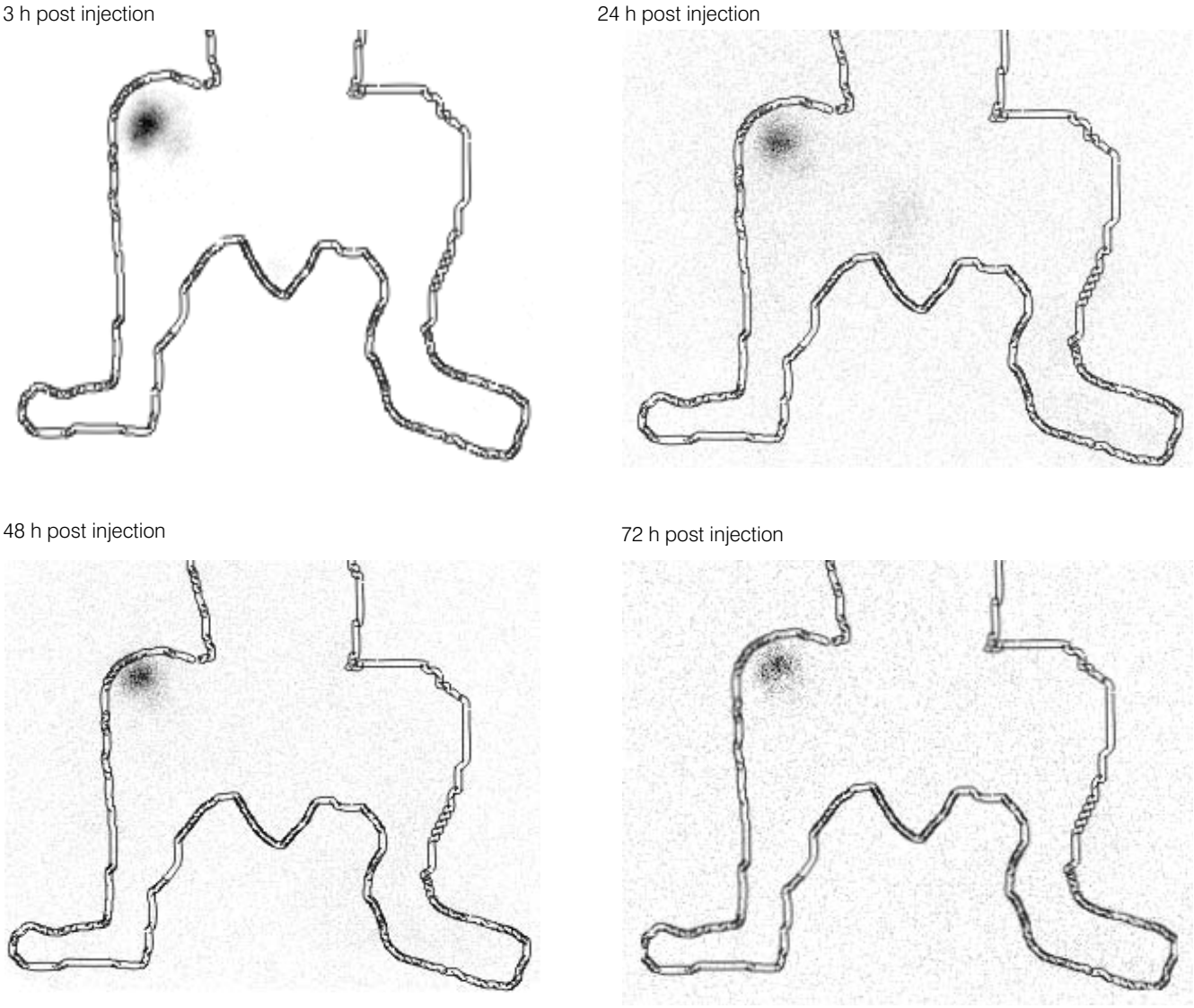

Figure 2. Static images of the knee of a rabbit taken at 3, 24, 48 and 72 h after injection. Differences in background are due to the automatic cutoff level setting of the camera software. Rabbit contours were generated from the acquisition data by using the InterView© software.

Table 1. Average treated/control values (cpm/cpm) in knee joints of treated rabbits

Animal	Treated/Control	SD
Rabbit A	12.10	2.47
Rabbit B	10.07	1.25
Rabbit C	9.96	1.02
Rabbit D	9.25	1.06
Mean	**10.34**	**0.87**

For radiation synovectomy, the radiocolloid particle size should be between 2 and 30 μm [21]. If the particle size is < 1 μm, leakage from the synovial space can occur and the material would not be phagocytosed by the synovial tissue. Our measurements, with the method of laser scattering to determine particle size, revealed that 99% of the ^{188}Re-tin colloid particles produced by the reaction at RT have adequate size to prevent their outflow from the joint. Analysis of the TLC results showed, that although the calculated labeling efficiency was 97.3% when the reaction mixture was heated at 100°C, smaller particles were present which made the preparation unsuitable for intra-articular administration. As the reaction product at 100°C yielded a less uniform pattern with more particles smaller than the desired size, we decided to use the RT product in the animal experiments even though its labeling efficiency determined by ITLC was only 63%. In contrast, the scintigraphic images indicated that the ^{188}Re-tin colloid was well retained in the synovial space for up to 72 h in arthritic rabbits with AIA, and no leakage was visualized. No sign of distribution similarity could be detected by scintigraphy in rabbits receiving intra-articular $^{188}ReO_4^-$ eluate and rabbits treated with intra-articular ^{188}Re-tin colloid solution. We presumed that that during the reaction of ^{188}Re tin colloid production, the unbound part of ^{188}Re would diffuse out of the joint. Retention of the radiopharmaceutical in the treated knee joint was confirmed by a time-independent, nearly constant activity ratio for the treated and control knees. As leakage could not be observed, our hypothesis is that different forms of ^{188}Re, probably reduced rhenium oxides were also produced by the reaction and

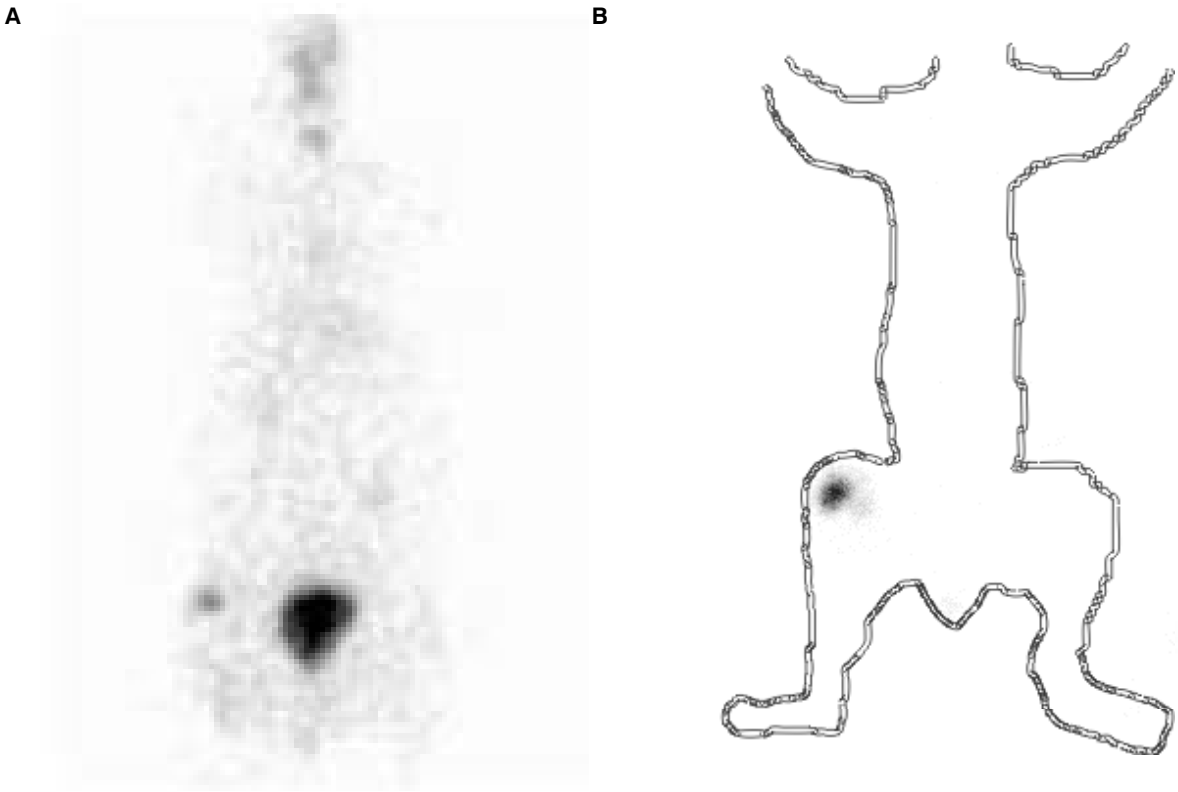

Figure 3. Comparison of biodistributions of the same amount and activity of intra-articular perrhenate eluate (**A**) and ^{188}Re-tin colloid solution (**B**) in rabbit, 3 h post injection.

Table 2. Residence times of ^{188}Re-tin colloid in rabbit blood and knee joint

Animal	Rabbit A	Rabbit B	Rabbit C	Rabbit D	Mean ± SD
Blood residence time [h]	0.89	1.15	1.41	1.21	**1.16 ± 0.21**
Knee residence time [h]	9.30	11.83	10.74	10.72	**10.64 ± 1.04**

Table 3. Absorbed doses in rabbit synovial surface and knee bone surface, calculated from the ROI acquisition data, and absorbed dose/activity data extrapolated to adult humans from rabbit calculations

Dose values from rabbit experiments(Gy)					
Animal	**Rabbit A**	**Rabbit B**	**Rabbit C**	**Rabbit D**	**Mean ± SD**
Synovial surface (250 cm^2)	3.51	3.94	3.33	4.21	**3.74 ± 0.40 Gy**
Knee bone surface	0.70	0.79	0.67	0.84	**0.75 ± 0.08 Gy**
Adult human absorbed doses extrapolated from rabbit data					
Organ					**Dose (mGy/MBq)**
Kidneys					0.02
Liver					0.02
Lungs					0.02
Red marrow					0.03
Bone surface					**0.28**
Synovial surface					**76.60**
Knee bone surface					**15.30**
Whole body					0.09
Effective dose					0.03

bound to the synovial membrane or taken up by synovial cells. Experiments to prove the presence of the above reduced rhenium oxides are continued in our laboratory. However, it is fundamental that a constantly higher ratio of labelled colloid be present in the reaction product as only this assures stable pharmacokinetics and thus dosimetric planning in patients. The histologic effects observed in our study are consistent with the recent observations of Wang et al. [22]. The formation of fibrous connective tissue and thinner synovial membranes was observed only in the treated arthritic knees, presumably as a consequence of the beta irradiation emitted by the intra-articularly injected ^{188}Re-tin colloid.

Conclusions

In conclusion, our results indicate that a ^{188}Re-tin colloid can be a safe and effective agent for radiation synovectomy; a suitable dose is delivered to the synovial membrane of the human knee not harming the deeper structures such as the bone. Further modifications are required in the preparation protocol in order to increase the labeling of colloid particles in the desired size interval.

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