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IN VIVO COMPARATIVE STUDY OF HYDROXYAPATITE LABELED WITH DIFFERENT RADIOISOTOPES: EVALUATION OF THE SCINTIGRAPHIC IMAGES

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Abstract – Radyosinovectomy (RSV) is a radiotherapeutic modality where a β-emitting radionuclide is administered locally by intra-articular injection on the form of a colloid or radiolabeled particulate. RSV is a well-accepted therapeutic procedure in inflammatory joint diseases and has been successfully employed for more than 50 years as a viable alternative to surgical and chemical synovectomy. The aim of this work is to compare the in vivo stability of hydroxyapatite labelled with ^{177}Lu, ^{90}Y and ^{153}Sm. All radionuclides were labelled with high yield and were retained in the joint for 7 days, showing stability and usefulness as tools in the RSV treatment. A similar retention of the products in the muscle was observed when the particles were administrated in the muscle. However, the pure form of the radionuclides were rapidly cleared from the blood and accumulated in the liver when injected i.v.. Although ^{153}Sm-HA is already available for nuclear medicine procedures and clinical studies with ^{90}Y-HA have been developed, ^{177}Lu-labeled RSV agents will be economically more viable and has not been studied yet. Its favorable characteristics contribute to follow, to predict and asses the success of RSV by bone scintigraphy studies.

Key words: Hydroxiapatite, radyosinovectomy, radiolabeling, rheumatoid arthritis.

INTRODUCTION

Radionuclide therapy (RNT) employing open sources of radiotherapeutic agents is fast emerging as an important part of nuclear medicine, primarly due to development of sophisticated molecular carriers . In order to develop effective radiopharmaceuticals for therapy, it is essential to carefully consider the choice of appropriate radionuclides as well as the carrier moiety with suitable pharmacokinetic properties that could result in good in vivo localization and desired excretion. The major criteria for the choice of a radionuclide for radiotherapy are suitable decay characteristics, easy of production and amenable chemistry. As regards to decay characteristics, physical half-life of the radionuclide should match with the biological half-life of the radiopharmaceutical. The energy of the particulate emission should be compatible to the volume of the lesion to be irradiated and at the same time should result in minimal dose delivery to the tissues surrounding the site of localization (9). Radionuclide with ideal properties for radiotherapy application has been applied in the production of radiopharmaceutical used in therapeutical protocols for cancer treatment, bone pain palliation and radiation synovectomy (RSV).

It is estimated that about 3 % of the population worldwide is affected by rheumatoid arthritis which manifestis mainly in the synovium. Because of the pain and debilitation, patients afflited by this degenerative disease are grateful even for symptomatic relief without a permanent cure. The aim of radiation synovectomy is to reduce pain, improve mobility and preserve joint function, resulting in better quality of life for the patient (8). The procedure of RSV requires only the injection into the synovial cavity of a radiopharmaceutical with the appropriated nuclear, chemical, and biochemical characteristics (4) and the radionucleotide should

Abbreviations: **RSV,** radiosynovectomy; **HA,** hydroxyapatite; **RNT,** Radionuclide therapy; **^{177}Lu,** lutetium-177 isotope; **^{90}Y,** yttrium-90 isotope; **^{153}Sm,** samarium-153 isotope.

be attached to particles that are sufficiently small to be phagocytosed , but not so small that they might leak of the joint before phagocytosis occurs (5).

Histological studies after RSV have shown reduction of the number and the size of the synovial villi with decreased hyperaemia in the early phase, trough thickening of the synovium often occurs. Later on, the sclerosing and fibrosing processes of the synovial villous stroma predominate, togheter with minimum diffuse damage of the articular cartilage. Both filtration and resorption of the synovial fluid are reduced. A few months after treatment with the radioisotope, mononuclear cell infiltration in the synovium has disappeared and if the treatment is effective the synovium is fibrosed (7).

Particulates labeled with b- emitting radionuclides such as ^{153}Sm, ^{165}Dy, ^{166}Ho, ^{186,188}Re have been studied for applicationin synovectomy. One of the most useful particulate employed is the Hydroxyapatite (HA) $[Ca_{10}(PO_4)_6(OH)_2]$, as a major chemical constituint of skeletal bone matrix. HA is converted into Ca and PO_4 ions in the body and is completely eliminated over a period of six weeks (6).

^{177}Lu is a very potential radioisotope for in vivo therapy, because of its favourable decay characteristics: decays with a half-life of 6.71 d by emission of β^- particles with E_{max} of 497 keV (78.6 %), 384 keV (9.1 %) and 176 keV (12.2 %), to stable ^{177}Hf. It also emits g photons of 113 keV (6.4 %) and 208 keV (11 %), that contributes for imaging the in vivo localization with a gamma camera. The long half-life of ^{177}Lu provides logistic advantage for facilitating supply to places far away from the reactors (9) ^{177}Lu could be very effective in radiation synovectomy of medium size joints.

^{90}Y is a radioisotope with good physical characteristics, ideal for intraarticular radiation synovectomy of large joints. It is a pure beta-emitter, E_{max} 2.3 MeV, half-life of 2.7 d and maximum tissue penetration of nearly 11mm with approximately 80 % of the energy deposited in the first 4 – 5 mm (3).

^{153}Sm has a half-life of 46.3 hr, maximum beta-energy of 0.81 MeV and an average soft-tissue penetration of 0.8 mm. It also presents a spectrum of gamma decay including a 20 % abundant 130 keV photon (1).

The aim of this work is to compare the in vivo stability of hydroxyapatite labeled with ^{177}Lu, ^{90}Y and ^{153}Sm in order to determine the influence of the radionuclide on biological pattern.

MATERIAL AND METHODS

Labeling procedure of ^{90}Y-HA

In a conical glass vial containing 40 mg of HA from Bio-Rad®, with particles in the desirable size range (20 mm) in 0.80 mL sterile water, 74 - 2,590 MBq of ^{90}Y in citrate form was added (using $^{90}YCl_3$ from Nordion®). 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 2 – 3 mL of sterile saline solution (pH = 6.0), sealed and autoclaved for 30 minutes at 121º C for further quality control tests (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) to a suspension of 20 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 and 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. Finally, the ^{177}Lu-HA was suspended in 2 - 3 mL sterile saline solution, autoclaved for 30 minutes at 121º C and used for further studies (radiochemical and biological determination).

Labeling procedure of ^{153}Sm-HA

The preparation of ^{153}Sm-HA was performed by adding citric acid monohidrates to $^{153}SmCl_3$ (in saline solution, molar ratio Sm:citrate of 1:2). After 30 minutes at room temperature, the final solution was incubated with 40 mg of HA and mixed with 750 mL of water. The vial was sealed, rotated swirl at room temperature for 30 minutes. Then, the product was rinsed with 20 mL of saline solution, centrifuged at 1000 rpm for 8 minutes. The supernatant (free ^{153}Sm) was separated from the precipitate carefully (labeled HA particles). The final precipitate (^{153}Sm-HA) was resuspended in 2 – 3 mL of sterile saline solution, sealed and autoclaved for 30 minutes at 121º C for further quality control tests.

Labeling yield determination

The labeling yield was determined by centrifugation for all the preparations. The reaction solutions were vortexed thoroughly and centrifuged at 2000 rpm for 5 minutes at the end of the reaction. Subsequently, the supernatants were carefully separated and the activity was measured in both: supernatant (free ^{90}Y, ^{177}Lu and ^{153}Sm) and pellet (particles of HA labeled), in a dose calibrator. From these data, the percentage of radiolabeling yield of ^{90}Y / ^{177}Lu / ^{153}Sm-HA were determined. The “in-vitro” stability was studied to determine the leaching of ^{90}Y, ^{177}Lu or ^{153}Sm activity from the radiolabeled HA at room temperature. The final products were suspended in 1.0 mL of saline solution and stored at room temperature for 7 days. Then, at different intervals 1, 2, 3 and 5 days, the suspensions were vortexed thoroughly and centrifuged, as mentioned before, to calculate the percentage of activity leaching in the supernatant.

Radiochemical purity preparations

The radiochemical purity of the ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA was determined by paper chromatography (Whatman® 3MM, 12 x 1.0 cm strips) using suitable eluting solvent, at 30, 120, 240 minutes after labeling, to assess the stability of the products. The strips were developed in 5 mM DTPA solution for ^{177}Lu-HA, 0.9 % saline solution for ^{90}Y-HA and ammonia:methanol:water (3:2:3) for ^{153}Sm-HA. Similar tests were carried out with $^{90}YCl_3$ and $^{177}LuCl_3$ and $^{153}SmCl_3$. It was observed in paper chromatography using 5 mM DTPA as the solvent, that ^{177}Lu-HA remained at origin (Rf = 0), whereas unreacted $^{177}LuCl_3$ moved towards the solvent front (Rf = 0.8), due the formation of ^{177}Lu-DTPA complex. Similarly, the ^{90}Y-HA remained at the origin (Rf = 0) in saline solution, while the free ^{90}Y moved near the front (Rf = 0.9). The $^{153}SmCl_3$, using as solvent ammonia:methanol:water (3:2:3), remained at origin (Rf = 0), while the ^{153}Sm-HA moved to front (Rf = 0.7).

Particle size determination of the preparations

Aliquots of 0.3 – 0.5 mL of ^{90}Y- HA / ^{177}Lu-HA / ^{153}Sm-HA were filtered using membranes of different size (Millipore®) in sequence (12; 8; 5 and 3 mm) followed by flushing air. The percentages of activity retained on the filters and in the eluent were determined in a dose calibrator (CAPINTEC).

Biological distribution of ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA

The biological behavior of ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA was studied in *Wistar* rats weighing 250 – 300 g under ketamine anesthesia. Doses of 18.5 - 37 MBq / 0.1 mL were injected intra-articularly in one joint knee. Serial scintigraphic images were recorded in gamma-camera (Medical Imagem System - Mediso®) at 1 and 24 and 7 days to determine the retention and leakage of the activity from the knee (synovium).

RESULTS AND DISCUSSION

The labeling procedures included centrifugation and separation steps in sterile conditions are simples, the quality control tests are reproducible and minimum facilities are required. 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-HA; ^{177}Lu-HA and ^{153}Sm-HA, are summarized in table 1. It was observed a radiolabeling yield higher than 87 % after 4 hours of preparation, with a radiochemical purity >98 % in all labeling procedures. 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-HA / ^{177}Lu-HA / ^{153}Sm-HA, described in the experimental section (labelling yield determination) are listed in table 1. It was found after 4 hours of preparation a radiolabeling yield superior than 87 %, with a radiochemical purity superior than 98 % in all labeling procedures.

The *"in vitro"* stability studies showed that the ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA preparations in saline, were highly stable at room temperature during 7 days, with radiochemical purity higher than 99 % in this period of time. This was evident from the results obtained by chromatographic system and centrifugation, respectively, listed in table I.

The particles size distribution of the three compounds (^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA), as can be seen in Table II, indicated that more than 99 % of the particles presented a size > 12 µm. All samples show approximately the same distribution pattern and particles between 5 - 20 µm. According Pandey et al. are believed to be ideally suited for synovectomy, due minimizes extra-articular leakage by lymphatic or venous drainage (8-2).

Table 1. Labeling Yield and Radiochemical Purity of ^{90}Y / ^{177}Lu-HA / ^{153}Sm-HA

	Labeling Yield	**Radiochemical putity**
^{90}Y-HA	87.36 ± 1.98 %	99.03 ± 0.43 %
^{177}Lu-HA	92.78 ± 0.85 %	99.88 ± 1.77 %
^{153}Sm-HA	91.65 ± 2.30 %	99.80 ± 0.67 %

Table 2. Particles size distribution of ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA (n=6).

Pore size	**% of particles**		
	^{90}Y-HA	**^{177}Lu-HA**	**^{153}Sm-HA**
12 μm	99	99.9	99
10 μm	-	-	-
8 μm	-	-	-
3 μm	-	-	-
<3 μm	1	0.01	1

The scintigraphic image in Wistar rats, obtained at one hour after intravenous administration (37 MBq/ 0.1 mL) of $^{177}LuCl_3$, is shown in Figure 1. It was observed clearly the skeleton as the major site of accumulation of the injected dose. When $^{177}LuCl_3$ is injected into the knee joint (37 MBq / 0.1 mL), exhibited a different distribution one hour after (Figure 2), remaining almost all activity in the joint and a little part leak to the bone. Meanwhile, the total activity distributed in bone, after 4 and 24 hours of injection.

Thus, the bones and urine should be, and in fact are, the two biologic compartments where radioactivity is found in any significant amount. Excretion of activity in the urine occurs mostly during the first 48 hours and is insignificant. Bone localization accounts for approximately 4 – 5 % of the administered activity and for ≈ 65 % of total leakage (2).

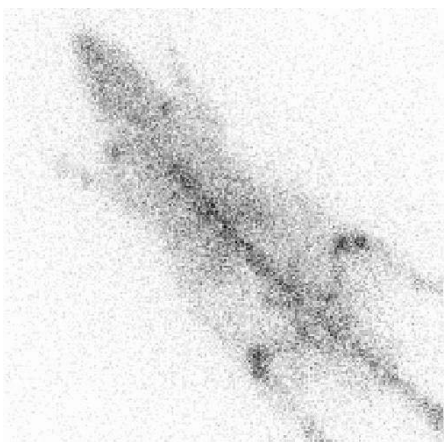

Figure 1. Gamma-camera image of $^{177}LuCl_3$, after intravenous injection.

Figure 2. Biological distribution of $^{177}LuCl_3$ injected in the knee joint.

The whole-body images recorded after ^{177}Lu-HA (37 MBq / 0.1 mL) administration in the cavity of the joint in Wistar rats after 7 days, prove the product remained intact in the knee even at least 7 days post-injection (Figure3).

Figure 3. Biological distribution of ^{177}Lu-HA.

The scintigraphic images of ^{153}Sm-HA following intra-articular injection in the knee joint of rats, one hour and 7 days did not present any detectable activity in any other organs until 7 days, confirming no extra-articular leakage of particles.

However, when administered $^{153}SmCl_3$ intravenously, a higher liver uptake was observed with less activity in bladder as recorded in figure 4.

Bremsstrahlung scintigrapy of ^{90}Y-HA in gamma camera were performed in Wistar rats, after 7 days of administration in the knee joint (Figure 5). No activity was found extra-articularly after this period of time.

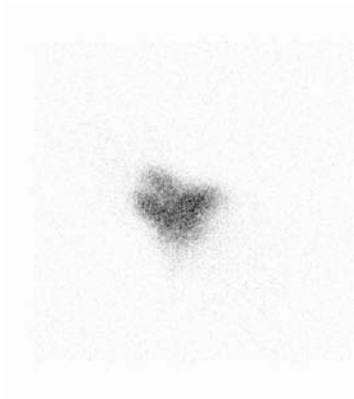

Figure 4. Image of $^{153}SmCl_3$ injected intravenously.

Figure 5: Gamma camera image of ^{90}Y-HA in Wistar rat.

Following intra-articular administration of labeled particulates, radioactivity may leave the knee joint by three routes. First may escape from the joint space intact, most commonly by way of lymphatic system after phagocytosis by macrophages, a second method of escape would involve breakdown of the particles within the joint space and liberation of the cation species of the metal ($Lu^{3+;}$ Sm^{3+} or Y^{3+}), free to diffuse through the joint capsule, the cation would be picked up by the venous drainage of the knee, and thus would give a biodistribution similar to that obtained by i.v. injection of ionic samarium or lutetium. And third, the breakdown to ionic form could follow by binding the cation specie to either to large biologic macromolecules, such as albumin, transferrin or small chelates. In each of this situations described above, a particular biodistribution would be seen. In the case of leakage of intact particles, the localization would occur in the either the regional lymph nodes or the reticuloendothelial system. In the second case, the free cation would behave as ionically administered yttrium, which is known to be a bone-seeking element. In the final case of binding to biologically active species, the yttrium would be found in the vascular system, if bound to large molecules, or be excreted rapidly in the urine, if bound to small species in chelated form (2).

The problem of radioactive leakage from the joint can be reduced by using radiopharmaceuticals with optimum particles size (5 – 20 μm) and choosing a radioisotope with a short half-life that minimizes the cumulative radiation dose to non-target tissue (8). It has been well established in previous woks that radiopharmaceutical particle size must be small enough to be phagocyted by the superficial cells of the synovium (< 40 μm) but not so small (> 2 μm), as to facilitate fast biological clearance by diffusion from the joint (10).

CONCLUSION

All the products described in the work present ideal characteristics for use in radiosynovectomy, each one for a determined intra-articular cavity. The methods of labeled and quality control are simples and easily, with high labeling yields as well as excellent radiochemical purity. The final products present stability up to 7 days at room temperature. Biological studies carried out in *Wistar* rats showed complete retention of injected radioactivity within the cavity of the knee for 7 days. These studies showed that ^{90}Y-HA, ^{177}Lu-HA and ^{153}Sm-HA offer potential as suitable agents in the management of RSV. Therefore, ^{177}Lu-labeled RSV agents will be economically more viable than the ^{90}Y-labeled analogues. 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 patients who have been refractory to medical management or in the hemophiliac patient with recurrent hemarthrosis

and synovitis who has failed medical therapy. The ^{153}Sm-HA and ^{90}Y-HA has been already commercially at the DIRF-IPEN - CNEN/SP being ideal for RSV. Although the modality has been used extensively in Europe for the past 25 years to treat rheumatoid arthritis in the knee joint, it has generated only modest research or clinical interest in the United States do date.

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Other articles in this theme issue include references (11-18).

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