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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.

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.