

# Optimization of Hydroxyapatite Particles labeling with Samarium-153 as a Therapeutic Agent for Radiation Synovectomy

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## ABSTRACT

**H**ydroxyapatite (HA) was studied as a particulate carrier for beta-emitting radionuclides in radiation synovectomy. Particles were labeled with  $^{153}\text{Sm}$  ( $T_{1/2} = 1.95$  days,  $\beta$ -energy = 810 (20%), 710 (50%), 640 (30%) keV,  $\gamma$ -energy = 103.2 (29.8%) keV, range of beta particles in tissue is 2.5 mm). Labeling efficiency was greater than 95% at pH 4-6. The compound is sterile and pyrogen free with its stability of 6 days. In vivo studies by intra-articular injection into the knee joints of normal rats showed the total cumulative leakage of  $^{153}\text{Sm}$  over 6 days was around 2 percentage injected dose (ID). The ease of preparation of  $^{153}\text{Sm}$ -HA, the high efficiency of labeling and low leakage from the joint make  $^{153}\text{Sm}$ -HA attractive for radiation synovectomy.

## INTRODUCTION

In severe case of chronic rheumatoid arthritis, surgical synovectomy can temporarily cease the disease process and provide significant relief of symptoms however the surgical techniques are complicated and invasive. If all of the inflamed synovium cannot be excised, regrowth of diseased synovium and recurrence of symptoms will eventually occur.

Chemical synovectomy has achieved limited acceptance because of systemic toxicity and local injury to articular cartilage.

Radiation synovectomy is an alternative to surgical synovectomy. This procedure con-

sists of intra-articular injection of the radionuclides in colloidal or particulate form then the inflamed synovium will be destroyed by the emitted beta particles<sup>(1)</sup>.

$^{153}\text{Sm}$  has a half-life of 1.95 days, it emits 3 beta-particles with energy 0.81, 0.71 and 0.64 MeV and 103.2 keV gamma ray which is useful for imaging.  $^{153}\text{Sm}$ -HA was selected as the therapeutic radiopharmaceutical for radiation synovectomy. The procedure of radiation synovectomy is simply injecting  $^{153}\text{Sm}$ -HA into the effected joint.

## MATERIALS AND METHODS

### *Preparation of $^{153}\text{Sm-HA}$ (2, 3, 4, 5)*

#### *Preparation of $^{153}\text{Sm-Citrate}$*

In order to determine the appropriate quantity of citric acid monohydrate, various quantity of citric acid monohydrate were added into  $^{153}\text{SmCl}_3$  (in normal saline). The compound were then stored in room temperature for 30 minutes. Final products were  $^{153}\text{Sm-citrate}$  solution in various mole ratios.

#### *Labeling of $^{153}\text{Sm}$ to HA particles*

In order to determine optimal quantity of HA in labeling procedure, various amount of HA (particle sizes range 10-40  $\mu\text{m}$ ) was added in 750  $\mu\text{l}$  water. Two hundred and fifty ml of  $^{153}\text{Sm-citrate}$  solution was then added and the vial was gently rotated at room temperature for 30 minutes, then the solution was transferred into a 15-ml centrifuge tube. Rinsed the precipitate with 4 ml saline solution, and centrifuged at 1000 rpm for 8 minutes. Precipitate (labeled HA particles) and supernatant (free  $^{153}\text{Sm}$ ) were separated and the percentage of labeling was calculated. Finally 2 ml of normal saline was added into the precipitate and the mixture was autoclaved at 121°C for 20 minutes.

To determine optimal specific activity of  $^{153}\text{Sm}$  various amount of inactive Sm ( $^{152}\text{Sm}$ ) was added into a fixed amount of  $^{153}\text{SmCl}_2$  before labeled to HA.

Varying pH of Sm-HA was also performed. Using 40 mg HA, add  $\text{H}_2\text{O}$  and 15 mCi of  $^{153}\text{Sm-Citrate}$ . And then adjust the pH. (pH range = 0 - 14).

### *Biodistribution studies in rats*

#### *Joint leakage studies*

Evidences of joint leakage were studied in three groups (5 rats in each group) of Sprague Drawley rats at 1, 3 and 6 days periods after injection. Each rat was injected intra-articularly with 0.15 ml of 15 - 40  $\mu\text{Ci}$  of  $^{153}\text{Sm-HA}$ . Rats were kept in metabolic cages and the total urine excreted was collected for each rat. At the end of time period for each group, rats were sacrificed and dissected. Samples from each organ were weighed and counted in MCA counter. The radioactivity uptake in organs were calculated.

#### *Control studies*

Control studies were performed in the same way as the joint leakage studies but using  $^{153}\text{Sm-citrate}$  instead of  $^{153}\text{Sm-HA}$ .

#### *Clinical trial*

Clinical trials were performed in 2 hospitals as follows:

- Department of Radiology, Division of Nuclear Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University.
- Rheumatic Disease Unit, Department of Medicine, Pramongkutklao Hospital

#### *Baseline Clinical assessment*

Following clinical data was assessed for baseline records in all participating patients.

- Visual analogue scale (VAS 1-100 mm) on the degree of joint pain at rest and during activity.
- Duration of morning stiffness (minutes)
- Functional capability of the dis-

eased joint in daily life.

- Joint circumference (cm.) or degree of joint swelling
- Joint tenderness score (range 0-3) using Ritchie articular index for the assessment of joint tenderness in RA
- Range of joint motion
- Presence or absence of joint pain on motion and joint instability.

### *Scintigraphic assessment*

#### 1. Bone scintigraphy

Three-phase bone imaging with 20 mCi  $^{99m}\text{Tc}$ -MDP was performed 1-2 weeks before radiation synovectomy.

#### 2. $^{153}\text{Sm}$ -HA scintigraphy

### *Injection procedure*

The injection was given in the nuclear medicine clinic. Aseptic technique and routine precautions were strictly followed in the handling and disposal of radioisotopes. Tip of 21-gauge needle was placed into articular space. Articular fluid was removed as much as possible before the radiopharmaceutical injection. This procedure was also to ensure that the needle was correctly in place.  $^{153}\text{Sm}$ -HA was injected into the joint. A mixture of 2 % xylocaine and 10 mg of triamcinolone acetonide was then added into articular space to make total volume fit for a particular joint and also to flush the needle. The minimum total volume of solution injected was 5 ml for knee joint and 2 ml for ankle, elbow and wrist joint. The mixture of xylocaine and triamcinolone acetonide was also helpful to minimize transient local reaction and effusion after injection. The patients remained in non weight-bearing position for 4 hours after the procedure. Patients were

then allowed to go home and advised to rest the injected joint on that day and could resume their normal activities on the following day.

### *Extra-articular activity analysis*

Anterior whole-body imaging was acquired immediately and 72 hours following injection using a single-headed gamma camera with a low-energy, all purpose parallel hole collimator with a 20% window centered at 103 keV for  $^{153}\text{Sm}$ . Extra-articular activity analysis was performed visually.

### *Intra-articular distribution analysis*

Anterior and lateral static images of the injected joint were performed immediately and 72 hours following injection. (SPECT images were acquired in some cases)

### *Follow-up and Clinical Interpretation*

Clinical results were assessed and recorded by the same method as baseline assessment to evaluate clinical responses at 1, 3, 6, 9, 12, 24 and 36 months.

### *Interpretation*

The results of systemic and joint statuses were evaluated on a simple scale as follows:

Excellent :

- Improved VAS in range of 80-100 mm
- No pain on motion
- Small or no palpable effusion
- No joint tenderness
- Either an improved or normal range of motion

Good :

- Improved VAS in range of 60-79 mm
- Slight or intermittent pain
- Small joint tenderness
- Improved range of motion

**Fair :**

Improved VAS in range of 20-59 mm  
 Reduction of pain but not abolished  
 Diminution of effusion but still in moderate volume  
 Moderate joint tenderness  
 Some improvement or maintenance of pretreatment range of motion

**Poor:**

Improved VAS in range of 0-19 mm  
 No changes or worsening in pain severity, joint tenderness and range of motion  
 Excellent and good were considered as success whereas fair and poor were considered as failure.

**RESULTS**

***Effect of Citric acid quantity on labeling efficiency.***

Citric acid acted as anti - coagulant and ligand transfer, the higher the quantity of citric acid added the less labeling yield (Table 1). Citric acid competed with Sm in binding with HA particles. However, it has to be added to increase stability of the compound. In this experiment we used mole ratio of Sm : Citric acid of 1 : 2.

**Table 1** *Effect of Citric acid quantity (mole) on labeling efficiency*

Sm : Citric acid	1:0	1:2	1:6	1:10	1:100	1:400	1:800	1:2,000
% labeling	99.86	99.74	98.48	91.98	76.71	46.40	23.13	7.90

***Effect of HA quantity on labeling efficiency***

Fix quantity of <sup>153</sup>Sm but vary HA quantity from 2.0 to 150.0 mg, the labeling efficiency was greater than 99% when HA quantity was greater than 20 mg (Table 2). Forty mg of HA is used in routine production to assure the high labeling efficiency.

**Table 2** *Effect of HA quantity on labeling efficiency*

mgHA	2.0	3.0	6.0	6.6	7.5	8.5	10.0	12.0	15.0	20.0	30.0	60.0	100.0	150.0
%labeling	79.8	84.0	84.6	84.8	88.1	89.9	90.0	90.9	91.2	99.2	99.4	99.7	99.8	99.8

***Effect of pH on labeling efficiency***

At low pH (pH < 4), the labeling efficiency decreased and some of HA particles were dissolved. At high pH (pH > 6) the labeling efficiency also decreased as shown in Table 3. The labeling efficiency was greater than 98% while pH is 4-6.

**Table 3** *Effect of pH on labeling efficiency*

pH	0	2	4	5	6	11	12	14
% labeling	8.66	84.31	99.95	99.95	99.98	94.84	95.37	96.22

**Effect of <sup>153</sup>Sm-specific activity on labeling efficiency**

The lower the specific activity of <sup>153</sup>Sm, the less the labeling efficiency was noted. But even as low as 1.6-2.9 mCi/mg the labeling efficiency was still greater than 99% (specific activity of <sup>153</sup>Sm produced at OAEP usually as high as 90 mCi/mg Sm), as shown in Table 4.

**Table 4** Effect of <sup>153</sup>Sm-specific activity on labeling efficiency

Specific activity (mCi/mg)	80	2.9	2.2	1.6	0.96	0.33
%Labeling	99.7	99.62	99.69	99.73	91.94	30.11

**Stability of <sup>153</sup>Sm-HA**

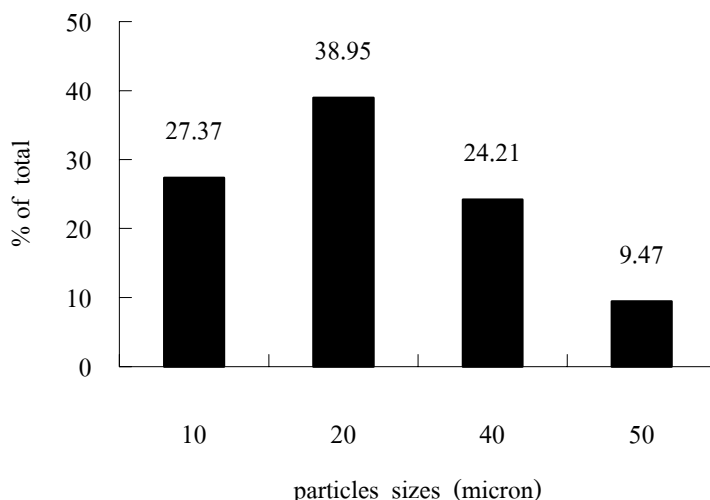
High stability of <sup>153</sup>Sm-HA in normal saline upto 6 days was shown in Table 5.

**Table 5** Stability of <sup>153</sup>Sm – HA

Days	0	1	2	3	4	5	6
% Labeling	99.10	99.70	99.90	99.80	99.96	99.97	99.94

**Distribution of HA particle size**

HA particle size was measured using haemocytometer. Ninety percent of HA have particles size of 10-40 µm (Fig. 1) which are suitable for articular space localization.



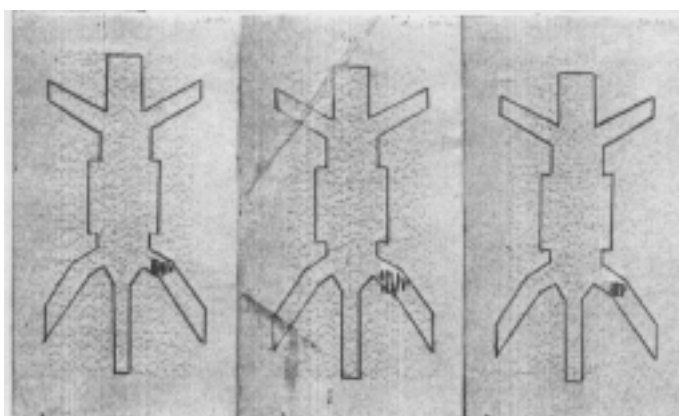
**Fig. 1** Frequency (%) distribution of HA particles as a function of particles size (micron)

**Biodistribution studies in rats**

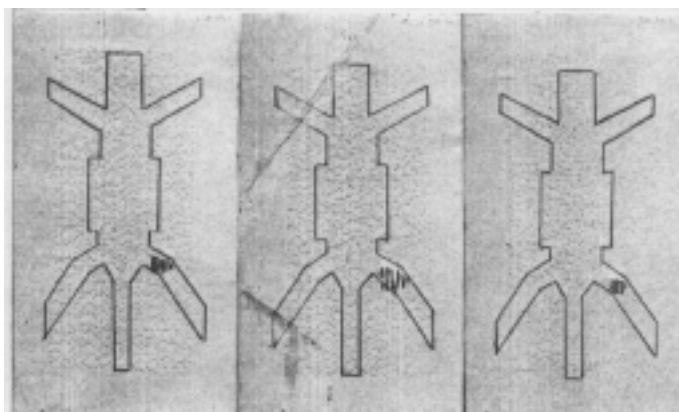
The distribution of radioactivity of  $^{153}\text{Sm}$ -HA leaks from the joint to organs during 1, 3 and 6 days post injection is low compared to  $^{153}\text{Sm}$ -citrate as shown in table 6 and figure 2-3.

**Table 6** Percentage injected dose (%ID) of  $^{153}\text{Sm}$ -HA and  $^{153}\text{Sm}$ -citrate in each group of normal rats

Days post injection	$^{153}\text{Sm}$ -HA			$^{153}\text{Sm}$ -citrate		
	1	3	6	1	3	6
Knee joint %ID	8.54	19.55	3.85	26.70	43.05	24.43
cumulative extra-leakage %ID	0.22	0.23	1.83	32.28	15.44	34.42



**Fig. 2** Distribution of  $^{153}\text{Sm}$  - HA in rats in 1, 3 and 6 days post injection (knee joint). (scan by Berthold TLC Scanner II with  $2\pi$  counting head an autochrons x/y plotter)



**Fig. 3** Distribution of  $^{153}\text{Sm}$  - Citrate in rats in 1, 3 and 6 days post injection (knee joint). (scan by Berthold TLC Scanner II with  $2\pi$  counting head an autochrons x/y plotter)

**Clinical results**

Clinical results are still in the evaluating process.

## DISCUSSION AND CONCLUSION

HA particles were easily labeled with Samarium-153 and gave the labeling efficiency of greater than 95%. It follows the characteristic of the ideal particulate agent that it has high binding affinity to a relevant beta emitter<sup>(2)</sup>.

HA particles could be prepared from common chemicals<sup>(3, 5)</sup> and formed into particles of desired size range by spray drying instruments but the process (to control particle size) was not so easy. HA particles in various size range from 10-40  $\mu\text{m}$ <sup>(2, 3, 4, 6, 7)</sup> are commercially available.

The primary disadvantage of radiation synovectomy procedure is the undesirable radiation dose delivered to non target organ due to the leakage of radioactive material from the injected joint<sup>(7)</sup>.

In this experiment the biodistribution of <sup>153</sup>Sm-HA in rats showed the low activity leakage from the injected joint was low (~2% ID cumulative) which meant the labeled <sup>153</sup>Sm-HA remained tightly by in vivo while the leakage in control studies (<sup>153</sup>Sm-citrate) was so high (~30% ID cumulative) in 6 days post injection. The activity leaked from the injected joint was mainly found in bone and liver.

It was noted that %ID of <sup>153</sup>Sm-citrate retained at the injected joint seems to be higher than <sup>153</sup>Sm-HA. This might be caused by the difficulty of intra-articular injection in rats (some sedimentation and retention of some larger particles in the injection apparatus).

Radionuclides used for radiation synovectomy<sup>(1, 3, 6, 8)</sup> should emit beta particles with sufficient energy for a maximum tissue penetration of 5-10 mm, short half-life, little or no gamma emission, minimal gamma ray

emission can be used to monitor leakage of radioactivity from the joint<sup>(9)</sup>.

Yttrium-90, Gold-198, Phosphorus-32 and Dysprosium-165<sup>(1, 3, 6, 8)</sup> were used for radiation synovectomy in other studies. Those radionuclides had some advantage and disadvantage as mentioned earlier. Samarium-153 was used in this study eventhough its maximum range in tissue is only 2.5 mm and therapeutic range is 0.7 mm. The particles were engulfed by the synovial lining and distributed throughout the tissue where they retained and ultimately degraded<sup>(10)</sup>. This presumed that even a weak beta emitting radionuclide, Samarium-153 could be effective in treatment of a large joint<sup>(3)</sup> and also Samarium-153 was routinely produced at the research reactor, Office of Atomic Energy for Peace (OAEP).

Radiolabeling of HA with <sup>153</sup>Sm is simple and provides good yield of labeled particles. There are some factors that affect the properties of <sup>153</sup>Sm-HA such as pH, specific activity of <sup>153</sup>Sm and quantity of HA. Either too low or too high pH decrease percentage labeling of <sup>153</sup>Sm-HA while high quantity of HA increases percentage labeling. The <sup>153</sup>Sm-HA showed high in vitro stability in saline up to 6 days at pH 4-6.

### **Product specifications :**

- Injected dose ~15 mCi/2 ml.
- The particle sizes range from 10 - 40 micron.
- The pH is 4 - 6.
- The percent labeling is greater than 95.
- The product is sterilized and pyrogen free.

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