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A comparative study of ^{131}I and ^{177}Lu labeled somatostatin analogues for therapy of neuroendocrine tumours

E.B. de Araújo *, J.S. Caldeira Filho, L.T. Nagamati, E. Muramoto, M.T. Colturato, R.M. Couto, P.B. Pujatti, J. Mengatti, C.P.G. Silva

Radiopharmacy Directory, Nuclear and Energetic Research Institute (IPEN), Av. Lineu Prestes, 2242, Cidade Universitária, Butantã, CEP 05508-000, São Paulo, Brazil

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ABSTRACT

This work analysed the influence of the chelating group and radioligand on somatostatin analogues *in vivo* and *in vitro* properties. The presence of DOTA in the radioiodinated peptide produced a labeled analogue with similar blood kinetics and biodistribution to ^{177}Lu-DOTATATE and with lower abdominal uptake than ^{131}I-TATE. In addition, ^{131}I-DOTATATE showed significative tumour uptake, despite not so persistent after 24 h. ^{131}I-DOTATATE can represent a cost-effective alternative to lutetium labeled peptide for neuroendocrine tumours therapy.

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1. Introduction

Neuroendocrine tumours (NETs) are a heterogeneous group of neoplasms which are characterised by the ability to produce biogenic amines and polypeptide hormones. These tumours come from endocrine glands, like pancreas, and usually origin metastases in the respiratory and gastrointestinal tract (Rufini et al., 2006).

In recent years, a number of new developments in targeted therapies have emerged (Oyen et al., 2007) and the presence of peptide receptors and transporters at the cell membrane of several NETs constitutes the basis of the clinical use of specific radiolabeled ligands. Because the majority of NETs express somatostatin receptors (SSTr), the introduction of radiolabeled somatostatin (SST) analogues for peptide receptor imaging and radiotherapy of neuroendocrine cancer have become a primary focus of interest in nuclear medicine (Kreening et al., 1992).

In order to visualise SST receptor-containing tumours, a long-acting SST analogue was required because the native SST has a half-life in blood of only 3 min due to rapid enzymatic degradation (Lamberts et al., 1987). Many efforts were made in order to find a radiolabeled selective analogue with a longer duration of action using synthetic SST peptide derivatives.

^{123}I-labeling Tyr^3-octreotide (Tyr-OC) was the first compound to be used for imaging of SST receptor-positive tumours in animals. However, some properties limited the use of this radiopharmaceutical, like relative low radiochemical yield and high uptake on liver and intestines after 30 min of the administration (Bakker et al., 1990).

Octreotide has been conjugated with diethylene-triamine-pentaacetic acid (DTPA) and labeled with ^{111}In, showing an improved biodistribution when compared with the initial analogue. Today, ^{111}In-DTPA-D-Phe^1-octreotide has become the dominant radiolabeled SST analogue for visualisation of SSTr-expressing tumours (Kreening et al., 1992; Kwekkeboom et al., 2001).

Octreotide has been also conjugated with the macrocyclic chelator DOTA (1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetra-acetic acid), resulting in tracers that are suitable for a variety of clinical applications. In addition, replacement of C-terminal threonil of the octapeptide with the natural amino acid threonine, changing octreotide to octreotate, increased SSTr-2 affinity and tumour uptake (Kwekkeboom et al., 2001).

SST analogues also can be labeled with beta-emitting therapeutic radioisotopes. Newer therapeutic approaches involve the use of the beta-emitter yttrium-90 conjugated via DOTA to Tyr-OC. The therapeutic potential of ^{90}Y-DOTA-Tyr-OC has been

* Corresponding author. Tel.: +55 1131 339547.
E-mail address: ebaraujo@ipen.br (E.B. de Araújo).

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evaluated in some clinical protocols (Bodei et al., 2003; Waldherr et al., 2002) representing a remarkable therapeutic drug and an effective alternative to chemo and biotherapies. However, kidney toxicity remains the major concern in repeated administrations.

Alternatively to ^{90}Y, 6.7 day half-life ^{177}Lu has emerged as a promising short-range β emitter for target radiotherapy. The mean range of ^{177}L β particles ($E\beta_{max} = 497$ keV) is 670 μm, making this radionuclide ideal for treating micro-metastatic disease. Because it also emits γ rays (208 keV, 11% abundance), imaging of ^{177}Lu labeled endoradiotherapeutic agents is possible (Zalutsky, 2003).

The therapeutic potential of ^{177}Lu labeled SST analogues was evaluated in comparative studies with either ^{111}In or ^{90}Y labeled molecules (Kwekkeboom et al., 2001; de Jong et al., 2005).

The SST analogue DOTA-Tyr^{3}-octreotate (DOTATATE) has a ninefold higher affinity for the SSTr subtype 2 as compared with DOTA-Tyr-OC. Labeled with ^{177}Lu, this compound was shown to be very successful in achieving tumour regression (Kwekkeboom et al., 2003; Forrer et al., 2005). In addition, SST derivatives labeled with ^{90}Y and ^{177}Lu can be combined to treat patients with tumours of various sizes with non-homogenous receptor distribution, to achieve higher cure rates (de Jong et al., 2005).

Despite good clinical therapeutical results obtained with ^{90}Y and ^{177}Lu labeled SST derivatives, these radionuclides are not easily available. ^{90}Y are produced by high cost ^{90}Sr-^{90}Y generator while ^{177}Lu only can be obtained in adequate specific activity by thermal neutron bombardment using enriched targets in medium to high flux reactor, making the production of ^{177}Lu restricted (Pillai et al., 2003).

^{131}I, with a half-life of 8.1 days, has been the most frequently used radionuclide for therapeutic applications, and has a mean range of 910 μm. Although ^{131}I has a tissue range that is well suited to the treatment of small tumours, it also emits a 364 keV γ ray in 81% abundance which is not ideal for conventional or single photon emission tomographic imaging devices (Zalutsky, 2003).

The experience gained with radioiodinated SST ligands showed that the diagnostic and therapeutic usefulness of them was limited by their unfavourable kinetics, *in vivo* deiodination and resulting dosimetry. Additionally, they exhibited low tumour retention, which was often attributed to fast intracellular degradation of the tracers and subsequent extracellularisation (Wester et al., 2002; Bakker et al., 1996).

DOTA chelating group are not involved in the radioiodination of DOTATATE. Labeling occurs by the introduction of the radioiodine in the aromatic residue of tyrosine like in the labeling of Tyr^{3}-octreotate (TATE) with radioiodine. However, the presence of the DOTA chelating group may change the lipophilicity of the peptide itself and influence in the stability and biological distribution of the radioiodinated peptide.

In this work, we optimised the labeling of DOTATATE and TATE with ^{131}I and the labeling of DOTATATE with ^{177}Lu to produce high radiochemical yield radiopharmaceuticals and evaluate the influence of the chelating group and of the radioligand on *in vitro* stability and *in vivo* behaviour of the SST labeled derivatives.

2. Materials and methods

2.1. Reagents

DOTATATE was provided by piChem and the TATE by Anaspec (EUA). Na^{131}I and $^{177}LuCl_3$ were obtained from Nordion (Canada) and IBD (Holland), respectively. All other reagents were purchased from Sigma-Aldrich.

2.2. Preparation of the radiotracers

2.2.1. Labeling of TATE and DOTATATE with radioiodine (^{131}I)

The labeling of TATE and DOTATATE with Na^{131}I was optimised using Chloramine T method (Breeman et al., 2001). A solution of 10 μg of peptide in 40 μL of PBS (0.1 M phosphate-buffered saline pH 7.5) was transferred to a reaction vial. After the addition of the Chloramine T solution (5 μg/5 μL) and 5–10 μL of radioiodine solution (7.4–111 MBq), the cap was carefully vortexed and the reaction was allowed to proceed for 3 min at room temperature. The reaction was interrupted by the addition of the sodium metabisulfite solution (10 μg/5 μL).

2.2.2. Labeling of DOTATATE with lutetium (^{177}Lu)

The labeling of DOTATATE (10 μg) with $^{177}LuCl_3$/0.05 N HCl was performed in acetate buffer 0.4 M pH 4.5 (Breeman et al., 2003). All reagents were prepared with Chelex 100 treated metal free water. The labeling reaction was allowed to proceed for 30 min at 90 °C.

2.3. Quality control

Radiochemical purity was determined by HPLC (Waters) with radioactivity (Packard) detection, using RP C_{18} column (4.2 × 50 mm, 5 μm-Waters) flow rate of 0.5 mL/min with a linear gradient of 40–80% (v/v) methanol in 50 mM sodium acetate buffer (pH 5.5) for 20 min and the composition was maintained for another 25 min (Bakker et al., 1996; Kwekkeboom et al., 2001). Free radioiodine was also determined by horizontal zone electrophoresis (Amershan) on Whatman 1 paper, 0.05 M barbital buffer, pH 8.6, using a field of 300 V for 40 min. Instant thin layer chromatography (ITLC) was applied to determine free lutetium, with citrate/citric acid buffer pH 5.0 as solvent (Rf of labeled peptide was 0.3–0.4 and Rf of free lutetium was 0.9–1.0) (Kwekkeboom et al., 2001).

2.4. Purification

Reaction mixtures were purified on pre-activated Sep-Pak C_{18} reversed phase extraction cartridge (Waters) eluted with 5 mL of distilled water to remove free radionuclide and 2.5 mL of ethanol 96% to elute the labeled peptide (Bakker et al., 1990). The solvent was evaporated under a gentle stream of nitrogen and the dry residue was dissolved in 2–5 mL of PBS or saline.

2.5. Stability in plasma

The stability of radiolabeled analogues was evaluated by the incubation on human plasma at 37 °C and radiochemical purity was determined 1, 4 and 24 h by ITLC-SG as previously described.

2.6. Biological studies

Animal experiments were performed in compliance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, second Edition.

Biological distribution studies were developed in *Swiss* mice and *Nude* mice bearing AR42J rat pancreatic tumour.

^{131}I-DOTATATE, ^{131}I-TATE and ^{177}Lu-DOTATATE (0.74 MBq/0.1 mL PBS) were injected in the tail vein. The thyroids of the animals were not blocked. The animals were sacrificed 1, 4 and 24 h after the dose administration for iodine labeled peptides and 1, 4, 24 and 48 h after dose administration for lutetium labeled peptide. The percent injected dose/organ (% ID/organ) and percent injected dose/gram (% ID/g) were determined.

2.7. In vitro internalisation assay in AR42J cells

AR42J cells (approximately 1×10^6) were distributed in centrifuge tubes (triplicate for each time, for specific and non-specific binding) containing 1 mL of internalisation buffer (F12 K medium containing 50 units/mL penicillin, 50 μg/mL streptomycin and 0.2% BSA). Non-specific internalisation was assessed by addition of octreotide 5.93×10^{-9} mol. After 1 h incubation at 37 °C, the radioligand (6.9×10^{-12} mol) was added and the incubation allowed for 0.25, 0.5, 1 and 2 h, when the cells were extensively washed with PBS to remove free radioligand and incubated for 5 min in 2 mL ice-cold glycine buffer pH 2.8. After removal of the acid buffer, cells were rinsed once with an additional 1 mL acid buffer and once with PBS. This acid buffer treatment enables dissociation of surface-bound ligand. Cells were then solubilised in 1 mL 1N NaOH and transferred to tubes for quantification of internalised radioactivity in a gamma counter. The radioactivity from combined acid fractions was also determined. Results were expressed as percent binding to 10^6 cells and as percent internalised (Hofland et al., 1996; Reubi et al., 2000; Froidevausx et al., 2002).

2.8. Statistical analysis

The results are expressed as MEAN $\pm$ SD and statistical analysis was performed using the Student's *t*-test for paired data with one-tailed distribution.

3. Results

3.1. Labeling procedures

3.1.1. Labeling of TATE and DOTATATE with radioiodine (^{131}I)

Different molar peptide to radionuclide ratios were investigated in order to obtain mono-iodinated peptides to be applied in the biodistribution studies, considering that the di-iodinated peptide no longer binds to the SSTr, as previously reported (Bakker et al., 1996).

Electrophoresis analysis showed radiochemical purity of 98.4 $\pm$ 0.3% for ^{131}I-TATE and 95.4 $\pm$ 0.5 for ^{131}I-DOTATATE in a molar peptide to radionuclide ratio of 2.73. After Sep-Pak purification procedure, the radiochemical purity determined on ethanol fraction was 99.3 $\pm$ 0.1% of ^{131}I-DOTATATE.

The labeling of DOTATATE using peptide to radionuclide ratio of 2.73, produced high radiochemical yield (Table 1), represented by one peak in HPLC profile with retention time (RT) of 22.73 min (Fig. 1A). With a molar peptide to radionuclide ratio of 0.54, a second radiochemical species was observed in HPLC profile (RT 24.9 min, Fig. 1B), probably related to the di-iodinated form of the peptide. Using very low peptide to radionuclide ratio (0.045) different species were found in HPLC profile (Fig. 1C).

3.1.2. Labeling of DOTATATE with lutetium (^{177}Lu)

High radiochemical yields (98.9 $\pm$ 0.3%) were obtained for reaction mixtures containing 10 μg of DOTATATE and 7.4 MBq of $^{177}LuCl_3$ employing acetate buffer pH 4.5 as reaction medium. After Sep-Pak purification procedure, the radiochemical purity of the ethanol fraction increased to 99.9 $\pm$ 0.01%.

Table 1
Radiochemical purity of ^{131}I-DOTATATE in ITLC-SG chromatographic system labeled with different molar peptide to radionuclide ratios ($n = 4$)

Molar peptide/radionuclide ratio	Radiochemical purity (%)
2.73	95.5 $\pm$ 0.9
0.54	93.7 $\pm$ 1.0
0.27	57.9 $\pm$ 0.6
0.045	2.6 $\pm$ 0.5

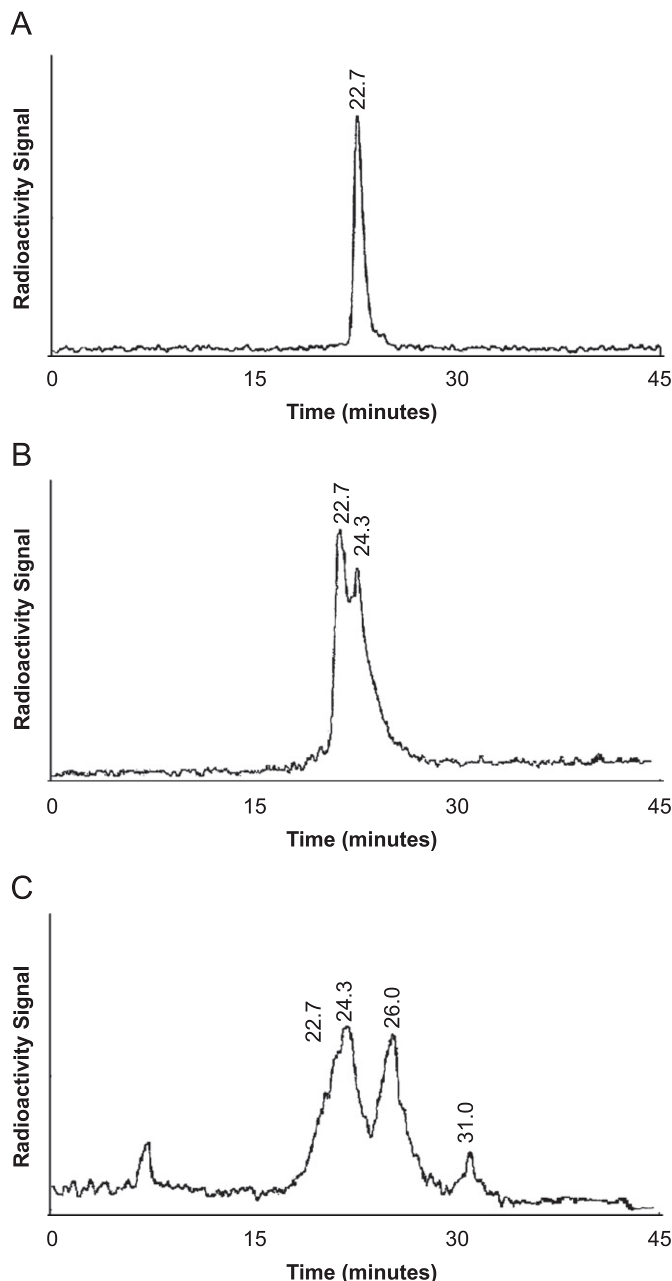

Fig. 1. HPLC profile of DOTATATE radioiodinated with different molar peptide to radionuclide ratios: (A) molar peptide to radionuclide ratio of 2.73, (B) molar peptide to radionuclide ratio of 0.54 and (C) molar peptide to radionuclide ratio of 0.045.

Fig. 2 shows a typical HPLC profile for ^{177}Lu labeled mixture. The labeled peptide (RT = 21.7 min) can be separated from free lutetium (RT = 7.3 min) and from unlabeled peptide (RT = 19.14 min in UV HPLC spectrum, not presented).

3.2. Stability of the preparations in human plasma

Either ^{131}I- or ^{177}Lu labeled peptides remained stable after 24 h (Table 2), showing that these preparations can be used as a tool for *in vivo* studies.

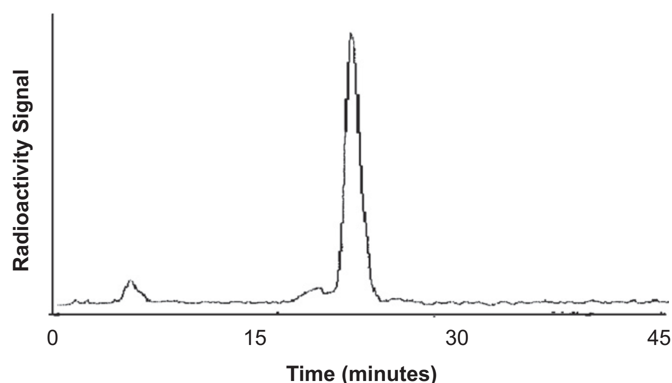

Fig. 2. HPLC profile of ^{177}Lu-DOTATATE after 24 h of labeling and storing at room temperature (RT of free lutetium was 7.3 min and of labeled peptide was 21.7 min).

Table 2
Stability of radiolabeled peptides in human plasma at 37 °C ($n = 3$)

	Radiochemical purity (%)			
	Immediately	1 h	4 h	24 h
^{131}I-TATE	98.4±0.3	98.4±0.5	96.8±1.0	95.8±0.1
^{131}I-DOTATATE	95.4±0.5	93.8±0.8	92.4±0.6	91.0±0.6
^{177}Lu-DOTATATE	98.9±0.3	Not measured	Not measured	98.7±0.5

3.3. *In vivo studies*

Biological distribution studies were performed with radioiodinated peptides obtained in a peptide to radionuclide ratio of 2.73, with radiochemical purity superior than 97%. At this condition, Sep-Pak purification procedure was not necessary. However, like other radioiodinated peptides and proteins labeled on tyrosine residues, it is important to study the possibility of *in vivo* dehalogenation.

The radioiodinated peptides exhibited fast blood clearance (Fig. 3) and kept stable *in vivo*, considering low thyroid uptake, observed specially to the DOTA iodinated compound (Table 3). The biodistribution pattern of ^{131}I-TATE in *Nude* mice xenografted with AR42J cells (Table 4) was similar in magnitude to that obtained for ^{123}I-Tyr-OC in rats (Bakker et al., 1990) and for ^{123}I-Tyr-OC and ^{123}I-RC-160 in tumour bearing rats (Breeman et al., 1993), and for ^{125}I-TOC in *Nude* mice bearing AR42J rat pancreatic tumour (Wester et al., 2002), with relatively high liver and intestinal uptake. In contrast ^{131}I-DOTATATE was predominantly excreted by the kidneys and the uptake on tumour and pancreas (that presents high density of SST receptors) was higher than ^{131}I-TATE, especially after 1 h of the dose administration (Table 4).

The ^{177}Lu-DOTATATE was mainly excreted by kidneys (Table 5) and the uptake of this radiopharmaceutical on kidneys was lower than ^{131}I-DOTATATE (Fig. 4), which can result in a comparatively less radiation damage to the kidneys.

As expected, the uptake and retention of radioactivity in tumour was notably higher at 1 h and 24 h for ^{177}Lu-DOTATATE, when compared to the radioiodinated analogues, due to intracellular trapping of the radionuclide or radiolabeled metabolites (Wester et al., 2002). The uptake on pancreas and adrenals was higher for ^{177}L labeled peptide, particularly after 24 h. Tumour to tissue ratios were also optimised, particularly tumour:blood and tumour:muscle ratios (Table 6).

3.4. *Internalisation assay*

The molecular basis of radiolabeled SST derivatives used in imaging and therapy is receptor-mediated internalisation of

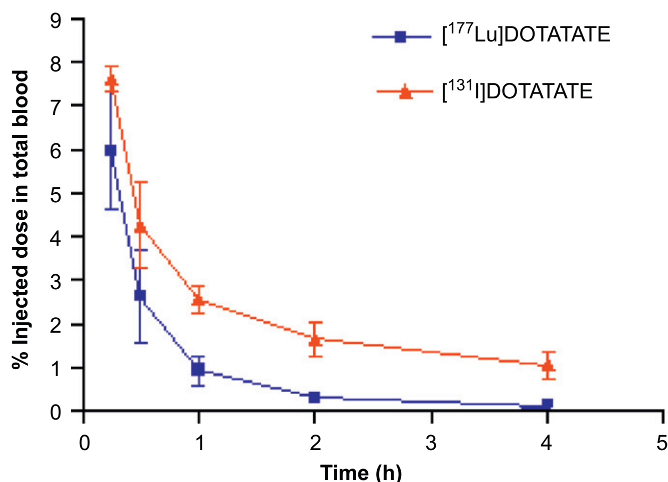

Fig. 3. Blood kinetics of ^{131}I- and ^{177}Lu-DOTATATE.

radiolabeled forms and retention in lysosomes, rendering possible the *in vivo* visualisation or therapy. The internalisation study showed that DOTATATE labeled with lutetium and iodine presented similar percentage of internalisation (about 50%) in AR42J tumour cells after 1 h of incubation (Fig. 5). This percentage decreased more significantly after 2 h for the ^{131}I-DOTATATE, as expected, considering the intracellular metabolisation of the radioiodinated peptide but remained superior to 45%. The total binding expressed as percent binding per 10^6 cells are similar for both compounds after 2 h of incubation (Fig. 6).

4. Discussion

^{177}Lu-DOTATATE has been applied in clinical protocols for therapy of neuroendocrine tumours (Kwekkeboom et al., 2003; Forrer et al., 2005; de Jong et al., 2005). The favourable biodistribution kinetic of the compound, that presents fast blood clearance, results in rapid and effective uptake in the tumour. The compound is mainly excreted by the kidneys, which constitutes the target organ for dosimetric considerations.

The physical properties of the ^{177}Lu are particularly attractive to irradiate small tumour mass and the presence of a low energy gamma emission allows the acquisition of scintigraphic images before and after therapy.

Despite the favourable physic characteristics of the ^{177}Lu, the production of this radionuclide with high specific activity, as required for peptide labeling procedures, only can be performed with moderate to high neutron flux nuclear reactor, and enriched targets (Pillai et al., 2003). In addition, the logistic of ^{177}Lu distribution not always favourable associated to the high cost of the production may restrict the use of ^{177}Lu for many countries, particularly those distant from the production centre.

On the other hand, ^{131}I is a well known radionuclide frequently applied in therapeutic procedures in Nuclear Medicine. Despite the gamma emission of high energy can be considered as a disadvantage in therapeutical procedures, this beta emitter radionuclide can be obtained in high specific activity and low costs when compared to the ^{177}Lu.

First labeling procedures for radioiodination of SST analogues like Tyr-OC and TATE resulted in low radiochemical yields and the biodistribution were not favourable due to the high hepatic-intestinal uptake that compromised the visualisation of tumours in abdominal region. Additionally, radioiodinated SST derivatives showed low tumour uptake when compared to the SST derivatives labeled with

Table 3
Tissue distribution of ^{131}I-TATE and ^{131}I-DOTATATE in *Swiss* mice (% ID/g) ($n=6$)

Tissue	^{131}I-TATE			^{131}I-DOTATATE		
	1 h	4 h	24 h	1 h	4 h	24 h
Blood	3.2±0.3	0.8±0.1	0.1±0.01	2.7±0.3	1.2±0.1	0.02±0.004
Liver	2.3±0.8	0.4±0.1	0.2±0.03	0.7±0.1	0.4±0.1	0.09±0.01
Spleen	0.8±0.3	0.4±0.1	0.1±0.04	0.6±0.1	0.3±0.04	0.06±0.02
Stomach	3.5±1.7	1.9±1.0	0.09±0.03	3.2±0.4	2.0±0.6	0.2±0.05
Int. (small)	12.3±4.4	1.3±1.0	0.05±0.01	1.5±0.1	0.6±0.1	0.4±0.2
Int. (large)	1.0±0.6	14.8±0.8	0.1±0.03	0.4±0.1	2.1±0.2	1.9±0.8
Kidneys	12.5±0.5	9.8±3.5	3.8±1.2	12.1±0.8	9.8±1.0	1.6±0.2
Sk. Muscle	0.5±0.2	0.13±0.06	0.02±0.002	0.3±0.02	0.1±0.03	0.03±0.01
Brain	0.08±0.01	0.02±0.01	0.01±0.001	0.08±0.03	0.04±0.01	0.006±0.003
Heart	0.7±0.1	0.2±0.1	0.05±0.02	0.5±0.1	0.2±0.1	0.02±0.003
Lung	1.6±0.4	0.5±0.1	0.01±0.02	0.8±0.4	0.5±0.2	0.05±0.005
Thyroid[a]	0.5±0.1	1.1±0.2	0.9±0.3	0.6±0.1	1.2±0.2	0.3±0.06
Adrenals[a]	0.02±0.01	0.007±0.004	0.002±0.0002	0.012±0.003	0.008±0.001	0.0014±0.0005
Pancreas	0.8±0.2	0.2±0.1	0.02±0.008	1.1±0.5	0.8±0.1	0.03±0.01

Values are mean±SD.
[a] In thyroid and adrenal the results were expressed as % ID/organ.

Table 4
Tissue distribution of ^{131}I-Tyr^3-octreotate and ^{131}I-DOTA-Tyr^3-octreotate in *Nude* mice bearing AR42J rat pancreatic tumour (% ID/g) ($n=4$)

Tissue	^{131}I-TATE		^{131}I-DOTATATE	
	1 h	24 h	1 h	24 h
Blood	2.1±0.5	0.09±0.03	2.9±0.3	0.1±0.01
Liver	2.1±0.7	0.2±0.03	1.3±0.1	0.2±0.01
Int. (small)	8.8±1.9	0.06±0.001	2.8±0.6	0.07±0.02
Sk. muscle	0.4±0.1	0.02±0.01	0.4±0.2	0.04±0.01
Thyroid[a]	0.3±0.1	0.5±0.03	0.5±0.2	1.2±0.3
Adrenals[a]	0.02±0.01	0.002±0.001	0.02±0.004	0.003±0.001
Pancreas	0.8±0.1	0.04±0.001	1.1±0.3	0.05±0.01
Tumour	1.1±0.4	0.2±0.1	1.7±0.01	0.1±0.01

Values are mean±SD.
[a] In thyroid and adrenal the results were expressed as % ID/organ.

Table 5
Tissue distribution of ^{177}Lu-DOTATATE in *Nude* mice bearing AR42J tumour (% ID/g) ($n=3$)

Tissue	^{177}Lu-DOTATATE (% ID/g)			
	1 h	4 h	24 h	48 h
Blood	2.4±0.1	0.2±0.1	0.06±0.01	0.05±0.01
Brain	0.1±0.05	0.05±0.03	0.04±0.04	0.02±0.01
Lung	2.3±0.3	1.1±0.8	0.6±0.3	0.4±0.1
Heart	0.7±0.2	0.2±0.04	0.1±0.02	0.08±0.01
Spleen	0.8±0.1	0.4±0.1	0.3±0.1	0.2±0.04
Liver	0.9±0.3	0.7±0.05	0.4±0.1	0.4±0.04
Stomach	5.9±1.4	3.8±0.5	2.1±0.4	1.4±0.1
Sk. muscle	0.3±0.1	0.09±0.04	0.03±0.01	0.03±0.01
Kidneys	10.8±0.8	10.0±2.0	4.4±1.7	2.5±0.6
Int. (small)	1.8±0.5	1.4±0.1	0.3±0.01	0.2±0.01
Int. (large)	1.0±0.4	5.2±0.4	0.6±0.1	0.5±0.1
Adrenal[a]	1.1±1.2	1.6±1.5	2.1±0.4	1.1±0.2
Pancreas	8.4±2.9	3.9±1.3	1.6±0.2	0.9±0.3
Bone	1.3±0.8	1.6±0.5	1.3±0.3	1.7±0.3
Tumour	2.4±0.8	1.2±0.3	0.8±0.3	0.6±0.1

Values are mean±SD.
[a] In adrenal the results were expressed as % ID/organ.

radiolanthanides and metallic radionuclides, considering that the introduction of the radioiodine in the Tyrosine residue make the derivative susceptible to enzymes inside the tumour cells.

Despite the disadvantages associated to the use of radioiodinated SST derivatives, this study demonstrated that the

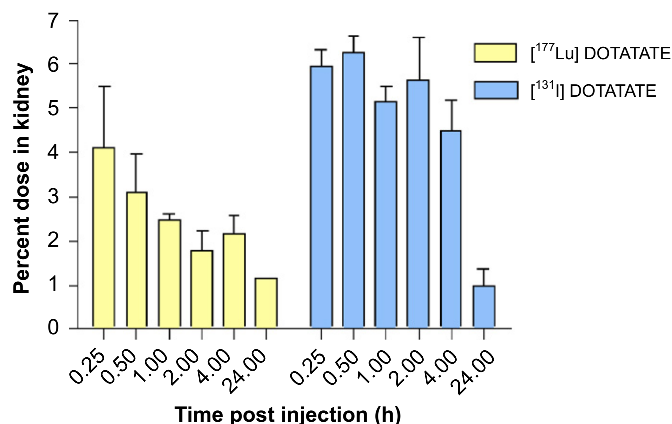

Fig. 4. Renal uptake of ^{131}I- and ^{177}Lu-DOTATATE in *Swiss* mice. Both radiopharmaceuticals were mainly excreted by kidneys, but ^{177}Lu-DOTATATE uptake by this organ was lower than ^{131}I-DOTATATE, which can result in a comparatively less radiation damage.

Table 6
Selected organ to tissue ratios (% ID/g) of labeled compounds in *Nude* mice bearing AR42J tumour

	^{131}I-TATE		^{131}I-DOTATATE		^{177}Lu-DOTATATE	
	1 h	24 h	1 h	24 h	1 h	24 h
Tumour:blood	0.52	2.11	0.59	1.05	1.03	13.8
Tumour:liver	0.53	1.06	1.29	0.87	2.66	2.08
Tumour:muscle	2.97	7.50	4.11	3.33	7.65	27.6

labeling of DOTATATE with ^{131}I resulted in a labeled peptide with biodistribution pattern very similar to ^{177}Lu-DOTATATE, that means, fast blood clearance and elimination by renal tract.

The presence of DOTA chelating group, not involved on labeling procedure with radioiodine, represents a structural modification of the peptide that probably altered the lipophilicity and contributed to reduce liver and intestinal uptake, when compared to the ^{131}I-TATE.

According to Bakker et al. (1996), who described the influence of the molar peptide to radionuclide ratio on radiochemical yield the Tyr-OC, a high percent of the mono-iodinated peptide was obtained with a molar peptide to radionuclide ratio of 43 (a great excess of peptide over radionuclide). In the same study, using

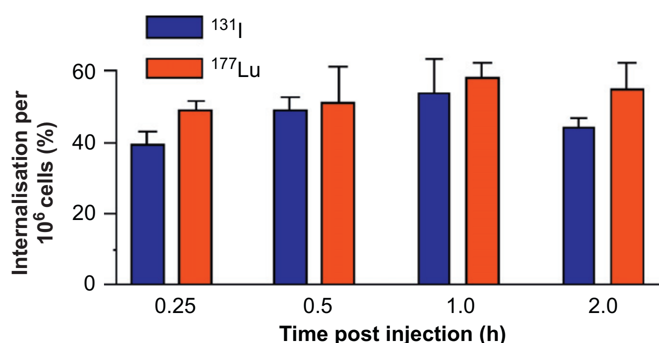

Fig. 5. Internalisation of ^{131}I- and ^{177}Lu-DOTATATE by AR42J pancreatic tumour cells. DOTATATE labeled with both radionuclides presented similar internalisation after 1 h of incubation.

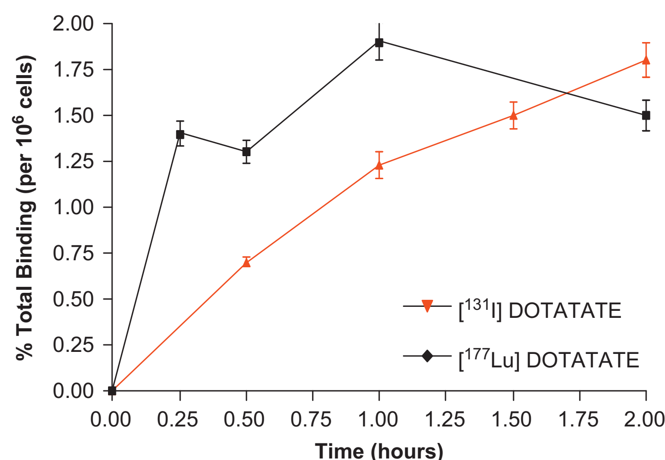

Fig. 6. Binding of both radiopharmaceuticals to AR42J rat pancreatic tumour cells.

molar peptide to radionuclide ratio of 3.5, the percent of di-iodinated peptide increased to 25% and the peptide radiolabeling yield varied from 31 to 93% making the Sep-Pak elution necessary to purify the peptide.

Our study showed that ^{131}I-DOTATATE can be obtained in high specific activity. Using a peptide to radionuclide ratio of 2.73, only the mono-iodinated peptide was produced, in high radiochemical yield that prevents the purification step on Sep-Pak cartridge. Additionally, the radioiodinated DOTATATE presented high stability in plasma and *in vivo* with low thyroid uptake representing low percent of dehalogenation.

Comparative competition studies using AR42J cells (Froidevausx et al., 2000) resulted in higher IC_{50} values for DOTA-Tyr-OC when compared to Tyr-OC or OC itself (2.44 against 0.33 and 0.91, respectively). The same study showed that the presence of the yttrium in the structure (Y-DOTA-OC) increased the IC_{50} (6.48) and that little modifications in the peptide structure (OC, Tyr-OC, vapreotide or lanreotide) altered their affinity for tumour cells.

Similar study using SST derivatives labeled with ^{99m}Tc by different bifunctional chelating agents (Decristoforo and Mather, 1999) showed the influence of labeling method and peptide structure on biodistribution. Small alterations in the sequence and number of amino acid residues on peptide structure may influence the biodistribution and the affinity by specific cell receptors.

Reubi et al. (2000) also showed that the biodistribution of the SST derivative is dependent of the radionuclide. In this way, radiolabeling with indium, yttrium or gallium resulted in specific interaction with the different types of SSTr (hsst1-5).

In our study ^{131}I-DOTATATE showed a favourable kinetic distribution and a significantly tumour uptake, despite not being so persistent in tumour after 24 h when compared to ^{177}Lu-DOTATATE ($\alpha = 5\%$). In addition, *in vitro* studies with pancreatic tumour cells (AR42J) showed similar percent of internalisation for both compounds, reaching almost 50% after 1 h of incubation. The permanence of the radiopharmaceuticals inside the cells is higher for the ^{177}Lu-labeled peptide, probably as a result of enzymatic metabolism of the iodinated peptide.

The compound ^{131}I-DOTATATE can be prepared in a simple and fast way, considering the availability of the radionuclide and the very good radiochemical yield obtained by direct electrophilic substitution using an appropriated molar peptide to radionuclide ratio to obtain the mono-iodinated form of the labeled peptide. This mono-iodinated species seems to present *in vivo* stability toward dehalogenation. The labeled ^{131}I-DOTATATE also presented favourable biodistribution, compatible with the use in the therapy of neuroendocrine tumours, like fast blood clearance and low uptake in non-target organs, particularly liver and intestines.

However, some unfavourable aspects concerning the biodistribution of ^{131}I-DOTATATE can be considered. The renal uptake of the ^{131}I-DOTATATE, relatively higher than the lutetium derivative ($\alpha = 5\%$) can result in a comparative superior dosimetry for this organ. Alternatively, the use of amino acid infusions, prescribed for therapeutic use of SST labeled derivatives with lutetium and yttrium can minimise the toxic effect over the kidneys (Bodei et al., 2003). Additionally, the lower tumour uptake of ^{131}I-DOTATATE when compared to the ^{177}Lu labeled peptide ($\alpha = 5\%$), particularly after 24 h of the administration, can reduce the therapeutic potential of the radiopharmaceutical. The superior physical half-life of ^{131}I (8 days) when compared to ^{177}Lu (6 days) as well as the superior energy of the beta particles of the ^{131}I can probably contribute to counterbalance the lower tumour uptake.

Some additional dosimetric studies will be proposed in order to elucidate the therapeutic potential of the ^{131}I-DOTATATE.

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