PREPARATION AND BIOLOGICAL BEHAVIOUR OF SAMARIIUM-153-HYDROXYAPATITE PARTICLES FOR RADIATION SYNOVECTOMY

M.G. AGRUELLES, I.S. LUPPI BERLANGA, E.A. TORRES, G.A. RUTTY SOLÁ, G. RIMOLDI

Comisión Nacional de Energía Atómica, Centro Atómico Ezeiza, Buenos Aires, Argentina

Abstract

The preparation and labelling procedures of $^{153}$Sm-hydroxyapatite ($^{153}$Sm-HA) are described in this paper. Hydroxyapatite (HA) was prepared and studied as a radiosynovectomy agent. HA particles were prepared from the reaction of calcium nitrate and ammonia phosphate at high pH. Samarium-153 labelling was done in two steps with citric acid. A series of experimental conditions, such as: specific activity, citric acid mass, radioactive solution volume, in-vitro stability, have been carried out. Radiolabelling efficiency was greater than 95%. In vitro studies showed high stability (>99%). Animal studies showed a good retention in the synovium, with a very low extra-articular leakage over 6 days after administration.

1. INTRODUCTION

Rheumatoid Arthritis (RA) is an ubiquitous incapacitating disease that places substantial demands on health care resources [1]. The characteristic disease manifestations of RA are joint pain, swelling and reduced mobility as a result of the synovial tissue inflammation. It causes destruction of joint structures with deformation and loss of function. Radiosynovectomy is a radiation therapy used over 30 years for palliation pain and swelling [2]. It consists of intra-articular injection of beta-emitting radionuclide in colloidal or particulate form, which gets in contact with synovium. Phagocytic cells absorb some of the injected dose, which is transmitted to the synovium. If the amount of radioactivity injected is large enough the tissue will be destroyed. Regenerated tissue will be asymptomatic for 2-5
years [3]. Compared with surgical synovectomy, the radiation therapy is simpler, less traumatic and the hospitalization time is shorter; the cost is lower and the duration of relief is comparable.

Ideal radionuclide must be a short-lived beta-emitter with no or low gamma-ray emission. Since radioactivity leakage needs time, short half-life nucleus is required. Thus decay occur without leaking and extra-articular irradiation is avoid. New radiosynovectomy agents are designed with desired characteristics: biodegradable, high affinity with target organ and high in vivo stability [4].

2. MATERIALS AND METHODS

2.1. Radionuclide

$^{153}$Sm chloride was produced in RA-3 reactor (Centro Atómico Ezeiza) by irradiation of 98.7% $^{152}$Sm$_2$O$_3$ via $^{152}$Sm(n,$\gamma$)$^{153}$Sm. The target material was dissolved in diluted nitric acid to a concentration of 5 mg/ml. It was then put inside a quartz ampoule and carried to dryness by heating under dry nitrogen flow. The sealed ampoule was irradiated for 36 hours, at a thermal neutron flux of $7.10^{13}$ n/cm$^2$s. Irradiated target was dissolved in HCl 0.1 N to get it as chloride, with a specific activity about of 5.55-11.10 GBq (150-300 mCi) $^{153}$Sm/mg Sm$_2$O$_3$.

2.2. Hydroxyapatite preparation

Hydroxyapatite particles were prepared from the reaction of calcium nitrate and ammonia phosphate at high pH [5.6]; 0.33 mol of Ca(NO$_3$)$_2$ was dissolved in 300 ml of water. The solution was adjusted at pH 12 by addition of concentrated ammonia and diluted to 600 ml. A (NH$_4$)$_2$HPO$_4$ solution (0.2 mol in 500 ml), similarly brought to pH 12 and diluted to 800 ml) was added, drop by drop, stirring vigorously. A voluminous precipitate was formed. The reaction mixture was gently boiled for 10 minutes. The precipitate was allowed to settle and
the supernatant solution was separated by decantation. The precipitate was rinsed with hot water, dried at 150 °C and heated for an hour at 240 °C to remove the ammonium nitrate. By strong heating at 800 °C for an hour, the product becomes largely anhydrous and hardened.

2.3. Particles size

Particle size range was studied using light microscopy. With an eyepiece graticule, the diameter of each particle in 100 consecutive fields from each sample (magnification x10) was recorded.

A process of sieving using sieves of 200 and 400 mesh was carried out. The portion of the sample retained on sieve 200 mesh (range over 75 µm) was discharged.

2.4. Labelling

Labelling was done in two steps:

(a) $^{153}$Sm-citrate was prepared by adding sufficient citric acid monohydrate to the $^{153}$SmCl$_3$ 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 minutes.

(b) The radioactive solution (370 MBq) was added to 1ml of particulate suspension (10 mg), stirring continuously (30 min, 37 °C).

2.5. Labelling efficiency

The radioactive mixture was transferred to a centrifuge tube using 4 ml of saline to rinse, centrifuged at 1000 rpm for 5 minutes. The supernatant was then transferred to another tube. Measurements of radioactivity were made and labelling efficiency was calculated as percentage of initial activity.

2.6. In vitro stability

In vitro stability studies were performed by incubating particles in normal saline and 1% human serum albumin solution over 48 hours at 37 °C with agitation. At different times
radiolabelled particles were centrifuged at 1000 rpm for 5 min. and activity in the particles and supernatant was measured.

2.7. Animal model

Normal rabbits were used as models to evaluate in vivo stability of radiolabelled HA particles. The studies were performed in New Zealand rabbits. Male and female rabbits weighing about 4 kg were used. Prior the administration microparticles were resuspended in 2 ml of saline (or glucose 5% in order to avoid the decantation) and autoclaved for 20 min at 121 °C. Each rabbit was injected intra-articulary (into the left posterior knee joint) with 0.2 ml containing 37 MBq (1 mCi) of \textsuperscript{153}Sm-HA. Images were obtained with a gamma camera using a high-resolution collimator (500.000 counts were measured with a 128x128 pixels matrix). The percent-injected dose in blood, urine and different organs was calculated daily over a period of 6 days. After that the animals were killed and the tissues were counted.

3. RESULTS

The yield of the hydroxyapatite synthesis was always greater than 80%. The particle size distribution showed a range of 5 \( \mu \text{m} \) to 50 \( \mu \text{m} \) (Table I).

<table>
<thead>
<tr>
<th>% of microparticles</th>
<th>Size (( \mu \text{m} ))</th>
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<tbody>
<tr>
<td>18</td>
<td>5-15</td>
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<td>35</td>
<td>15-25</td>
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<td>5</td>
<td>45-55</td>
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TABLE I. Size distribution HA-microparticles
Labelling efficiency over time.

Fig. 1. In vitro stability of radiolabelled HA particles

Effect of Sm-153-citrate mass on labelling efficiency of HA particles.

Fig. 2. Effect of $^{153}$Sm-citrate mass on labelling efficiency of HA particles

Labelled particles showed to be stable more than 48 h. No dissociation of activity from the particles was observed (Fig. 1). HA particles retained more than 99% from the original labelling at the studied conditions. No changes in particle size were observed.

Labelling did not depend on the presence of emulsifiers or microparticle pre-treatment. Though, it was highly dependent on complex mass as can be seen on Fig. 2. The labelling
efficiency was greater than 99% when the $^{153}$Sm-citrate quantity was less than 7 μg/10 mg of particles. On the other hand, the labelling efficiency was less than 50% when $^{153}$Sm-citrate mass was 75 μg.

The sedimentation velocity was lower when the particles were dispersed in glucose 5% solution. This is an important factor because if the sedimentation occurs very fast, many particles remain into the syringe and the administration is difficult.

Respect to animals' studies, no extra-articular localization of activity was detected by whole-body scans. All organs showed insignificant accumulation of $^{153}$Sm activity. The principal observed fact over the whole study, was the permanence of the injected product in the joint.

4. DISCUSSION

Hydroxyapatite microparticles preparation method was reached. Reproducible method was with high yield Size could be measured by optic microscopy. Particles size ranged from 5 to 50 μm, although it was difficult to estimate size bellow 5 μm.

Radiolabelling of HA particles with Sm-153 is simple to perform with high yields and radiolabelled-HA particles demonstrate high in vitro stability. Furthermore, labelling showed to be independent from $^{153}$Sm specific activity but was highly dependent on $^{153}$Sm-citrate mass.

The leakage of Sm-153 up to 6 days post administration in the joint was very low. Stability experiments proved labelled particles were stable over 48 h.

Finally, biological behaviour was the expected one.
REFERENCES


