Chemistry and Biological Behavior of Samarium-153 and Rhenium-186-Labeled Hydroxyapatite Particles: Potential Radiopharmaceuticals for Radiation Synovectomy

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Hydroxyapatite (HA), a natural constituent of bone, was studied as a particulate carrier for beta-emitting radionuclides in radiation synovectomy. Particles were radiolabeled with ^{153}Sm or ^{186}Re and their in vivo safety was investigated following intra-articular injection into knees of normal rabbits and rabbits with antigen-induced arthritis (AIA). Radiolabeling efficiency was greater than 95%; in vitro studies showed minimal (≤1%) loss of activity from particles over a 6-day period with ^{153}Sm-labeled HA and about 5% loss of activity over a 5-day period with ^{186}Re-labeled HA. The total cumulative extra-articular leakage of ^{153}Sm over 6 days was 0.28% in normal rabbits and 0.09% in AIA rabbits. Leakage of ^{186}Re from the joint was 3.05% over a 4-day period with 80% of extra-articular activity found in the urine. Histopathological evaluation of treated knees showed that HA particles are distributed throughout the synovium, embedded in the synovial fat pad. The ease and efficiency with which this HA carrier is labeled, coupled with observed extremely low leakage rates from the joint, make radiolabeled HA particles an attractive candidate as a radiation synovectomy agent for evaluation in rheumatoid arthritis patients.

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Approximately 1% of adults in the United States have definite or probable rheumatoid arthritis by current diagnostic criteria (*1*). The major cause of pain, as well as physical disability in these patients, is destruction of diarthrodial or synovial joints. This inflammatory response is modulated by synoviocytes, lymphocytes and macrophages in the synovium. Radiation synovectomy is the ablation of inflamed synovium by means of an intra-articular injection of a beta-emitting radionuclide (Table 1) in colloidal or particulate form (*2–18*). This technique has been used extensively in Europe for more than 25 yr. A major problem associated with use of radiocolloids has been excessive leakage (5%–25%) of radionuclides (*4*) possibly due to the relatively small size of colloids used. This problem was partly overcome by using radioactive particles or aggregates that were 1–20 μm in size (*19*). Ferric hydroxide macroaggregates (FHMA) were known to be taken up by synovial tissue and metabolized by synovial enzymes (*20,21*). Extra-articular leakage of radionuclides when labeled to FHMA was variable (1%–13%) (*6*). We have recently shown that although radiolabeled FHMA particles have significant advantages when compared to colloidal preparations for radiation synovectomy, FHMA may not be the ideal carrier system for long-lived radionuclides with low specific activities (*22*).

Recently, a number of radiolabeled microspheres

TABLE 1
Radionuclides Proposed for Use in Radiation Synovectomy

Radionuclide	Half-life (days)	Decay	Energy (MeV)	Range in tissue (mm)
^{153}Sm	1.95	β^-	0.70	2.5
		γ	0.08	
^{198}Au	2.7	β^-	0.96	3.6
		γ	0.41	
^{186}Re	3.75	β^-	1.07	3.7
		γ	0.14	
^{32}P	14.3	β^-	1.70	7.9
^{166}Ho	1.125	β^-	1.84	8.5
		γ	0.08	
^{165}Dy	0.0958	β^-	1.30	5.7
		γ	0.09	
^{90}Y	2.7	β^-	2.2	11.0

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TABLE 2
Extra-articular Leakage (%ID) of Non-HA Particle-Bound Radioactivity: Control Studies in Normal Rabbits

Organ	Time (hr)	$^{186}ReO_4^-$	^{186}Re-HEDP	^{153}Sm-citrate
Urine	0–24	82.2	53.1	3.5
	24–48	1.1	7.0	0.6
	48–72	0.9	2.9	0.6
Blood	0–24	0.020	0.96	0.06
	24–48	0.004	0.68	0.04
	48–72	0.009	0.52	0.03
Other organs	72			31.5
Total Leakage;	3 days	84.3	65.1	35.6

(*23–25*) were introduced as potential carriers of beta-emitting radionuclides. These agents all exhibit unacceptable extra-articular leakage of radionuclides. Although radiation synovectomy appears to be efficacious, its safety is suspect and the challenge to find an appropriate radiopharmaceutical still remains. We have developed a new class of agents (*26–28*) for radiation synovectomy using particles made from hydroxyapatite (HA), a natural constituent of bone and biologically compatible. In this report, we evaluate the safety of ^{153}Sm and ^{186}Re-labeled HA particles in normal rabbits and rabbits with antigen-induced arthritis (AIA).

MATERIALS AND METHODS

Preparation of Radiopharmaceuticals

Hydroxyapatite Particles. Spherical and porous hydroxyapatite (HA) particles varying in size between 15 μm and 40 μm were supplied by CeraMed Corporation (Denver, CO). Particles were prepared by initially forming a precipitate from the reaction of $Ca(NO_3)_2$ and $(NH_4)_3PO_4$ at high pH (*29*). The precipitate was suspended in aqueous solution and subjected to a spray-drying process to produce HA particles of controlled size and range. Relative size distribution of particles used in these studies is shown in Figure 1. At least 90% of HA particles were in the range of 20–40 μm.

Rhenium-186-HEDP-HA. Radiolabeling of HA particles with two steps. Preparation of ^{186}Re-hydroxyethylidenediphosphonate (HEDP) was followed by the incubation of ^{186}Re-HEDP with the HA particles.

Rhenium-186-HEDP was prepared by adding 1 ml containing 1.11 GBq (30 mCi) of a Na[^{186}Re]ReO_4 solution (6.18–10.06 GBq (167–272 mCi) ^{186}Re/mg $NaReO_4$, (Mallinckrodt Medical, Inc., St. Louis, MO) to a vial containing a lyophilized mixture of Na_2HEDP (10 mg), $SnCl_2{\cdot}2H_2O$ (3.5 mg), and gentisic acid (3 mg). The resultant solution was autoclaved for 20 min at 121°C. Radiochemical purity (labeling efficiency) of ^{186}Re-HEDP was determined by two different ITLC methods. The first method used 0.01 *M* HEDP in saline on a silica gel ITLC to separate free ^{186}Re-perrhenate and ^{186}Re-HEDP from reduced hydrolysed ^{186}Re species (ReO_2). The second method used methyl ethyl ketone (MEK) on a silica gel ITLC to determine the amount of free ^{186}Re-perrhenate in the preparation.

Rhenium-186-labeled HA particles were prepared by sequential addition of the following materials to a vial containing 40 mg HA particles: N_2-purged saline (750 μl), ^{186}Re-HEDP (0.074–0.11 GBq (2–3 mCi), 100 μl), 20% Triton-X 100 in water (50 μl) and $SnCl_2{\cdot}2H_2O$ (100 μl of a 4 mg/ml solution in N_2-purged water). The mixture was purged with additional N_2 and then stirred for 60 min at room temperature. Contents of the vial were transferred to a 15-ml centrifuge tube using 4-ml N_2-purged saline to rinse. Radiolabeled particles were separated from free ^{186}Re activity via centrifugation (8 min at 1000 rpm) to determine labeling efficiency. Labeled HA particles were then resuspended in 1–5 ml of saline.

Samarium-153-HA. Samarium-153 was supplied by the University of Missouri-Columbia Research Reactor (MURR) as ^{153}Sm-chloride in 0.1 N HCl (5.55–10.36 GBq (150–280 mCi) ^{153}Sm/mg Sm_2O_3). Preparation of ^{153}Sm-labeled HA particles was done in two steps. Preparation of ^{153}Sm-citrate was followed by incubation of ^{153}Sm-citrate with HA particles.

Samarium-153-citrate was prepared by adding sufficient citric acid monohydrate to the above ^{153}Sm-chloride solution to give a concentration of 15 mg/ml citric acid in 0.1 N HCl. The mixture was allowed to stand at room temperature for 30 min.

The HA particles were labeled by adding 250 μl (0.555 GBq (15 mCi)) of the above ^{153}Sm-citrate solution to a vial containing 40 mg HA in 750 μl of water. The vial was sealed and contents were gently agitated via rotation for 30 min at room temperature. Radiolabeled particles were transferred to a 15-ml centrifuge tube using 4-ml saline to rinse. Radiolabeled particles were separated from free ^{153}Sm activity via centrifugation (8 min at 1000 rpm) to determine labeling efficiency. Labeled HA particles were then resuspended in 5 ml of saline and autoclaved for 20 min at 121°C.

In Vitro Stability of Radiolabeled HA Particles

The in vitro stability studies were performed by incubating labeled particles in 2 ml of either saline or diluted human synovial fluid at room temperature. Frozen synovial fluid was diluted 1:1 with saline (to reduce viscosity). At various times (up to three half-lives of the radionuclide), radiolabeled particles in the incubating fluid were centrifuged at 1000 rpm for 8 min and activity in the pellet and supernatant was measured. Stability of the HA particles radiolabeled with ^{153}Sm or ^{186}Re is shown in Figure 2.

Animal Model

Normal rabbits and rabbits with antigen-induced arthritis (AIA) were used as models to evaluate the in vivo stability and safety of

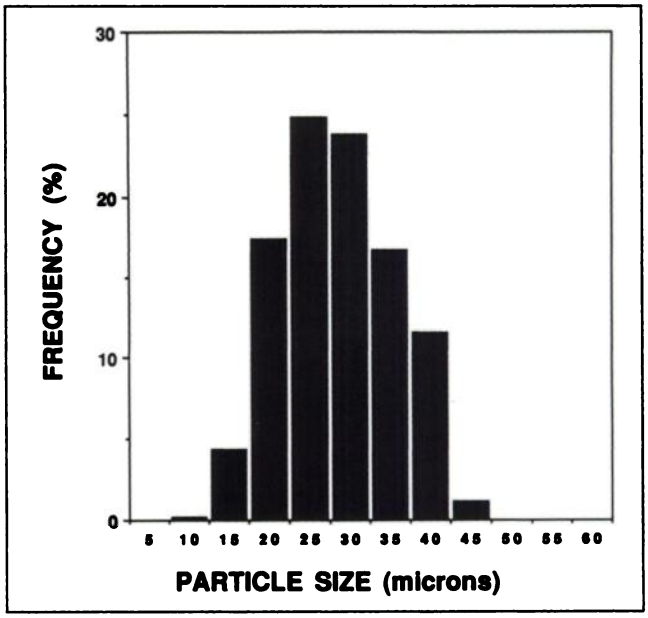

FIGURE 1. Frequency (%) distribution of hydroxyapatite particles as a function of particle size (μm).

radiolabeled HA particles. A joint lesion that closely resembles rheumatoid arthritis in man was developed in normal rabbits by Dumonde and Glynn (*30*). The model is called antigen-induced arthritis (AIA) as described by Steinberg et al. (*31*). In summary, New Zealand white rabbits (n = 14) weighing 3–4 kg were sensitized twice during a 6-wk period with 20 mg of ovalbumin given intradermally. At the end of this period, the animals received an intra-articular injection of ovalbumin in order to induce arthritis in the knees. Antigen-induced arthritis (synovitis) was achieved within 6–7 days.

Control Studies

To determine the biological fate of radionuclides that are injected intra-articularly but are not bound to HA, control studies were performed in three groups of normal rabbits (n = 3 per group). Each rabbit in a group was injected intra-articularly with 18.5 MBq (0.5 mCi) of one of the following three radiochemicals: ^{153}Sm-citrate, $^{186}ReO_4^-$ or ^{186}Re-HEDP. Daily blood samples were obtained over a period of 3 days. Animals were kept in metabolic cages and the total urine excreted within 3 days was collected in three separate containers. At the end of 3 days the animals were killed and organs were removed and weighed. Samples from each organ were weighed and counted in a well counter. The percent injected dose in blood, urine and different organs was calculated.

Joint Leakage Studies

Safety studies were first performed with ^{153}Sm-HA particles in normal rabbits (n = 12) and then in AIA rabbits (n = 10). Samarium-153-labeled HA particles (11.1–22.2 MBq (0.3–0.6 mCi)) were injected into one knee joint of a rabbit. Daily blood samples and total urine excreted were obtained over a period of 3–6 days. At the end of 3 days, six animals from the control group and four from the AIA group were killed and the remaining animals were killed at the end of 6 days. Percent injected dose in major organs, total urine and circulating blood was calculated. Extra-articular leakage (EAL) was calculated as the sum of all activities in major organs, total urine excreted and the activity remaining in the circulating blood at the time of sacrifice.

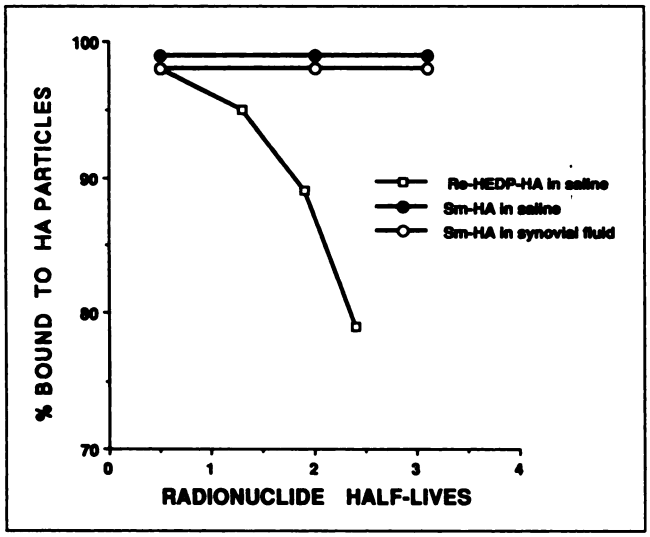

FIGURE 2. The in vitro stability of radiolabeled hydroxyapatite particles in saline and synovial fluid as a function of radionuclide half-life.

TABLE 3
Extra-Articular Leakage (%ID) of ^{153}Sm in Normal Rabbits

Organs	3 days (n = 6)	6 days (n = 6)
Blood	0.0004 ± 0.0001	0.0014 ± 0.0005
Liver	0.012 ± 0.003	0.029 ± 0.012
Kidney	0.004 ± 0.003	0.004 ± 0.002
Lung	0.070 ± 0.115	0.004 ± 0.006
Bone	0.005 ± 0.002	0.14 ± 0.07
Marrow	0.003 ± 0.002	0.005 ± 0.002
Muscle	0.0005 ± 0.0004	0.047 ± 0.094
Spleen	0.0001 ± 0.0001	0.0001 ± 0.0001
Lymph nodes	0.0004 ± 0.0002	0.0006 ± 0.0007
Urine (total)	0.068 ± 0.040	0.049 ± 0.007
Cumulative leakage	0.16 ± 0.16	0.28 ± 0.19

In a pilot study, safety studies were also performed with ^{186}Re-HEDP-HA in AIA rabbits (n = 4).

Histopathology

Following the animals' death, rabbit knees were carefully removed and specimens for synovial examination were placed in 10% neutral buffered formalin. Surrounding soft tissues were dissected and specimens were bisected on a small power band saw. Specimens were then placed in a solution of 20% formic acid made 20% in neutral buffered formalin to which 1 kg of washed weak cationic exchange resin (Dowex HCS-5) was added per 10 liters of solution. Baseline specimen radiographs were obtained and serial radiographs were then taken on a daily basis until decalcification was complete. The specimens were then trimmed, washed in running tap water for 1 hr and paraffin processed. Sections were cut at 5 μ, stained with hematoxylin-eosin, examined histologically and photographed with a Leitz Orthoplan 2 research microscope and a Leitz Vario-Orthomat 2 camera (Ernst Leitz, Wetzlar, Germany).

When specimens were examined for hydroxyapatite carrier particles, they were processed without decalcification. Specimens were placed in 10% neutral formalin and surrounding soft tissues dissected, and the specimens were bisected on a small power band saw. After an additional 24 hr in formalin, specimens were dehydrated in alcohol and xylene and immersed in methyl methacrylate monomer (MMM) for three successive changes of solution over a 24-hr period. Specimens were finally placed in polymerized MMM. Resultant blocks were cut at 5 μ on a Riechert polycut S sledge microtome (Jung Riechert, Vienna, Austria) and stained with hematoxylin-eosin, trichrome and Von Kossa silver stains. Examination and photography was performed as in the decalcified sections.

RESULTS

Radiopharmaceuticals

Labeling Efficiency. The hydroxyapatite particles selected for these studies are readily labeled with either ^{186}Re or ^{153}Sm. Labeling efficiency is always greater than 95%.

In Vitro Stability. Stability of the ^{153}Sm-HA preparation was studied in normal saline and in synovial fluid over 6 days. In saline, no dissociation of activity from the particles was observed. Similarly, in synovial fluid, 99% of activity remained bound to HA particles over the same period. The ^{186}Re-HEDP-HA preparation was studied only in normal saline; it showed higher dissociation rates com-

TABLE 4
Extra-Articular Leakage (%ID) of ^{153}Sm in AIA Rabbits

	3 days (n = 4)	6 days (n = 6)
Blood	0.0002 ± 0.0002	0.0003 ± 0.0001
Liver	0.007 ± 0.003	0.012 ± 0.007
Kidney	0.004 ± 0.001	0.002 ± 0.001
Lung	0.001 ± 0.001	0.010 ± 0.002
Bone	0.026 ± 0.006	0.039 ± 0.025
Marrow	0.001 ± 0.001	0.006 ± 0.004
Muscle	0.008 ± 0.014	0.008 ± 0.007
Spleen	0.0002 ± 0.0001	0.0001 ± 0.0001
Lymph nodes	0.0003 ± 0.0001	0.0001 ± 0.0001
Urine (total)	0.004 ± 0.002	0.011 ± 0.001
Cumulative leakage	0.05 ± 0.02	0.09 ± 0.04

TABLE 5
Extra-Articular Leakage (%ID) of ^{186}Re in AIA Rabbits

	Re-HEDP-HA (n = 4)	
Organs	2 days	4 days
Blood	0.02	0.03
Liver	0.01	0.02
Kidney	0.05	0.05
Lung	0.003	0.008
Bone	0.040	0.070
Marrow	0.010	0.020
Muscle	0.020	0.030
Spleen	0.0001	0.0002
Lymph nodes	0.0001	0.0003
Urine (total)	1.67	2.82
Cumulative leakage	1.82	3.05

pared to ^{153}Sm-HA. At 5 days, there was 5% unbound ^{186}Re activity that increased to 20% by 9 days. In some experiments (data not shown) it was observed that ^{153}Sm-HA exhibited high stability in an initially acidic medium (pH 3) over a period of several days. The pH of the medium, however, gradually increased during this period, probably due to the natural buffering capacity of HA. Under extremely acidic conditions (pH 1-2), however, radiolabeled HA particles and HA itself, would not be stable.

Animal Studies and Control Studies

The extra-articular leakage of radionuclides following injection of radiochemicals (not bound to HA particles) into knee joints of normal rabbits is summarized in Table 2. Results are expressed as the percent of injected dose (%ID) in the total blood or total urine (collected during a 24-hr period). A larger amount of ^{186}Re activity (82%) was excreted in urine within 24 hr of administration of ^{186}Re perrhenate when compared to 53% of excreted activity following the ^{186}Re-HEDP injection. In contrast, less than 5% of ^{153}Sm activity was excreted in urine within 72 hr after ^{153}Sm-citrate administration. With ^{186}Re, the total leakage in urine and blood over a period of 3 days was more than 65% of injected dose. Samarium-153-citrate, on the other hand, exhibited a total of only 5% leakage in urine and blood. Based on tissue distribution studies and organ counting, 32% of the ^{153}Sm activity was retained in the body, of which a significant amount (25%) was found in bone. These control studies show that more than 60% of the ^{153}Sm activity is retained within the joint space while most of ^{186}Re activity leaks from the joint.

Joint Leakage Studies

Samarium-153-HA Particles. The extra-articular leakage of ^{153}Sm activity following injection of ^{153}Sm-HA particles into the knee joint is summarized in Table 3 (normal rabbits) and Table 4 (AIA rabbits). Total cumulative leakage in normal rabbits is 0.16% in 3 days and 0.28% in 6 days. In AIA rabbits, the extra-articular leakage is 0.05% in 3 days and 0.09% in 6 days. All organs showed insignificant accumulation of ^{153}Sm activity. The ^{153}Sm activity that leaked from the knee joint was mostly seen in liver and bone.

Rhenium-186-HEDP-HA Particles. In a small pilot study involving four AIA rabbits, the safety studies were performed with ^{186}Re-HEDP-HA particles. Two animals were killed after 2 days and the other two after 4 days. The results are shown in Table 5. The cumulative leakage in 2 days is 1.82% and in 4 days is 3.05%. Most ^{186}Re activity that leaked from the knee joint (>80%) was excreted in urine.

Histopathology

Histopathology of a normal rabbit knee is shown in Figures 3A and 3B and that of an AIA rabbit is shown in Figures 4A and 4B. The normal rabbit knee joint shows (Fig. 3A) articular cartilage of normal thickness with smooth surface and clean joint space. The AIA knee reveals (Fig. 4A) a hypercellular synovial pannus consisting of vascularized fibroconnective tissue, inflammatory cells and proliferative synoviocytes filling the joint space overlying the articular cartilage and eroding it laterally.

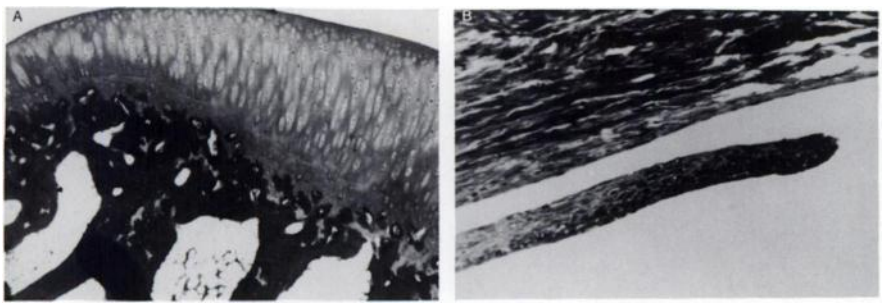

FIGURE 3. Histopathology of a normal rabbit knee joint. (A) A section through the distal femur showing articular cartilage of normal thickness with smooth surface and clean joint space (63×). (B) The detail of normal synovial tuft at the margin of joint capsule showing a fibrovascular core that has an almost inapparent synovial surface lining. Underneath the free tuft is a portion of fibrous joint capsule with a similar thin layer of inapparent synoviocytes (157×).

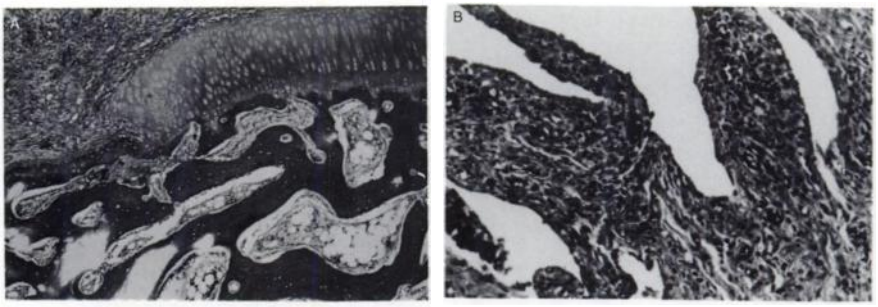

FIGURE 4. Histopathology of a rabbit knee with AIA infection. (A) A hypercellular synovial pannus consisting of vascularized fibroconnective tissue, inflammatory cells and proliferative synoviocytes filling joint space overlying the articular cartilage and eroding it laterally (63×). (B) Proliferation of fibrous tissue, blood vessels and synoviocytes to cause increased thickness of the synovial membrane. The synovial villi also contain an admixture of acute and chronic inflammatory cells.

The normal rabbit knee with HA particles is shown in Figures 5A and 5B. The synovial fat pad (Fig. 5A) shows deposits of HA particles. Figure 5B is a polarized photograph revealing moderately birefringent crystals of HA particles lodged in the synovium. Figure 6A and 6B show HA particles in the knee joint of a rabbit with AIA infection. The patellar fat pad (Fig. 6A) has large, dark aggregates of HA particles embedded throughout the synovium.

DISCUSSION

The HA particles used in this study appear to be a very attractive carrier for use in radiation synovectomy. HA is readily prepared from common chemicals and can be formed into particles of the desired size range in a controlled process. HA is a natural substance known to be biodegraded into calcium and phosphate ions. For many years, it has been used successfully as a coating for implants in joint arthroplasty and for dental reconstruction. This success is attributed to the biocompatibility of HA with soft tissue. Particles used in this study, however, differ from those used in dental procedures in that these particles are not subjected to a sintering process that hardens and toughens material. Unsintered HA particles are more susceptible to biological degradation. A recent study in normal rabbits shows that HA particles are indeed totally biodegraded and are no longer present in rabbit knees 6 wk postadministration (*18*).

Radiolabeling of HA with ^{186}Re and ^{153}Sm is simple to perform and provides excellent yields of labeled particles. The two-step procedure described herein (preparation of an intermediate radiochemical, followed by labeling of the hydroxyapatite particles) offers a fundamental advantage over previous one-step reactions that have been used to prepare radiocolloids. In the two-step procedure, the particulate carrier can be carefully prepared and controlled to have desired properties (size, density, porosity, biodegradability, etc.) before radiolabeling occurs; then the radiolabeling process can be independently optimized and controlled. In the one-step procedure, neither chemistry of the particle formation or the radiolabeling process can be independently controlled; the final product is always the result of balancing and compromising conditions for particle formation and radiolabeling.

The ^{153}Sm-HA particles demonstrate high in vitro stability in either saline or synovial fluid up to several half-lives of the radionuclide. The ^{153}Sm-HA particles also exhibited high stability at low pH (pH 3) over several days (unpublished results). The ^{186}Re-HEDP-HA particles, however, exhibit significant loss of label over time. The difference in stability between the two labeled species can be attributed to the chemical properties of each radionuclide. Samarium is a basic oxide (i.e., it yields alkaline solutions when added to water) and such +3 elements tend to form insoluble hydroxides and phosphates at physiological pH. Since hydroxyapatite itself is a natural buffer at around pH 7, samarium remains bound to the HA particle as insoluble species and cannot be washed off the particle until the HA particle itself dissolves. In vivo, any free ^{153}Sm binds predominantly and not to bone marrow.

Rhenium, on the other hand, is either an acidic or basic oxide depending on its oxidation state. Rhenium in the +4/+5 oxidation state, as in Re-HEDP, is a basic oxide. Therefore, as with samarium, rhenium remains bound to the HA particle. Rhenium-HEDP, however, is only stable in the presence of excess ligand and reducing agent. Without these, rhenium is easily oxidized to the +7 oxidation state, forming an acidic oxide. In aqueous media, these elements tend to exist in anionic forms (e.g., perrhenate, ReO_4^-) which are soluble and mobile at physiological pH. Thus, the ^{186}Re label washes off the HA particles with time

FIGURE 5. Normal rabbit knee with hydroxyapatite (HA) particles. (A) The synovial fat pad shows deposits of HA crystals (9.6×). (B) A polarized photograph shows moderately birefringent crystals of HA particles lodged in the synovium (125×).

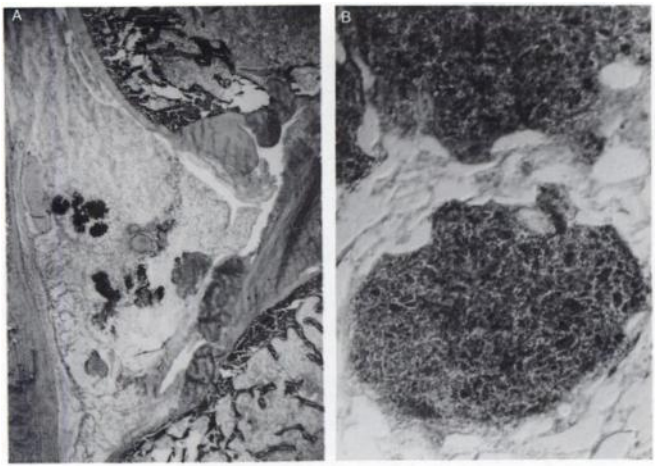

FIGURE 6. Rabbit knee with AIA infection with HA particles. (A) The patellar fat pad has large, dark aggregates of HA particles (9.6×). (B) A detail of microcrystalline aggregates of HA particles (125×, differential interference contrast microscopy).

because the rhenium presumably undergoes oxidation to ^{186}Re-perrhenate which has no affinity for HA. This type of in vivo oxidation process has been demonstrated for ^{186}Re-HEDP, a radiopharmaceutical in development for the relief of pain associated with metastatic bone disease (*32*).

The control studies performed with the ^{153}Sm and ^{186}Re compounds *not* bound to the HA particles provide information on the distribution of radioactivity that would occur upon leakage of these compounds or their reaction products from the joint. Samarium-153-citrate is retained to a greater extent in the knee than either ^{186}Re-HEDP or ^{186}Re-perrhenate. This observation is consistent with the basic oxide concept discussed above since samarium(III) forms insoluble hydroxides within the synovium. The ^{153}Sm activity that does leak from the joint is found mainly in bone, an observation consistent with what is known about bone uptake of free lanthanides. Rhenium, on the other hand, prefers the +7 oxidation state (an acidic oxide) in vivo; hence the ^{186}Re-HEDP (which undergoes oxidation to Re-(VII)) and ^{186}Re-perrhenate injected into the joint are expected to leak from the joint and appear in urine. This is indeed what is observed. Rapid renal excretion of leaked ^{186}Re tends to minimize any radiation exposure to normal organs caused by leakage.

The leakage of ^{153}Sm up to 6 days (three half-lives) postintra-articular administration of labeled HA is remarkably low and not too different between normal and AIA rabbits. This observation is in agreement with previous reports that found the leakage of ^{165}Dy-FHMA to be similar in both animal models (*12*). Rhenium-186 dissociates from the HA particles to a greater extent than ^{153}Sm (three orders of magnitude) which is consistent with the in vitro stabilities that show 20% loss of ^{186}Re from HA when ^{186}Re-HEDP-HA is incubated in normal saline. Although the extra-articular leakage of ^{186}Re is greater, more than 90% of leaked activity is excreted in urine.

Leakage rates found in this study are the lowest reported to date in the literature. Other carriers for ^{153}Sm and ^{186}Re have not been evaluated for periods longer than one half-life and even then have shown poor retention of activity in the injected joint (*24,25*). Macroaggregates of ferric hydroxide (FHMA), the carrier which has been most extensively studied for radiation synovectomy, has shown leakage rates of more than 1% over 24 hr when labeled with ^{165}Dy (*12*). The extreme short half-life of this isotope (2.3 hr), however, minimizes the amount of time during which leakage of radioactivity can occur. However, this short half-life also limits the practical utility of this radiopharmaceutical.

An important observation in these studies stems from histological analyses of joints injected with HA particles. Results show that HA particles are distributed throughout the synovium, suggesting that a high-energy beta particle may not be necessary for therapeutic treatment of a large inflamed joint when bound to HA particles. A weak beta-emitting radioisotope such as ^{153}Sm could be effective in treatment of a large joint because the HA carrier would be able to deliver beta radiation more evenly throughout the synovium.

Techniques described herein can be readily applied to a wide variety of therapeutic radioisotopes. The HA particles used in these studies can be labeled with any element that forms a basic oxide, such as ^{166}Ho (or any beta-emitting lanthanide for that matter) or ^{90}Y. Other beta-emitting radioisotopes can be bound to HA particles via chelating ligands that have high affinity for hydroxyapatite (such as diphosphonates). This carrier system has the potential of introducing into the medical community a new class of radiation synovectomy agents that can be designed for different-sized joints depending on beta energy of the radioisotope and desired dose to the inflamed tissue. Of the two radiopharmaceuticals studied here, ^{153}Sm-labeled HA particles show great promise in treatment of rheumatoid arthritis patients. Its short half-life, its extremely low leakage from the joint and its even distribution throughout the synovium make this agent attractive for use in radiation synovectomy.

REFERENCES

1. Harris ED Jr. Rheumatoid arthritis: the clinical spectrum. In: Kelley WN, Harris ED, Ruddy S, Sledge CB, eds. *Textbook of rheumatology*. Philadelphia: W.B. Saunders, 1981:930.
2. Ansell BM, Crook A, Mallard JR, Bywaters EGL. Evaluation of intra-articular colloidal ^{198}Au in the treatment of persistent knee effusions. *Ann Rheum Dis* 1963;22:435–439.
3. Oka M, Rekonen A, Routsi A, Seppala O. Intra-articular injection of ^{90}Y resin colloid in the treatment of rheumatoid knee joint effusions. *Acta Rheumatol Scand* 1971;17:148–160.
4. Gumpel JM, Beer TC, Crawley JCW, Farran HEA. Yttrium-90 in persistent synovitis of the knee—a single centre comparison: the retention and extra-articular spread of four ^{90}Y radiocolloids. *Br J Radiol* 1975;48:377–381.
5. Oka M. Radiation synovectomy of the rheumatoid knee with yttrium-90. *Ann Clin Res* 1975;7:205–210.
6. Williams ED, Caughey DE, Hurley PJ, John MB. Distribution of yttrium-90 ferric hydroxide colloid and gold-198 colloid after injection into the knee. *Ann Rheum Dis* 1976;35:516–520.
7. Spooren P, Johannes J, Rasker JJ. Synovectomy of the knee with ^{90}Y. *Eur J Nucl Med* 1985;10:441–445.
8. Davis MA, Chinol M. Radiopharmaceuticals for radiation synovectomy:

evaluation of two Yttrium-90 particulate agents. *J Nucl Med* 1989;30:1047–1055.
9. Winston MA, Bluestone R, Cracciolo A III, Blahd WH. Radioisotope synovectomy with ^{32}P-chromic phosphate, kinetic studies. *J Nucl Med* 1973;14:886–889.
10. Onetti CM, Gutierrez E, Hleba E, Aguirre CR. Synoviorthesis with ^{32}P-colloidal chromic phosphate in rheumatoid arthritis. *J Rheumatol* 1982;9:229–238.
11. Howson MP, Shepard NL, Mitchell NS. Colloidal chromic phosphate ^{32}P synovectomy in antigen-induced arthritis in the rabbit. *Clin Orth Rel Res* 1988;229:283–293.
12. Sledge CB, Noble J, Hnatowich DJ, Kramer R, Shortkroff S. Experimental radiation synovectomy by ^{165}Dy ferric hydroxide macroaggregate. *Arth Rheum* 1977;20:1334–1342.
13. Sledge CB, Zuckerman JD, Zalutsky MR, et al. Treatment of rheumatoid synovitis of the knee with intraarticular injection of dysprosium 165-ferric hydroxide macroaggregates. *Arth Rheum* 1986;29:153–159.
14. Sledge CB, Zuckerman JD, Shortkroff S, et al. Synovectomy of the rheumatoid knee using intra-articular injection of dysprosium-165 ferric hydroxide macroaggregates. *J Bone Joint Surg* 1987;69A:970–975.
15. Zuckerman JD, Sledge CB, Shortkroff S, Venkatesan P. Treatment of antigen-induced arthritis in rabbits with dysprosium-165-ferric hydroxide macroaggregates. *J Orth Res* 1989;7:50–60.
16. Chinol M, Vallabhajosula S, Gordon RE, Klein MJ, Goldsmith SJ. Holmium-166-FHMA: a new agent in radiation synovectomy [Abstract]. *J Nucl Med* 1989;30:856.
17. Chinol M, Vallabhajosula S, Zuckerman JD, Gordon RE, Klein MJ, Goldsmith SJ. Radiation synovectomy using Ho-166-FHMA in rabbits with antigen-induced arthritis [Abstract]. *J Nucl Med* 1990;31:780.
18. Shortkroff S, Mahmood A, Sledge CB, et al. Studies on Ho-166-labeled hydroxyapatite: a new agent for radiation synovectomy [Abstract]. *J Nucl Med* 1992;33:937.
19. Noble J, Jones AG, Davis MA, Sledge CB, Kramer RI, Livni E. Leakage of radioactive particle systems from a synovial joint studied with a gamma camera: its application to radiation synovectomy. *J Bone Joint Surg* 1983;65A:381–389.
20. Ball J, Chapman JA, Muirden KD. The uptake of iron in rabbit synovial tissue following intra-articular injection of iron dextran. *J Cell Biol* 1964;22:351–364.
21. Muirden KD, Peace G, Rogers K. Clearance of ^{59}Fe-labeled erythrocytes from normal and inflamed rabbit knee joints. *Ann Rheum Dis* 1969;28:630–636.
22. Chinol M, Vallabhajosula S, Zuckerman JD, Goldsmith SJ. In vivo stability of ferric hydroxide macroaggregates (FHMA). Is it a suitable carrier for radionuclides used in synovectomy? *Nucl Med Biol* 1990;17:479–485.
23. Mumper RJ, Mills BJA, Ryo UY, Jay M. Polymeric microspheres for radionuclide synovectomy containing neutron-activated Holmium-166. *J Nucl Med* 1992;33:398–402.
24. Ehrhardt GJ, Lattimer JC, Day DE, Stapleton J. Evaluation of activable, degradable glass ^{153}Sm microspheres for radiation synovectomy [Abstract]. *J Nucl Med* 1991;32:945.
25. Ehrhardt GJ, Zinn K, Jia W, et al. Microspheres of human serum albumin labeled by a specific linker technology for use in radiation synovectomy [Abstract]. *J Nucl Med* 1991;32:964.
26. Chinol M, Vallabhajosula S, Goldsmith SJ, Deutsch K, Chinen L, Deutsch E. Evaluation of new radiopharmaceuticals for radiation synovectomy (RS) [Abstract]. *J Nucl Med* 1991;32:963.
27. Deutsch K, Chinen L, Deutsch E, Chinol M, Vallabhajosula S, Goldsmith SJ. The chemistry of two new classes of agents for radiation synovectomy [Abstract]. *J Nucl Med* 1991;32:945.
28. Brodack JW, Chinen LK, Deutsch E, Deutsch KF. Studies on the radiolabeling of hydroxyapatite particles for use as radiation synovectomy agents [Abstract]. *J Nucl Med* 1992;33:980.
29. Hayek E, Newesely H. Pentacalcium monohydroxyorthophosphate (hydroxyapatite). *Inorg Synth* 1963;7:17.
30. Dumonde DC, Glynn LE. The production of arthritis in rabbits by an immunological reaction to fibrin. *Br J Exper Path* 1962;43:373–380.
31. Steinberg ME, McCrae CR, Berselli RA, Cram B. Intra-articular 5-fluorouracil in antigen-induced arthritis. *J Bone Joint Surg* 1971;53A:514–522.
32. Maxon HR, Thomas SR, Hertzberg VS, et al. Rhenium-186 hydroxyethylidene diphosphonate for the treatment of painful osseous metastases. *Semin Nucl Med* 1992;22:33–40.