2007 International Nuclear Atlantic Conference - INAC 2007
Santos, SP, Brazil, September 30 to October 5, 2007
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-02-1

HYDROXYAPATITE LABELLED WITH Y-90 OR LU-177 FOR RADIOSYNOVECTOMY

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ABSTRACT

Radiation synovectomy (RS) is a method of treatment by intra-articular application of β-emitting radioisotopes. There are several radionuclides available for this treatment such Y-90; Sm-153; Dy-165; Ho-166; Lu-177 and Re-188/186. The radiopharmaceuticals have been show to be effective and safe for this procedure. Hydroxyapatite particles (HA) are regarded as one of the most suitable carriers for applications in RS, and labeling with ^{177}Lu or ^{90}Y has been envisaged. Owing his favorable decay characteristics, the ^{177}Lu has been used in radiosynovectomy of small-sized joints, and the ^{90}Y has been the most useful radionuclide for applications in knee joint. The present study describes the preparation, labeling and quality control of HA labeled with both radionuclides. The labeling process was carried out using $^{90}YCl_3$ from Nordion® and $^{177}LuCl_3$ from IDB®. The percentage of labeling was determined by measuring the activity of both particles (^{90}Y-HA and ^{177}Lu-HA) and in the supernatants (^{90}Y / ^{177}Lu free). The radiochemical purity was performed to assess the stability of the ^{90}Y / ^{177}Lu-HA by paper chromatography system. Filters of different size were used for particle size determination. The labeling yield was higher than 87.0%. The final product presents a radiochemical purity > 98.9%, with particles range > 12 μm and stability of 5 days at room temperature. A scintigraphic image by gamma-camera was performed in rats to evaluate the extra-articular leakage from the knee. Both preparations appear suitable for RS, with a low extra-articular leakage and no acute toxicity in the clinical study using ^{90}Y-HA.

1. INTRODUCTION

Synovectomy by intra-articular application of a β-emitting radioisotopes in colloidal form or radiation synovectomy (RS) was introduced in 1952 for treatment of an inflamed synovial membrane [1]. Since that time, a large number of radionuclides have been studied and their usefulness and clinical efficacy were analyzed in several clinical trials. Since 1968, the term radiosynoviorthesis (RSV) has been used, meaning a restoration of the synovial membrane by the use of radionuclides, and has been applied to relieve pain and inflammation from rheumatoid arthritis (RA) for more than 40 year [2]. RSV has been pursued as an effective alternative to chemical and surgical synovectomy in cases of RA and other inflammatory arthropathies such as osteoarthritis and hemophiliac arthropathy. Today RSV is an alternative or even supplementary therapeutic approach for the treatment of patients suffering RA, improve mobility and preserve joint function, resulting in better quality of life for the patient [3]. Advances in this area have been facilitated by the availability of a wide range of beta emitting radionuclides and the feasibility of incorporating into bio-degradable particles and

colloids. Several gamma and beta-emitting radionuclides such as ^{165}Dy; ^{199}Au; ^{32}P; ^{153}Sm; ^{166}Ho; $^{186/188}$Re and ^{90}Y have been tested as potential nuclides for RS. Therefore, an ideal agent for RSV would be one in which the radionuclide is irreversible attached to pre-formed particles of appropriate size. The ideal size of such particles is reported to be 2 – 10 μm, so that they are small enough to be phagocytosed, but not so small that they may leak out of the cavity before being phagocytes resulting in high doses delivered to non-target organs. In addition, the particles should be biodegradable, and the biological half-life of such particles should be longer than the physical half-life of the radionuclide tagged with them [4].After an injection of a beta emitting radiopharmaceutical into the joint space, some of the injected radioactivity is absorbed by phagocytic lining cells along the synovial surface. The local high energy irradiation results in sclerosis and fibrosis of the inflamed synovial membrane, achieving significant reduction of pain and joint effusion in approximately 70 % of the patients. Thus, RS holds considerable promise for the growing field of therapeutic Nuclear Medicine. The procedure requires only the injection into the synovial cavity of a radiolabeled compound with the appropriate nuclear, chemical, and biochemical characteristics [1].

Yttrium-90 is often believed to be among the most useful of the radionuclide that have been considered for therapeutic applications in knee joint, half-life of 64.1 h and beta rays of high-energy 2.3 MeV, with no gamma rays, and decays to a stable daughter (^{90}Zr) [5]. Lutetium-177 is considered to be a promising radionuclide for use in radiation synovectomy of small-sized joints owing to its favorable decay characteristics [$t_{½}$=6.73 days, $E_{\beta max}$= 0.49 MeV, E_{γ}= 113 KeV (6.4%), 208 KeV (11%)] [4].

The Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is one of the preferred particulates, constituent of bone matrix, it is the natural substance known to be biodegraded into calcium and phosphate ions (Ca^{2+} and PO_4^{3-}) by natural metabolic processes and eliminated over a period of six weeks, thereby providing excellent biocompatibility. For many years, hydroxyapatite (HA) has been used successfully as a coating for implants in joint arthroplasty and for dental reconstruction and can be synthesized easily with the desired particle size [4].

The aim of this work is to establish the labeling procedure, the quality control analysis and the stability of HA labeled with two different radioisotopes Y-90 and Lu-177 for radiation synovectomy.

2. MATERIALS E METHODS

2.1 Labeling procedure of ^{90}Y-HA

The process was carried out using $^{90}YCl_3$ from Nordion®, according to Couto et al. [6]. In a conical glass vial containing 40 mg of HA from Bio-Rad®, with particles in the desirable size range (20 μm) in 0.80 mL sterile water, was added 74 - 2,590 MBq of ^{90}Y in citrate form. The vial was sealed and mixed for 30 minutes at room temperature. The suspension was centrifuged at 2000 rpm for 5 minutes, the liquid was discarded and the precipitated resuspended with 5 mL of 0.9 % saline solution. The final precipitate (^{90}Y-HA) was resuspended in 5 – 8 mL of sterile saline solution (pH = 6.0), sealed and autoclaved for 30 minutes at 121ºC for further quality control tests.

2.2 Labeling procedure of ^{177}Lu-HA

The preparation of ^{177}Lu-HA was performed by adding 0.1 - 0.2 mL of $^{177}LuCl_3$ from IDB-Holland® (74 - 370 MBq) containing 50; 100; 200 and 400 μg of Lutetium oxide (carrier) to a suspension of 5 – 40 mg of HA in 0.8 mL of 0.9 % saline solution, after the addition of 0,1 mL of 0.5 M $NaHCO_3$ buffer (pH=9). The reaction mixture was vortexed; the pH was adjusted to 7.0 using 0.1 M HCl and mixed continuously at room temperature for 30 minutes. Subsequently, the reaction mixture was centrifuged at 2000 rpm for 5 minutes. The supernatant was separated from the precipitate carefully. The precipitate were washed further using 1 mL of 0.9 % saline solution on each occasion, to ensure the removal of free ^{177}Lu activity in case of possible leaching from the labeled particles. Finally, the ^{177}Lu-HA was suspended in 3 - 5 mL sterile saline solution, autoclaved and used for further studies (radiochemical and biological determination).

2.3 Labeling yield of ^{90}Y-HA and ^{177}Lu-HA

The labeling yield was determined by centrifugation. The reaction solution was vortexed thoroughly and centrifuged at 2000 rpm for 5 minutes at the end of the reaction. Subsequently, the supernatant was carefully separated and the activity was measured in both: supernatant (free ^{90}Y or ^{177}Lu) and pellet (particles of HA labeled with ^{90}Y or ^{177}Lu), in a dose calibrator. From these data, the percentage of radiolabeling yield of ^{90}Y / ^{177}Lu-HA were determined. The *"in-vitro"* stability was studied to determine the leaching of ^{90}Y or ^{177}Lu activity from the radiolabeled HA at room temperature. The final product was suspended in 1,0 mL of saline solution and stored at room temperature for 5 days. Then, at different intervals 1; 2; 3 and 5 days, the suspension was vortexed thoroughly and centrifuged, as mentioned before, to calculate the percentage of activity leaching in the supernatant. This procedure was repeated at different time intervals and in this manner verified the radiochemical purity, too.

2.4 Radiochemical purity of ^{90}Y-HA and ^{177}Lu-HA

The radiochemical purity of the ^{90}Y-HA and ^{177}Lu-HA was confirmed by paper chromatography using suitable eluting solvent. Aliquots of the reaction mixture (5 μl) were applied at 1.0 cm from the lower end of Whatman® 3MM chromatography paper strips (12 cm x1.0 cm) at 30; 120; 240 minutes after labeling, to assess the stability of the products. The strips were developed in 5 mM DTPA solution (^{177}Lu-HA) and 0.9 % saline solution (^{90}Y-HA) as the mobile phases, dried, divided into segments of 1 cm each and the radioactivity associated with each segment was measured in a NaI(Tl) solid scintillation counter. Similar tests were carried out with $^{90}YCl_3$ and $^{177}LuCl_3$. It was observed in paper chromatography using 5 mM DTPA as the solvent, that ^{177}Lu-HA remained at origin (R_f = 0), whereas unreacted $^{177}LuCl_3$ moved towards the solvent front (R_f = 0.8), due the formation of ^{177}Lu-DTPA complex. In the mean time, the ^{90}Y-HA remained at the origin (R_f = 0) in saline solution, while the free ^{90}Y moved near the front (R_f = 0.9).

2.5 Particle size determination of ^{90}Y-HA and ^{177}Lu-HA

Aliquots of 0.3 – 0.5 mL of ^{90}Y- HA / ^{177}Lu-HA were passed through filters (Millipore®) of different size in decrease order (12; 8; 5 and 3 μm) followed by flushing air. The percentage of activity retained on the filters and the filtrate was determinate in a dose calibrator (CAPINTEC).

2.6 Biological distribution of ^{90}Y-HA and ^{177}Lu-HA

The biological behavior of ^{90}Y-HA and ^{177}Lu-HA was studied in *Wistar* rats weighing 250 – 300 g under ether anesthesia. Doses of 18.5 - 22.2 MBq /0.1 mL were injected intra-articularly in one knee of the joint. Serial scintigraphic images were recorded in gamma-camera (Medical Imagem System - Mediso®) at 4; 24; 72 and 120 hours to determine the retention and leakage of the activity from the knee (synovium).

3. RESULTS

The percentage of radiolabeling yield determined by measuring the radioactivity associated with the pellet and the supernatant and the radiochemical purity determined by paper chromatography system of ^{90}Y / ^{177}Lu-HA, described in the experimental section (2.3) are listed in table 1. It was found after 4 hours of preparation a radiolabeling yield > 87 %, with a radiochemical purity > 98 % in both labeling process.

Table 1. Labeling Yield and Radiochemical Purity of ^{90}Y / ^{177}Lu-HA

^{90}Y-HA		^{177}Lu-HA	
Labeling Yield	Radiochemical purity	Labeling Yield	Radiochemical purity
(87.36 ± 1.98) %	(99.03 ± 0.43) %	(92.78 ± 0.85) %	(99.88 ± 1.77) %
(n = 6)			

"In vitro" stability studies showed that the ^{90}Y-HA and ^{177}Lu-HA preparations were highly stable after 5 days at room temperature, as were evident (>98 %) during this period of time (Figure 2). The radiochemical purity was confirmed by chromatography system and centrifugation. The typical paper chromatography patterns of ^{177}Lu-HA are illustrated in Figure 3. It was observed that ^{177}Lu-HA remained at the point of spotting (R_f = 0.0) and low detected radioactivity at R_f of $^{177}LuCl_3$ (<1%) was marked.

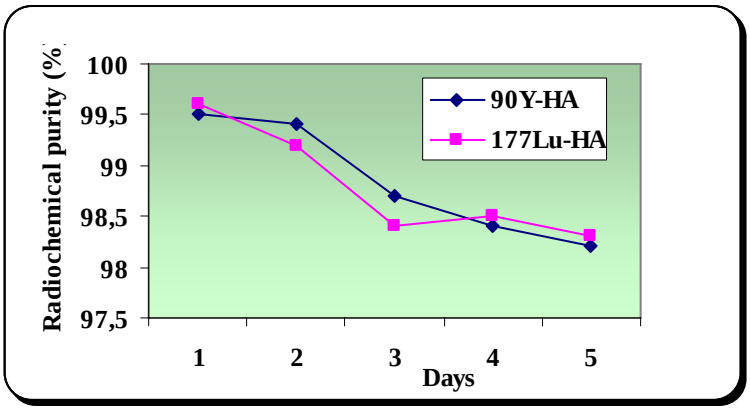

Figure 2. Stability of ^{90}Y-HA and ^{177}Lu-HA during 5 days

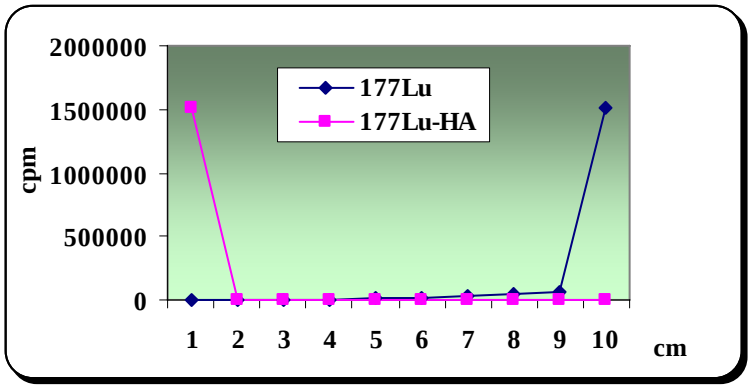

Figure 3. Paper chromatogram of ^{177}Lu-HA *(R_f=0)*

The particles size distribution during the production of ^{90}Y-HA and ^{177}Lu-HA were: 94.8 % (12 μm); 4 % (8 μm); 0.02 % (3 μm) and 95 % (12 μm); 5 % (8 - 3 μm), respectively. During the labeling process of ^{90}Y-HA and ^{177}Lu-HA, about 90 % of the particles were in the range of 12 - 15μm. According to Pandey et al. particles between 5 - 20μm are believed to be ideally suited [3].

Biological studies in rats of both compounds showed complete retention of radioactivity within the knee cavity for a least up 5 days as illustrated in Figure 4. The uptake and distribution are very similar for both compounds and the whole-body images did not show any detectable activity in any other organs. Both radiopharmaceuticals appear suitable for RSV.

Preliminary clinical study in patient was performed using 148 MBq of ^{90}Y-HA, to evaluate the "*in vivo*" stability of the compound or leakage of radioactivity from the joint, 4 and 24 hours after doses administration.

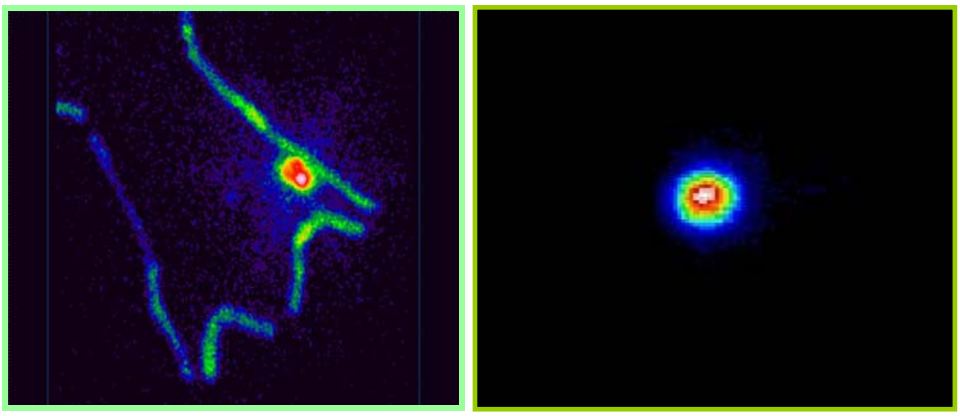

Figure 4. Gamma camera images of ^{177}Lu-HA (left) and ^{90}Y-HA (right), in *Wistar* rats after 5 days of injection.

4. CONCLUSIONS

The preparation of ^{90}Y-HA and ^{177}Lu-HA described in this work presented the main criteria of an ideal radiopharmaceutical for RSV: simple procedures for radiolabeling HA particles with ^{90}Y and ^{177}Lu in high labeling yields as well as excellent radiochemical purity using small amounts of HA, 40 and 5 mg, respectively. The radiolabeled particulates presented stability of 5 days at room temperature. Biological studies carried out in *Wistar* rats showed complete retention of injected radioactivity within the knee for up to 5 days. These studies showed that ^{90}Y-HA and ^{177}Lu-HA offer potential as suitable agents in the management of RSV. In the first clinical application, a low extra-articular leakage and no acute toxicity was observed using ^{90}Y-HA. Therefore, ^{177}Lu-labeled RSV agents will be economically more viable than the ^{90}Y labeled analogues. Its favorable characteristics contribute to follow, to predict and asses the success of RSV by bone scintigraphy studies. It appears as though RSV is an effective as well as cost-effective alternative to surgical synovectomy and is becoming the procedure of choice particularly in the hemophiliac patient with recurrent hemarthrosis and synovitis who has failed medical therapy.

ACKNOWLEDGMENTS

The authors are grateful to Antonio Carlos Freire and Natanael Gomes da Silva for help in carry out the biological distribution and gamma camera image.

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