Affinity profiles for human somatostatin receptor subtypes SST1–SST5 of somatostatin radiotracers selected for scintigraphic and radiotherapeutic use

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Abstract. In vivo somatostatin receptor scintigraphy using Octreoscan is a valuable method for the visualisation of human endocrine tumours and their metastases. Recently, several new, alternative somatostatin radioligands have been synthesised for diagnostic and radiotherapeutic use in vivo. Since human tumours are known to express various somatostatin receptor subtypes, it is mandatory to assess the receptor subtype affinity profile of such somatostatin radiotracers. Using cell lines transfected with somatostatin receptor subtypes sst1, sst2, sst3, sst4 and sst5, we have evaluated the in vitro binding characteristics of labelled (indium, yttrium, gallium) and unlabelled DOTA-[Tyr³]-octreotide, DOTA-octreotide, DOTA-lanreotide, DOTA-vapreotide, DTPA-[Tyr³]-octreotate and DOTA-[Tyr³]-octreotate. Small structural modifications, chelator substitution or metal replacement were shown to considerably affect the binding affinity. A marked improvement of sst2 affinity was found for Ga-DOTA-[Tyr³]-octreotide (IC₅₀ 2.5 nM) compared with the Y-labelled compound and Octreoscan. An excellent binding affinity for sst2 in the same range was also found for In-DTPA-[Tyr³]-octreotate (IC₅₀ 1.3 nM) and for Y-DOTA-[Tyr³]-octreotate (IC₅₀ 1.6 nM). Remarkably, Ga-DOTA-[Tyr³]-octreotate bound at sst2 with a considerably higher affinity (IC₅₀ 0.2 nM). An up to 30-fold improvement in sst3 affinity was observed for unlabelled or Y-labelled DOTA-octreotide compared with their Tyr³-containing analogue, suggesting that replacement of Tyr³ by Phe is crucial for high sst3 affinity. Substitution in the octreotide molecule of the DTPA by DOTA improved the sst3 binding affinity 14-fold. Whereas Y-DOTA-lanreotide had only low affinity for sst3 and sst4, it had the highest affinity for sst5 among the tested compounds (IC₅₀ 16 nM). Increased binding affinity for sst3 and sst5 was observed for DOTA-[Tyr³]-octreotide, DOTA-lanreotide and DOTA-vapreotide when they were labelled with yttrium. These marked changes in subtype affinity profiles are due not only to the different chemical structures but also to the different charges and hydrophilicity of these compounds. Interestingly, even the coordination geometry of the radiometal complex remote from the pharmacophoric amino acids has a significant influence on affinity profiles as shown with Y-DOTA versus Ga-DOTA in either [Tyr³]-octreotide or [Tyr³]-octreotate. Such changes in sst affinity profiles must be identified in newly designed radiotracers used for somatostatin receptor scintigraphy in order to correctly interpret in vivo scintigraphic data. These observations may represent basic principles relevant to the development of other peptide radioligands.

Key words: Somatostatin receptor subtypes – Receptor affinity – Gallium-labelled radioligands – Yttrium-labelled radioligands – Octreotate


Introduction

In vivo somatostatin receptor scintigraphy has been shown in the last decade to be a valuable method in humans for the visualisation of primary tumours and metastases which express somatostatin receptors, such as most neuroendocrine tumours. Although earlier studies had used radioiodinated octreotide as the radioligand [1], the gold standard for these investigations is presently indium-111 labelled DTPA-octreotide (Octreoscan) [2]. Despite good results with Octreoscan, in the last few years there have been several reports describing new, alterna-
tive somatostatin radioligands. Some have been synthesised with the aim of using them for the radionuclide therapy of tumours; one such example is DOTA-[Tyr³]-octreotide, which can be labelled with yttrium-90 and was shown to be a promising agent for this type of metabolic therapy [3–5]. A further ligand which is based on another synthetic backbone molecule, lanreotide, is DOTA-lanreotide (Mauritius, see Fig. 1), which has been claimed to compare favourably with Octreoscan for diagnostic purposes [6, 7]. Vapreotide (Fig. 1) and its DTPA derivative have also recently been described as alternative radiotracers [8, 9]. Furthermore, gallium-67 labelled DOTA-[Tyr³]-octreotide has recently been suggested to be a favourable alternative to Octreoscan [10]. Finally, molecules based on slightly modified octreotide, such as DTPA-[Tyr³]-octreotate (Fig. 1), have been shown in animal models to be more effective for the visualisation of tumours than Octreoscan [11].

The recent cloning of several somatostatin receptor genes has increased our understanding of somatostatin receptor structure and function. To date the human somatostatin receptor subtypes sst1, sst2, sst3, sst4 and sst5 have been cloned and partially characterised [12, 13]. All five receptor subtypes can functionally couple to the inhibition of adenylate cyclase and to several other second messenger systems. Pharmacological studies have shown that all five human subtypes bind somatostatin 14 and somatostatin 28 with high affinity, whereas the sst2 subtype preferentially binds the octapeptide octreotide with very high affinity. Conversely, sst1 and sst4 do not bind octreotide whereas sst3 has an intermediate affinity and sst5 a moderately high affinity for octreotide [12, 13]. It is important to know the affinity pattern of peptide analogues foreseen to be of value for oncological applications since we know that human tumours can express several of these somatostatin receptor subtypes, each subtype with a different pattern of expression depending on the individual tumour and on the tumour type [14, 15].

It is conceivable that small structural modifications of somatostatin analogues, including the introduction of a metal in the chelator, may affect the binding properties of the analogues for the various sst subtypes. It is therefore crucial to evaluate the somatostatin receptor subtype affinity profile of new radioligands intended for the in vivo scintigraphy of tumours, in order not only to select analogues with the most adequate and unique affinity profile but also to interpret correctly the in vivo scans of the tumours performed with such ligands. Our aim was therefore to evaluate the binding affinity profile of a number of somatostatin radioligands used in nuclear medicine, by testing their affinity in cells stably transfected with the five different human somatostatin receptor subtypes. Both established and new somatostatin radioligands were included in the study. Throughout this paper, the non-radioactive metal-chelator-peptide conjugates were used as competitors to determine IC₅₀ values utilising ¹²⁵I-[Leu⁸, d-Trp²², Tyr²⁵]-somatostatin 28 as radioligand. We assumed that – as generally agreed – the non-radioactive metals behave like their radioactive congeners.

Materials and methods

Peptides. The peptides studied in this investigation are listed in Table 1 and depicted in Fig. 1. Control peptides were somatostatin 28 (SS-28; Bachem, Switzerland), octreotide (Novartis, Basel, Switzerland) and the sst1-selective analogue Des-α- Tri-[Tyr³]-octreotide (CH28; J. Rivier, San Diego, USA; [16]). Vapreotide was a gift of Debiopharm (Lausanne). DOTA-lanreotide and DTPA-[Tyr³]-octreotate were provided by A. Srinivasan (St. Louis, USA).

Chemical synthesis. DOTA-[Tyr³]-octreotate was synthesised according to de Jong et al. [11]. DOTA-[Tyr³]-octreotide was synthesised using standard Fmoc (9-fluorenylmethoxycarbonyl) strategy [17] on tritylchloride resin. The protected peptide was cleaved from the resin, cyclised, deprotected and purified by preparative high-performance liquid chromatography (HPLC) analogous to the method of Arano et al. [18]. DTPA-octreotide, DOTA-octreotide, DOTA-[Tyr³]-octreotide, DOTA-lanreotide and DOTA-vapreotide and the In⁶⁷, Ga⁶⁹ and Y⁹⁰ complexes were synthesised according to the methods described previously [19, 20]. The following non-radioactive metal isotopes were used: ¹¹⁵In, ⁸⁹Y and ⁶⁹⁷Ga. The exact mass spectra (MS) and elemental analysis of DOTA-[Tyr³]-octreotide were as follows: electrospray-ionisation mass spectroscopy (ESI-MS): m/z=1421.7 [M+H]⁺ (16%), 711.7 [M+2 H]²⁺ (97%), 474.7 [M+3 H]³⁺ (100%); C₆₅H₉₂N₁₄O₁₈S₂·7H₂O·0.4AcOH·0.05TFA (1577.37): calcd C 50.18 H 6.81 N 12.40; found C 50.38 H 6.74 N 12.43; found C 50.38 H 6.74 N 12.40; amino acid analysis: Thr 0.86 (1), Cys 1.20 (2), Tyr 1.00 (1), Phe 0.99 (1), Lys 1.07 (1), Trp det. (1); purity (HPLC): >97%. The remaining peptides were characterised by ESI/MS spectra and their purity (>97% in all cases) was confirmed by reversed phase HPLC (RP-HPLC).

Determination of peptide concentrations and molar extinction coefficients. The determination of DOTA-peptide concentrations is based on knowledge of the exact elemental composition of DOTA-[Tyr³]-octreotide. The known peptide content allowed the determination of the molar extinction coefficient ε. This was confirmed by a labelling experiment using a defined excess of ⁸⁹Y(NO₃)₃ spiked with ⁹⁰YCl₃ as described below. The molar extinction coefficients of the remaining DOTA/DTPA-peptides were then determined as follows: Aliquots containing approximately 50 µg of the respective peptide in 35 µl 0.01 M CH₃COOH were diluted with 0.1 M CH₃COOH to a final volume of 500 µl and the absorbance at 280 nm was recorded. Sodium acetate buffer (20 µl, 0.1 M, pH 5.1) and a ⁸⁹Y(NO₃)₃ solution (32–36 µl, 2 mM) spiked with ⁹⁰YCl₃ (15 MBq/500 µl) were added to a 50 µg DOTA-peptide aliquot (35 µl 0.01 M CH₃COOH). The reaction mixture was heated for 25 min to 95°C and incubated at room temperature for further 15 min together with DTPA (20 µl, 1 mM) before being injected into an HPLC system which was coupled to a gamma detector (Hewlett Packard 1050 HPLC system with a flow-through Berthold LB 506 C1 gamma detector; column: Macherey Nagel, Nucleosil 120-C₁₈; flow 0.75 ml/min; eluents: A=0.1% trifluoroacetic acid (TFA) in H₂O and B=acetonitrile (MeCN); non-linear gradient: 0–5 min, 100% A; 25 min, 75% A; 30–35 min, 100% A). The