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IMPROVEMENT IN THE ^{111}In-DTPA-TYR^3-OCTREOTIDE AND ^{177}Lu-DOTA-TYR^3-OCTREOTATE PRODUCTION.

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ABSTRACT

Recent advances in receptor mediated-tumor imaging have resulted in the development of somatostain analogues, the biomolecular basis for the clinical use of these compounds in nuclear medicine for diagnostic and peptide receptor radionuclide therapy (PRRT). PRRT is a very good therapeutic option for patients with metastatic neuroendocrine gastroenteropancreatic (GEP) tumour. Clinical studies with different sst-positives tumors proved advantages of [^{177}Lu-DOTA-Tyr^3]octreotate (DOTATATE) for therapy. The aim of this work is to establish and validate the labeling, the quality control procedures of DTPA-Tyr^3-Octreotide (DTPA-Oct) and DOTA-Tyr^3-Octreotate (DOTATATE) labeled with In-111 and Lu-177, respectively, for routine production at Radiopharmacy Directory (DIRF) Brazil. Labeling were performed in a "glove-box" using $^{111}InCl_3$ (Nordion) and in hot-cell with $^{177}LuCl_3$ (IDB-Holland) at pH 4.5; using DTPA-Oct (Pichem) and DOTATATE (IDB-Holland) at room temperature and at 82-85 ºC for 30 minutes, respectively. The radiochemical purity was determined by ITLC-SG in 0.1 mol L^{-1} sodium citrate, pH 5.5 and by Sep-Pak silica cartridge. Sterility was performed by the microbiology procedures and pyrogen tests by the "in-vitro" Limulus test (LAL). The stability of both radiolabeled peptides was high even 72 hours under refrigeration. The radiochemical purities of the labeled compounds were confirmed by HPLC. Sterility and pyrogen tests were negative in all delivered vials. The efficient procedure to obtain ^{111}In-DTPA-Oct and ^{177}Lu-DOTATATE was confirmed in the first comparative clinical groups. The methods were validated and 46.287 GMBq of ^{111}In-DTPA-Oct and 1,193 GBq of ^{177}Lu-DOTATATE were distributed in 2008, to nuclear medicine services in Brazil.

1. INTRODUCTION

Peptides are used for diagnosis and/or therapy by the coupling of radionuclides. The choice of the radionuclides depends on the application of the radiolabeled tumor detection, treatment, or treatment planning. The molecular basis of the use of radiolabeled peptide in scintigraphy and radionuclide therapy is receptor mediated internalization and cellular retention of the radionuclide [1, 2].

Somatostatin (SST) is a cyclopeptide that has a broad inhibitory effect on the secretion of hormones such as growth hormone, glucagon and insulin. The finding that somatostatin inhibits hormone secretion of various glands led to the application in the treatment of diseases based on the instance overproduction of hormones by tumors. The native peptide itself is unsuitable for routine treatment, as after intravenous administration it has a very

short half-life due to rapid enzymatic degradation. Somatostatin receptors have been identified in different kinds of tumors such as neuroendocrine tumors and tumors of central nervous system, breast, lung and lymphatic tissue making these receptors potential targets for radionuclide diagnostics and therapy. These observations have served as the biomolecular basis for the clinical use of radiolabeled somatostatin analogues which, at present, are of great interest in nuclear medicine for diagnostic and peptide receptor radionuclide therapy (PRRT) applications [1, 2].

Octreotide, an octapeptide analog of somatostatin, has a longer biological half-life, which makes it more suitable for labeling and imaging. There are only a few treatment modalities for metastasized neuroendocrine gastro-enteropancreatic (GEP) tumors. Besides surgery, (chemo)-embolization, chemotherapy, and treatment with somatostatin analogs, PRRT offers therapeutic strategy, as a majority of GEP tumors possess somatostatin receptors (SSTRs). Up to date, five subtypes have been identified (sst1-5), whereof the SSTRs subtype 2 (sst2) is frequently over expressed on endocrine pancreatic tumors, carcinoids, small-cell lung cancer and on certain brain and breast tumors [1-3].

Octreotide can be radioiodinated or labeled with the radiometals. Initial radiolabeling methods were focused on direct labeling due its simplicity, which was relatively successful for the iodine radioisotopes (I-123, I-125 and I-131). For radionuclides such as, Indium (In-111), yttrium (Y-90) and lutetium (Lu-177), indirect labeling methods with bi-functional chelators are mandatory [4].

Bi-functional chelators are compounds that are able to form a stable complex with radionuclide, containing a reactive group that can be used for coupling to the peptide or monoclonal antibody [4].

In-111, Y-90 and Lu-177 have been the most frequently used radionuclides for targeted radiotherapy in various clinical trials over the past decade. Differences in the physical properties of these radionuclides, which are important for the effectiveness of the therapy and related emitted particles, particle energy and tissue penetration range, as illustrated in Table 1 [4].

Table 1. Physical characteristics of radionuclides

Radioisotope	Particles	Particle energy (keV)	Penetration (range)*	Half-life (days)
In-111	γ Auger electron	171 - 245 3 - 19	10 μm (<1)	2.8
Y-90	β	935	12mm (~600)	2.7
Lu-177	γ β	113 - 208 130	2mm (~100)	6.7

* ***Number of cells, based on an average tumor cell size of 20 μm; ~, approximately***

Besides γ-radiation, which makes In-111 suitable for imaging, it emits both Auger and conversion electrons with a medium-to-short penetration range (0.02 – 10 and 200 - 500μm, respectively). In-111 coupled via the chelator DTPA to D-Phe-octreotide, as ^{111}In-DTPA-

Octreotide was used in the first clinical trials in which patients with metastasized GEP tumors were treated with radiolabeled somatostatin analogues [5, 6].

Somatostatin analogs featuring a DOTA-chelator can be radiolabeled among others with the β^- radioisotopes, Y-90 and Lu-177 for PRRT. Analogs frequently used for therapy are: [DOTA-Tyr^3]-octreotide and [DOTA-Tyr^3]-octreotate. In the latter compound, the alcohol threoninol at the C-terminal of the octreotide has been replaced by the natural amino acid threonin. This alteration resulted in an analog: (Tyr^3-octreotate), which showed increased affinity for sst2, compared to both [Tyr^3]-octreotide and [Phe^1]-octreotide "in-vitro" and "in-vivo". Clinical studies on patients with different sst-positives tumors proved advantages of [^{177}Lu-DOTA-Tr^3]-octreotate for therapy. PRRT with radiolabeled somatostatin analogs was shown to be effective in patients with sst2-positive-size reduction, improving quality of life and survival [7, 8].

2. OBJECTIVE

The aim of this work was to establish and validate the labeling and the quality control procedures of DTPA-Tyr^3-Octreotide (DTPA-Oct) and DOTA^o-Tyr^3-Octreotate (DOTATATE) labeled with In-111 and Lu-177, respectively, for routine production at Radiopharmacy Directory (DIRF), IPEN - CNEN / SP, Brazil.

3. MATERIALS AND METHODS

3.1 Labeling procedure of ^{111}In-DTPA-Oct

The labeling was carried out under Good Manufacturing Practices (GMP) condition in a "glove-box", using $^{111}InCl_3$ from Nordion-Canada and octreotide conjugated with diethylenetriamine-pentacetic acid (DTPA), DTPA-Oct provided by Pichem. The relationship between $^{111}InCl_3$: peptide was 12.2 MBq μg^{-1}, in 0.4 mol L^{-1} sodium acetate pH 4.5. The vial was mixed for 30 minutes at room temperature, and after cooling, 0.1 mL of gentisic acid (4 mg mL^{-1} gentisic acid in sodium acetate buffer pH 4.5) was added. The volume was completed until a desirable radioactive concentration with a sterile saline solution and sterilized under aseptic conditions in a 0.22 μm Millipore filter. Three samples (0.1 mL) of ^{111}In - DTPA-Oct. were sent to perform quality control assays and the final product was kept at 2–8 ^oC. The product was fractionated under request and delivered to several hospital and nuclear medicine centers in packages containing dry-ice.

3.2 Labeling procedure of ^{177}Lu-DOTATATE

The process was performed in a "hot cell", using $^{177}LuCl_3$ in 0.05 mol L^{-1} HCl and [DOTA^o,Tyr^3]-octreotate (DOTATATE) with 42 mg mL^{-1} gentisic acid, 210 mg mL^{-1} ascorbic acid in buffer acetate pH 4.5, from IDB-Holland. The molar relation between $^{177}LuCl_3$ and DOTATATE was 2.1. The vial was kept at 82-85 ºC for 30 minutes, and after cooling, 0.5-1.0 mL of DTPA solution (3 mg mL^{-1} DTPA in sterile saline solution) was added. The volume was completed until a desirable radioactive concentration with a sterile saline solution and sterilized under aseptic conditions in a 0.22 μm Millipore filter. Three samples (0.1 mL) of ^{177}Lu-DOTATATE were analyzed and the final product was kept at

2-8 ^{o}C before quality control approval and, fractionated according to request and delivered in package containing dry-ice.

3.3 Quality Control – determination of radiochemical purity

Radiochemical purity and stability were determined by instant thin-layer chromatography (ITLC-SG) using 0.1 mol L^{-1} sodium citrate buffer pH 5.5 as solvent. Radiochemical purity was also determined by solid phase extraction (Sep-Pak, C-18), Waters cartridge. The free radionuclide was eluted with 5 mL of 0.1 mol L^{-1} sodium acetate buffer pH 5.5 and the labeled peptide with 5 mL of methanol.

An aliquot of 10 μL of DTPA-Octreotide conjugated with In-111, with in a 55 MBq mL^{-1} concentration was analyzed by high performance liquid chromatography (HPLC), Shimadzu LC-20AT Prominence with an automatic sample injector SIL-20A, using Shim-pack ODS column (250 x 4.6 mm, 5 μm) in λ=220 nm, with a flow rate of 1.0 mL min^{-1} and the gradient system consisted of a mixture of acetonitrile (ACN) and water with 0.1% trifluoacetic acid (TFA) for 30 minutes. Quantitative γ-counting was performed on a Bioscan, Model B-FC-3300. The retention time (R_t) of peptide and the labeled compound was compared.

3.4 Quality Control – microbiological and biological assays

The microbiological analyses were determined in different culture medium (tioglicolate and soybean casein triptcase broth) incubated at (22.5 ± 2.5) ºC and (33.5 ± 2.5) ºC. The apyrogenicity was evaluated using the "in-vitro" Limulus test (LAL).

The biological assay was performed in Wistar rats (250 g) injecting intravenously 0.370 MBq in 0.1 mL of ^{111}In-DTPA-Oct. The animals were maintained anesthetized and the scintigraphy images were obtained after 30 minutes in a Gamma Camera Nucline TH 22 Mediso. The animals were sacrificed after 1 hour and the organ activities measured in gamma counter Capintec. The results were expressed as % injected dose per gram in the different organs.

4. RESULTS

The characteristics of the final product are illustrated in Table 2. The labeling efficiency or radiochemical purity, determined in both chromatography systems, were higher than 98% in all batches, when the samples were under refrigeration (Table 3).

Table 2. Characteristic of the final product (^{111}In-DTPA-Oct and ^{177}Lu-DOTATATE)

Product	pH	Purity (%)	SA(MBq mg^{-1})	Sterility	Pyrogen
^{111}In-DTPA-Oct	4.5	99.39 ± 0.21	8.02 ± 5.92	negative	negative
^{177}Lu-DOTATATE	4.5	99.55 ± 0.02	28.38 ± 4.7	negative	negative

* ***SA – specific activity***

Table 3. Radiochemical purity of ^{111}In- DTPA-Oct and ^{177}Lu-DOTATATE, stored under refrigeration (n=20)

Time (hours)	^{111}In-DTPA-Oct (%)	^{177}Lu-DOTATATE (%)
Immediately	99.39 ± 0.21	99.55 ± 0.02
4	99.80 ± 0.57	99.53 ± 0.02
28	98.75 ± 0.02	99.30 ± 0.05
48	98.43 ± 0.11	99.41 ± 0.03
72	98.36 ± 0.07	99.22 ± 0.07

DTPA-Oct and ^{111}In-DTPA-Oct were analyzed in the same conditions and the HPLC chromatogram of the labeled peptide, illustrated in Fig. 1 showed R_t = 14.3 minutes.

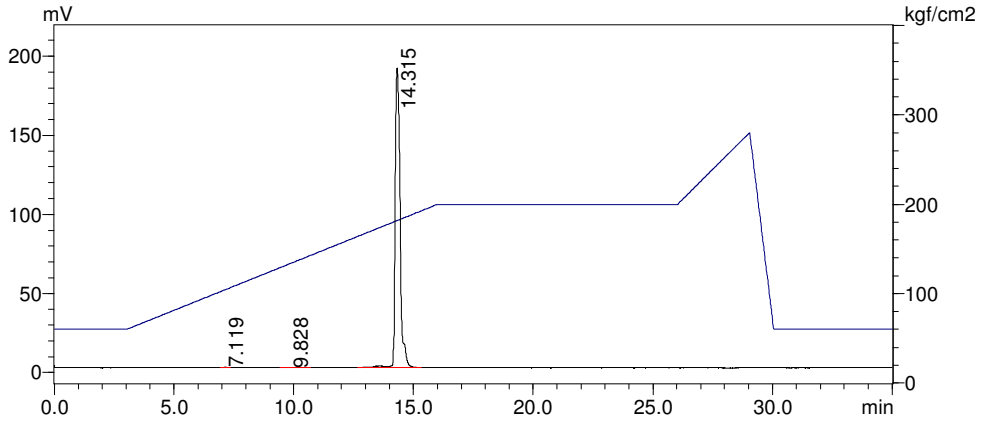

Figure 1. HPLC chromatogram of ^{111}In-DTPA-Oct (R_t = 14.3 min.). Radioactive concentration: 55.5 MBq mL^{-1}. Conditions: Shim-pack VP-ODS column (250 x 4.6 mm; 5 µm). Eluents A: water (0.1 % TFA); B: acetonitrile (0.1% TFA); gradient: 15% B for 3 minutes; 15 to 50% B in 13 minutes; 50%B for 10 minutes; 50 to 70% B in 3 minutes; 1.0 mL min^{-1} flow rate; 10 µL volume injection; detection in λ=220 nm and room temperature.

The stability of ^{177}Lu-DOTATATE and ^{111}In-DTPA-Oct was high even 72 hours under refrigeration with radiochemical purity >98.65% determined in both systems. Sterility and pyrogen tests were negative in all delivered vials (Table 2 and 3).

Fig. 2 represents a Wistar rat scintigraphy image in a Gamma Camera and in Table 4 presents the % activity in different organs.

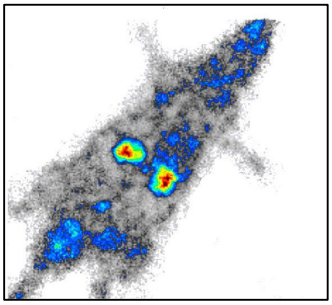

Figure 2. ^{111}In-DTPA-Oct scintigraphy image in a Wistar rat (1 hour)

Table 4. Biological distribution of ^{111}In-DTPA-Oct (% activity/organ)

Organ	(% activity/organ
Kidneys	19.30 ± 3.34
Liver	0.35 ± 0.08
Spleen	0.03 ± 0.01
Pancreas	0.30 ± 0.13
Lungs	0.26 ± 0.16

It was observed a normal distribution of the injected dose in specified organs and the scintigraphy image shows a significant uptake in the kidneys (Fig. 2, Table 4).

5. CONCLUSIONS

During 2008, 1.193 GBq of ^{177}Lu-DOTATATE were distributed with an specific activity (28.38 ± 4.70) MBq μg^{-1}, using an initial activity of (45,732 ± 3,574) MBq of $^{177}LuCl_3$ and (1,637 ± 266)µg of DOTA- Tyr^3-octreotate (DOTA-Octreotate) in 20 batches and 46,287 GBq of ^{111}In-DTPA-Oct with an specific activity of (8.25 ± 5.92) MBq mg^{-1}, using initial activity of (5,550 ± 740) MBq of $^{111}In\ Cl_3$.

The labeling and quality control procedures, under cGMP conditions, have been developed and validated at Radiopharmacy Directory of IPEN – CNEN/SP and the clinical application was successfully performed. Using the suitable methodology, it is possible to provide high quality peptide radiopharmaceuticals for diagnosis and clinical use for PRRT in Brazil.

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