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Comparison of receptor affinity of ^{nat}Sc-DOTA-TATE versus ^{nat}Ga-DOTA-TATE

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Abstract

BACKGROUND: ^{44}Sc as a positron emitter can be an interesting alternative to ^{68}Ga ($T_{½}$ = 67.71 min) due to its longer half-life ($T_{½}$ = 3.97 h). Moreover, the β^- emitter ^{47}Sc can be used for therapy when attached to the same biomolecule vectors. DOTA as a chelating agent has been proven suitable for the radiolabelling of peptides recognising tumour cell receptors *in vivo* with M^{3+} radiometals. DOTA-derivatized peptides have been successfully labelled with ^{90}Y and ^{177}Lu for therapy, and with ^{68}Ga for PET imaging. However, published data on ^{44}Sc-labelled DOTA-biomolecules as potential PET radiotracers are still very limited. The aim of this study was to compare the affinity of ^{nat}Ga- and ^{nat}Sc-labelled DOTA-TATE to somatostatin receptors subtype 2 expressed in rat pancreatic cancer cell line AR42J.

MATERIAL AND METHODS: The cold complexes of DOTA-TATE with ^{nat}Ga and ^{nat}Sc were synthesized and identified by HPLC and MS analysis and evaluated *in vitro* for competitive binding to cancer cell line AR42J expressing somatostatin receptors subtype 2 (sstr2).

RESULTS: The IC50 values calculated from the displacement curve of [^{125}I-Tyr^{11}]-SST-14 were: 0.20 ± 0.18, 0.70 ± 0.20, 0.64 ± 0.22 and 0.67 ± 0.12 for ^{nat}Ga-DOTA-TATE, ^{nat}Sc-DOTA-TATE, DOTA-TATE, and [Tyr^{11}]-SST-14 complexes, respectively, with the affinity lowering in the decreasing order: ^{nat}Ga-DOTA-TATE > DOTA-TATE > Tyr^{11}-SST-14 > ^{nat}Sc-DOTA-TATE.

CONCLUSIONS: The binding affinity of ^{nat}Ga-DOTA-TATE appeared higher than that of ^{nat}Sc-DOTA-TATE. Further *in vitro* and *in vivo* studies are needed to verify the influence of the chelated metal on the affinity and uptake of the respective radiolabelled compounds. This information might be crucial when the *in vivo* applications of peptides labelled with ^{68}Ga and ^{44}Sc for PET, as well as the use of ^{47}Sc for radiotherapy are considered.

Key words: scandium-44, gallium-68, PET tracers, receptor affinity, DOTA-derivatised peptides

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Introduction

In recent years peptide receptor radionuclide therapy (PRRT) has utilized synthetic peptides as vectors for radionuclides such as ^{90}Y and ^{177}Lu. This has been accomplished with the aid of positron emission tomography (PET), which involves the same vector biomolecules labelled with positron emitters, and was first demonstrated with ^{68}Ga-labelled somatostatin (SST) analogues for diagnostic imaging of neuroendocrine tumours [1]. Since then growing interest in other positron emitters obtained in generator systems has been observed [2, 3]. The increasing availability of new radionuclides with diagnostic and therapeutic properties offers new possibilities for individualized nuclear medicine options.

^{44}Sc is a positron emitter radionuclide [$E_{\beta+}$ max = 1475.4 keV, E_r = 1157.0 keV (99.9%)] with a half life of 3.97 hours, which can be utilized for diagnostics with ^{47}Sc as a matched pair for radiotherapy. Additionally, Grignon et al. [4] reported that ^{44}Sc is an interesting radionuclide for nuclear medicine imaging using $\beta^+ - \gamma$ coincidences. The use of ^{44}Sc with a half-life more than 3 times longer than that of ^{68}Ga ($T_{½}$ = 67.71 min) makes it an useful alternative for diagnostic purposes but also for dosimetry and

further therapy planning with the use of biomolecules labelled with the β^- emitting ^{47}Sc as radiotherapeutic agents [5]. The availability of ^{44}Sc is also increasing [3, 6].

The chemistry of Sc^{3+} is similar to that of the lanthanides, and the "lanthanide like" elements. Due to its small ionic radius it is also chemically similar to aluminium and gallium [7]. The thermodynamic stability constant of the 1,4,7,10-teraazacyclododecane-N,N',N',N'''-teraacetic acid (DOTA) complex with Ga^{3+} is in the range from 21.3 to 26.1 [8, 9], with Sc^{3+} it is 27.0 [10], while the following values of 26.7, 23.9, 29.2, 25.95 have been reported for Lu^{3+} [7, 8, 11, 12]. All of the above values are similar as indicated from the data reported by Viola-Villegas and Doyle [13]. Hence chelators developed for the complexation of gallium, and the lanthanides can also be used for the complexation of scandium.

Generally, small neuropeptides, such as somatostatin (SST) and gastrin releasing peptide (GRP)/bombesin (BN) analogues, labelled with γ - and/or β^--emitting radionuclides are investigated for their ability to bind to receptors which are overexpressed in a variety of malignant tumours [14–16]. The affinity of the designed chelator-peptide construct to these receptors may vary depending on the metal incorporated into the complex [17].

The published results of the comparative *in vitro* and *in vivo* study of DOTA-BN[2-14]NH_2 labelled with ^{90}Y and ^{177}Lu as well as with ^{44}Sc and ^{68}Ga revealed differences in the *in vitro* and *in vivo* behaviour of these complexes, which could be attributed to the influence of metal on the complex receptor affinity [18, 19]. The present study is focused on the *in vitro* binding affinity of the M^{3+} type radiometals of Sc and Ga complexed with [DOTA,Tyr^3,Thr^8] octreotide (DOTA-TATE), which is a clinically used somatostatin analogue [20, 21].

Material and methods

Chemicals

DOTA-TATE was purchased from piChem (Austria). [^{125}I-Tyr^{11}]-SST-14 and [Tyr^{11}]-SST-14 were purchased from Perkin-Elmer Life and Analytical Sciences (USA). All other chemicals and materials were used as supplied and were of analytical grade unless otherwise stated.

Cold complexes of the peptide with ^{nat}Ga and ^{nat}Sc were synthesized and identified by high pressure liquid chromatography (HPLC) and mass spectrometry (MS).

High pressure liquid chromatography (HPLC)

The HPLC system for the quality control of the cold complexes was equipped with UV-VIS detector. The analysis was performed using a reverse phase C-18 Luna column (Phenomenex, USA). The mobile phase was a gradient of 0.1% TFA (Trifluoroacetic Acid)/H_2O (Solvent A) and 0.1% TFA/Acetonitrile (ACN) (Solvent B). The elution scheme of solvent B was 0% for 2 min, increased to 40% from 2 to 9 min, remaining at 40% until 15 min, and then decreased to 0% in 3 min from 18 min to 23 remaining at 0%, at a flow rate of 0.6 ml/min.

Cold complexes of DOTA-TATE with ^{nat}Sc and ^{nat}Ga

The cold metal complexes of DOTA-TATE were synthesized and identified according to the previously described method [18]. Briefly, 100 μg of DOTA-TATE was dissolved in 250 μl ammonium acetate 0.4 M, pH 5, or 250 μl of ascorbic acid (100 mg/ml) was added. An appropriate amount of $^{nat}ScCl_3$ or $^{nat}GaCl_3$ solution (1 mg/ml in 0.05 M HCl) was added to obtain a molar ratio of DOTA-TATE to metal 1:5. The sample was incubated at 95°C for 25 minutes and left to cool down to room temperature. The cold complexes were analyzed by HPLC. The samples were purified from free metal by Solid Phase Extraction (SPE) using pre-conditioned C-18 columns (100 mg resin, Sep-Pak, Waters), and the mobile phase consisted of 5 ml of ethanol and 5 ml of 0.9% NaCl. The samples were loaded on the cartridge followed by 5 ml 0.9% NaCl (to elute non-bound ^{nat}Sc or ^{nat}Ga) and by 3 ml pure methanol (^{nat}Sc-DOTA-TATE or ^{nat}Ga-DOTA-TATE fraction). The cold complex fractions were then lyophilized under vacuum giving a light yellow powder in both cases. The purified samples were also analyzed by Electron Spray Ionization-Mass Spectrometry (ESI-MS).

In vitro studies

Cell culture

The rat pancreatic cancer cell line AR42J expressing somatostatin receptors subtype 2 (sstr2) was used for the *in vitro* experiments. The cell line was cultured in RPMI-1640 (Gibco Invitrogen) supplemented with 10% foetal calf serum (Gibco Invitrogen), antibiotics (streptomycin, 100 μg/ml; penicillin, 100 U/ml; Sigma Aldrich), and glutamax (Gibco Invitrogen). The cells were kept in a humidified atmosphere at 37°C in 5% CO_2. The cells were fed every 2 days and subcultured by trypsinization (0.05% Trypsin-EDTA, Gibco Invitrogen) when the cells covered about 80% of the surface in the flask.

Saturation curve of ^{125}I-Tyr^{11}-SST-14

A saturation receptor assay for [^{125}I-Tyr^{11}]-SST-14 was performed prior to the binding affinity studies in order to determine the minimum concentration required for the saturation of sstr. The cells were seeded in 24-well plates ($\sim 8 \times 10^4$ cells/well) 48 h before the day of the experiment. On the day of the experiment the cells were incubated at 37°C in 5% CO_2 atmosphere for 90 min in the presence of increasing concentration of [^{125}I-Tyr^{11}]-SST-14 (0, 20000, 40000, 60000, 80000, 100000, and 120000 cpm, corresponding to 0, 6.3, 12.4, 18.9, 25.0, 31.5 and 37.8 pM, each in triplicate). At the completion of incubation the supernatant was collected and the cells were rinsed twice with 0.5 ml of cold phosphate-buffered saline (PBS). The cells underwent lyses by addition of 1N NaOH and incubation at 37°C/5% CO_2 for 10 min. The radioactivity of the collected fractions was measured in order to determine the minimum required concentration. Experiments were performed in triplicate.

Competitive binding studies

The *in vitro* receptor binding affinity and specificity of Tyr^{11}-SST-14, DOTA-TATE and its cold complexes with ^{nat}Sc and ^{nat}Ga in AR42J cells were determined by a competitive displacement cell-binding assay using the iodinated analogue [^{125}I-Tyr^{11}]-SST-14 according to the method previously described [18]. Briefly, $\sim 8 \times 10^4$ cells/well were seeded in 24-well plates 48 h before the day of the experiment. On the day of the experiment the cells were incubated at 37°C in 5% CO_2 atmosphere for 1 h in the presence of 30,000–50,000 cpm of ^{125}I-[Tyr^{11}]-SST-14 and

increasing concentrations of the respective compound (from 1pM to 1μM), each in triplicate. Upon completion of the incubation, the reaction medium was aspirated and the cells were washed twice with cold PBS. The cells underwent lyses by addition of 1 N NaOH and incubation at 37°C/5% CO_2 for 10 min. The radioactivity of the collected fractions was measured in order to determine the IC_{50} value (inhibitory concentration, 50%).

Statistical methods

The results were analyzed by non-linear regression analysis using GraphPad Prism (version TM, GraphPad software, San Diego California, USA).

Results

Cold complexes of DOTA-TATE with ^{nat}Sc and ^{nat}Ga

The ESI-MS analysis of ^{nat}Ga-DOTA-TATE confirmed the presence of a single main complex at 764.2 $[m/z]^{2+}$, which was in agreement with the calculated value (MW = 1505.3). The respective ESI-MS analysis of ^{nat}Sc-DOTA-TATE also confirmed the presence of a main peak at 761.5 $[m/z]^{2+}$, being in agreement with the calculated value (MW = 1498.5). Figures 1 and 2 present the mass spectrum and the HPLC profile of ^{nat}Sc-DOTA-TATE, respectively. For the determination of the exact concentration of the cold complexes used for the *in vitro* assays, the BCA Protein Assay was used (BCA kit, Thermo Scientific). A calibration curve of DOTA-TATE of GMP grade was used for the determination of the concentration of DOTA-TATE (R&D grade), ^{nat}Sc-DOTA-TATE, and ^{nat}Ga-DOTA-TATE. The measured values were in good accordance with the calculated values.

Binding affinity studies

AR42J cancer cells were incubated with increasing concentrations of [^{125}I-Tyr^{11}]-SST-14 in order to measure specific radioligand binding at equilibrium so as to perform the displacement affinity study of the [Tyr^{11}]-SST-14, DOTA-TATE, ^{nat}Sc-DOTA-TATE and, ^{nat}Ga-DOTA-TATE. Based on the saturation curve, the minimum required amount of [^{125}I-Tyr^{11}]-SST14 in order for the receptors to be saturated was in the range of 9.5–15.8 pM (30,000–50,000 cpm), calculated according to the specific activity of the iodinated compound (2200 Ci/mmol).

The IC50 values calculated from the displacement curve of [^{125}I-Tyr^{11}]-SST-14 were: 0.20 ± 0.18, 0.70 ± 0.20, 0.64 ± 0.22 and 0.67 ± 0.12 for ^{nat}Ga-DOTA-TATE, ^{nat}Sc-DOTA-TATE, DOTA-TATE, and [Tyr^{11}]-SST-14 complexes, respectively, as listed in Table 1. The values indicate the following affinity pattern: ^{nat}Ga-DOTA-TATE > DOTA-TATE > Tyr^{11}-SST-14 > ^{nat}Sc-DOTA-TATE. The respective displacement curves are presented in Figure 3.

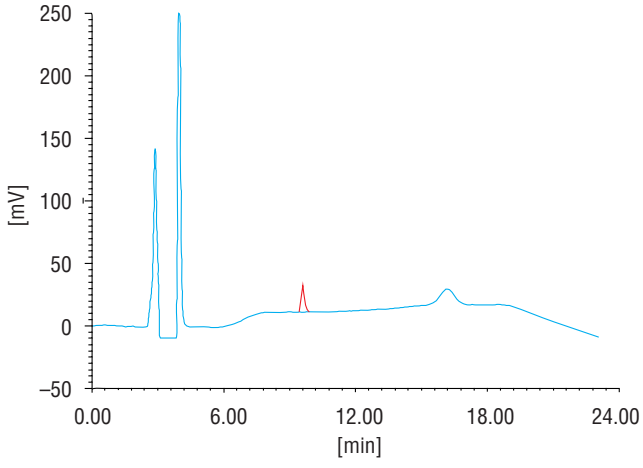

Figure 2. RP-HPLC analysis of ^{nat}Sc-DOTA-TATE (UV absorbance monitored at 220 nm).

Discussion

Majkowska-Pilip and Bilewicz [10] evaluated the tri and tetraaza ligands for formation of macrocyclic complexes with Sc and compared them with analogous complexes of ^{177}Lu and ^{68}Ga. The authors concluded that DOTA is the most suitable ligand for binding scandium radionuclides to biomolecules. Viola-Villegas and Doyle

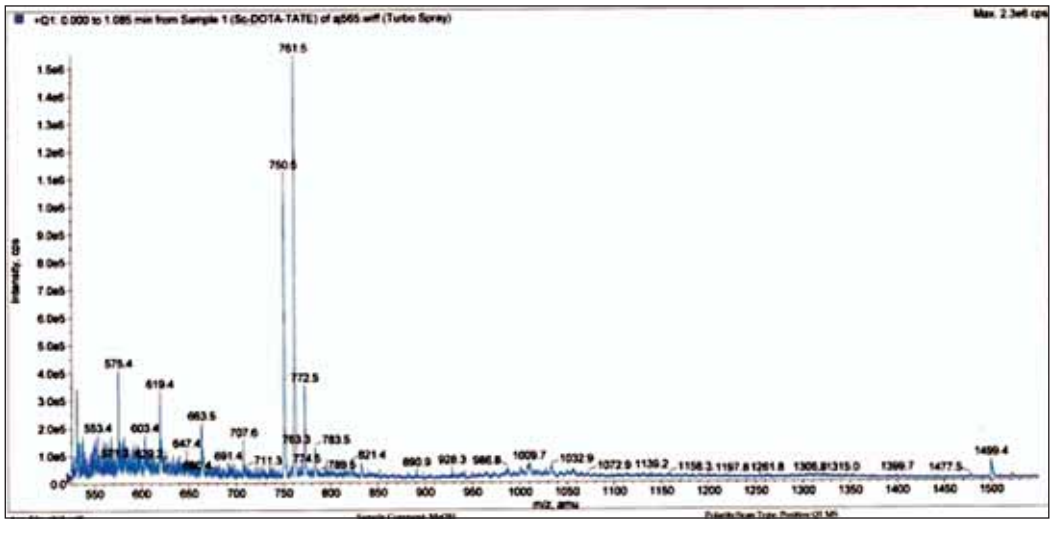

Figure 1. ESI-MS spectrum of ^{nat}Sc-DOTA-TATE.

Table 1. IC50 values from competitive binding assays in AR42J cells for the DOTA-TATE and its ^{nat}Sc and ^{nat}Ga complexes, along with [Tyr11]-SST-14. The IC50 values of the complexes of the GRP analogue DOTA-BN[2-14]NH2 in PC-3 are given for comparison

Derivative	IC50 ± SD (nM) [^{125}I-Tyr^{11}]-SST-14 AR42J cells	Derivative	IC50 ± SD (nM) ^{125}I-[Tyr^4]-BN PC3 cells
[Tyr^{11}]-SST-14	0.67 ± 0.12	DOTA-BN[2-14]NH$_2$	1.78 ± 0.12*
DOTA-TATE	0.64 ± 0.22	^{nat}Y-DOTA-BN[2-14]NH$_2$	1.90 ± 0.06*
^{nat}Ga-DOTA-TATE	0.20 ± 0.18	^{nat}Lu-DOTA-BN[2-14]NH$_2$	1.34 ± 0.11*
^{nat}Sc-DOTA-TATE	0.70 ± 0.20	^{nat}Ga-DOTA-BN[2-14]NH$_2$	0.85 ± 0.06**
		^{nat}Sc-DOTA-BN[2-14]NH$_2$	6.49 ± 0.13**

*Koumarianou et al. [18]; **Koumarianou et al. [19]

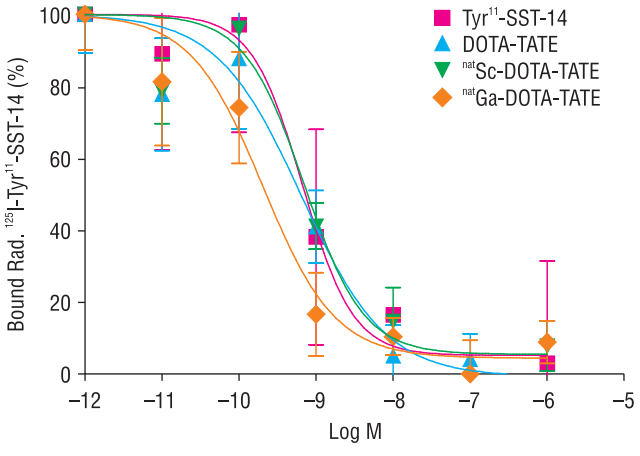

Figure 3. Displacement curves of [^{125}I-Tyr^{11}]-SST-14 from the competitive binding studies for Tyr^{11}-SST-14, DOTA-TATE, ^{nat}Sc-DOTA-TATE, and ^{nat}Ga-DOTA-TATE

[13] indicated that there are differences in the crystal structure of DOTA with Ga and Sc, suggesting at the same time a higher stability of Sc-DOTA complex. On the other hand, it has been shown previously by Reubi et al. [17] that DOTA can be a very efficient chelator for Y, Lu, and Ga when coupled to somatostatin analogues. The *in vitro* comparison indicated that not only the peptide sequence and conjugated chelator, but to a large extent also the metal involved in the complex formation influences the affinity of the molecule to the somatostatin receptors. Our previously published comparison of the *in vitro* and *in vivo* properties of another DOTA-conjugated peptide, ^{90}Y-DOTA-BN[2-14]NH$_2$, ^{177}Lu-DOTA-BN[2-14]NH$_2$, ^{44}Sc-DOTA-BN[2-14]NH$_2$, and ^{68}Ga-DOTA-BN[2-14]NH$_2$, revealed differences in the *in vitro* receptor affinity of these analogues (see Table 1) [18, 19]. These differences may be attributed to the structural changes in the radioligand molecule, which influence the interaction with the receptor. The introduction of a certain metal or its replacement by another one may provoke considerable alterations in the *in vivo* binding affinity of a peptide to cell receptors and may have an important impact on the *in vivo* biodistribution of these radiopharmaceuticals.

So far, there is no published data on ^{44}Sc-labelled peptides as PET tracer candidates in terms of *in vitro* and *in vivo* behaviour. Therefore, the main goal of this study was to evaluate the influence of the new radionuclide, ^{44}Sc, on the receptor affinity of another DOTA derivatized peptide which is already well established, such as the somatostatin analogue DOTA-TATE [20, 21]. The ^{68}Ga-DOTA-TATE was used in direct comparison since ^{68}Ga complexes with DOTA chelated somatostatin analogues have been showing improved affinity to somatostatin receptor subtypes [17]. Considering the rather short half-life of ^{68}Ga, the ^{44}Sc with 3.97 h half-life can be an interesting alternative for conjugation with biomolecules of longer metabolic half-life to allow late PET imaging.

Summary

In the present study ^{nat}Ga-DOTA-TATE showed slightly higher binding affinity to sst receptors of the AR42J cell line than ^{nat}Sc-DOTA-TATE, and the IC$_{50}$ values of the studied derivatives were decreasing in the order ^{nat}Ga-DOTA-TATE > DOTA-TATE > [Tyr^{11}]-SST-14 > ^{nat}Sc-DOTA-TATE. This relationship is in favour of Ga and is similar to the previously reported data for the DOTA-BN[2-14]NH$_2$ derivatives [18, 19]; however, the differences of affinity between Ga- and Sc-labelled DOTA-TATE are not so pronounced as they were in the case of DOTA-BN[2-14]NH$_2$. Therefore, further studies are needed to verify if the influence of Sc radionuclides on the peptide affinity to certain receptors is critical for their diagnostic or therapeutic utility.

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