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Labeling of DOTA-Tyr^3-octreotate with ^{177}Lu - Stability and Biodistribution Study

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Abstract

The labeling with ^{177}Lu and quality control procedures to produced DOTA (1,4,7,10-N,N´,N´´,N´´´, tetraazaciclododecane tetraacetic acid) coupled octreotate labeled peptide was evaluated.

The labeling was performed using 30µg of DOTA-Tyr^3-octreotate and 500 and 1110 MBq of ^{177}Lu, buffered with sodium acetate/acetic

acid 0.4M pH 4.5 for 20 minutes at 100ºC. The radiochemical purity was determined by ITLC na HPLC. Biological distribution studies were performed on *Nude* mices with tumours (AR42J rat pancreatic tumour cells) by invasive method.

The stability of the ^{177}Lu-DOTA-Tyr^3-octreotate was followed by 7 days, and in both labeling procedures, the radiochemical purity were superior than 98%. Biodistribution studies showed fast blood clearance and the kidneys were the critical organs. The uptake in tumour were significant after 24 hours and the labeled peptide showed high *in vivo* stability.

Key words: DOTA, octreotate, ^{177}Lu, biodistribuition.

Introduction

The advances in molecular biology opened the way for peptides in nuclear medicine. These biomolecules can be radiolabeled with an appropriated radioisotope to produce radiopharmaceuticals for diagnostic and therapeutic applications [1].

Among the peptides, somatostatin (sst) analogues have been intensively studied with positive clinical results[2-5]. The radioisotope with great perspectives to label these molecules for therapy application is ^{177}Lu. It has some properties such as physical half life of 6.7 days[6], ß$^-$ emission of 497 keV (78%), that can be absorbed in a small mass of tissue and the emission of gamma radiation (208,3 keV, 11%) wich allows simultaneous external detection, used for scintigraphy and dosimetric studies.

The aim of this work is to evaluate *in vitro* stability of DOTA-Tyr^3-octreotate labeled with ^{177}Lu and the biodistribution of the complex in *Nude* tumor bearing mice.

Material and methods

Labeling procedure

Labeling was performed using 30µg of DOTA-Tyr^{3-}octreotate (Pichem-Austria) and 500 and 1110 MBq of $^{177}LuCl_3$ in 0.05 N HCl, (MDS Nordion, Canada) for *in vitro* stability studies and 30µg of DOTA-Tyr^3-octreotate with 104 MBq of $^{177}LuCl_3$ to biological distribution studies. The reaction mixtures were buffered with sodium acetate/acetic acid 0.4 M pH 4.5. All reagents were prepared with Chelex 100 treated metal free water. The labeling reactions were allowed to proceed for 20 minutes at 100 ºC. The reaction mixtures were mantained at 7 - 8 ºC for stability studies. The conditions for radiolabeling were based in the procedures of Breeman et al[7].

Quality Control

The radiochemical purity was determined by instant thin layer chromatography (ITLC-SG), with citrate/citric acid buffer 0.1 M, pH 5.0 as the mobile phase. The stability was evaluated 48 and 168 hours after labeling reaction. The HPLC (Waters^{TM} 600 Controller with an UV/visible detector, 280nm and Radiomatic Flo-ONE\Beta radiodetector, Packard) was performed using C_{18} column (4.6 x 250 mm, 5µm, Waters), eluted with metanol:sodium acetate 0.06M, pH 5.5 gradient [5].

Biological Distribution Studies

Nude mice weighting 20.5 ±3.5g (n=3/group) were implanted subcutaneously in the back with 10^6 cells of the AR42J rat pancreatic tumor. Tumors are allowed to grow for about 4 weeks. These animals were injected in a tail vein with ^{177}Lu-DOTA-Tyr^3-octreotate diluted in NaCl 0.9% (0.74MBq/0.1mL). Biological distribution studies were performed by invasive method. At 1, 4, 24 and 48 hours after injection the animals were sacrificed. Blood and organs were colected and the radioactivity in these samples and in the diluted standard of the administered dose were determined using a gama counter (Packard).

Results

The complex was found to be stable with radiochemical purity superior than 98% (Table 1) for both activities (500 and 1110 MBq) when stored at low temperature. The biodistribution studies are showed in Table 2, results were expressed as percentage of the injected dose (ID) per tissue (%ID/tissue) and per gram of tissue (%ID/g).

The figure 1 and 2 shows the HPLC outline with a well defined peak in both radiolabeling conditions.

Table 1
Radiochemical purity of ^{177}Lu-DOTA-Tyr^3-octreotate (n=4).

Time (hours)	500MBq	1110MBq
0	99.4 ± 0.02%	99.46 ± 0.06%
48	98.84 ± 0.1%	98.77 ± 0.08%
168	98.8 ± 0.07%	98.3 ± 0.24%

Table 2
Biodistribution of ^{177}Lu-DOTA-Tyr^{3}-octreotate in *Nude* mice
(% ID/g ± SD) (n = 3).

Organs	1 hr	4 hr	24hr	48hr
Brain	0.10 ± 0.05	0.05 ± 0.03	0.04 ± 0.042	0.02 ± 0.007
Lung	2.33 ± 0.30	1.10 ± 0.81	0.61 ± 0.29	0.36 ± 0.13
Heart	0.68 ± 0.19	0.18 ± 0.04	0.09 ± 0.02	0.08 ± 0.01
Spleen	0.82 ± 0.15	0.43 ± 0.09	0.27 ± 0.06	0.24 ± 0.04
Liver	0.92 ± 0.26	0.69 ± 0.05	0.40 ± 0.05	0.36 ± 0.04
Stomach	5.93 ± 1.45	3.80 ± 0.55	2.06 ± 0.40	1.38 ± 0.14
Muscle	0.32 ± 0.11	0.09 ± 0.04	0.03 ± 0.004	0.03 ± 0.01
Kidneys	10.84 ± 0.82	9.99 ± 1.96	4.35 ± 1.79	2.54 ± 0.65
Small Intestine	1.77 ± 0.53	1.43 ± 0.08	0.26 ± 0.01	0.19 ± 0.01
Large Intestine	1.02 ± 0.43	5.16 ± 0.44	0.62 ± 0.08	0.50 ± 0.11
Adrenal	1.14 ± 1.18	1.65 ± 1.51	2.07 ± 0.44	1.06 ± 0.22
Pancreas	8.42 ± 2.94	3.95 ± 1.26	1.64 ± 0.21	0.90 ± 0.28
Bone	1.34 ± 0.79	1.59 ± 0.46	1.34 ± 0.28	1.67 ± 0.32
Tumor	2.45 ± 0.77	1.18 ± 0.32	0.83 ± 0.26	0.57 ± 0.09
Blood/mL	1.57 ± 0.10	0.16 ± 0.07	0.04 ± 0.006	0.03 ± 0.005

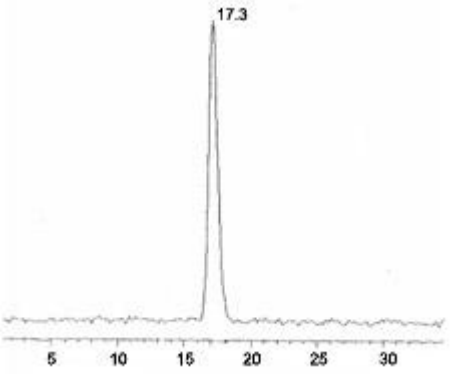

Fig.1 - HPLC outline of 30 µg -500MBq

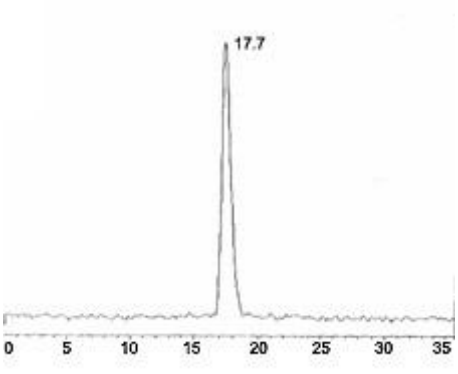

Fig. 2 - HPLC outline of 30 µg - 1110MBq

Discussion and conclusion

The interest in using somatostatin analogues labeled with radioisotopes to

receptor target therapy is increasing[8,9].

In this study, the complex ^{177}Lu-DOTA-Tyr^3-octreotate was obtained with high stability. Additional studies will be developed to evaluate radiochemical stability using labeling activities compatibles with therapeutical aplication.

Biological distribution studies showed the high uptake of the compound in tissues with high density of somatostatine receptors like adrenals and pancreas.

Blood clearance was fast and the kidneys are the critical organ, because the compound is mainly excreted in the urine. Bone uptake was very low, showing the great *in vivo* stability of the compound, considering that free luthetium presents high affinity to the bone tissue.

Tumour uptake of ^{177}Lu-DOTA-Tyr^3-octreotate was significant and evidences the applicability of the complex in tumour target therapy.

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