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Effects of adding an albumin binder chain on [^{177}Lu]Lu-DOTATATE

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ABSTRACT

Introduction: [^{177}Lu]Lu-DOTATATE peptide receptor radionuclide therapy is used for treatment of neuroendocrine tumours. We investigated whether prolonging blood residence time of [^{177}Lu]Lu-DOTATATE with albumin binders could increase tumour accumulation and tumour-to-kidney ratios for improved therapeutic efficacy.
Methods: DOTATATE and its derivatives with an albumin-binder motif (GluAB-DOTATATE and AspAB-DOTATATE) were prepared by solid-phase peptide synthesis. Binding affinities of the Lu-labeled peptides for human somatostatin receptor 2 (SSTR2) were measured with membrane competition binding assays. Compounds were radiolabeled with [^{177}Lu]$LuCl_3$ and purified by HPLC. SPECT imaging and biodistribution studies (1, 4, 24, 72, and 120 h) were performed in immunodeficient mice bearing AR42J pancreatic tumour xenografts.
Results: GluAB-DOTATATE and AspAB-DOTATATE were synthesized in 18.8% and 14.3% yields, while Lu-GluAB-DOTATATE and Lu-AspAB-DOTATATE were obtained in 86.5% and 50.0% yields, respectively. The compounds exhibited nanomolar binding affinity (K_i: 8.72–8.95 nM) for SSTR2. The ^{177}Lu-labeled peptides were obtained in non-decay-corrected isolated yields of ≥41%, with >96% radiochemical purity, and molar activities in the range of 314–497 GBq/μmol. In vivo, [^{177}Lu]Lu-GluAB-DOTATATE and [^{177}Lu]Lu-AspAB-DOTATATE had significantly higher blood activity at 1, 4 and 24 h compared to [^{177}Lu]Lu-DOTATATE. Tumour uptake of [^{177}Lu]Lu-DOTATATE was 21.35 ± 5.90%ID/g at 1 h and decreased to 10.10 ± 5.78%ID/g at 120 h. For [^{177}Lu]Lu-GluAB-DOTATATE tumour uptake increased from 21.89 ± 6.86%ID/g at 1 h to 24.44 ± 5.84%ID/g at 4 h, before decreasing to 12.02 ± 1.84%ID/g at 120 h. For [^{177}Lu]Lu-AspAB-DOTATATE tumour uptake was 11.12 ± 3.18%ID/g at 1 h, 18.41 ± 4.36% ID/g at 24 h, and decreased to 16.90 ± 8.97%ID/g at 120 h. Renal uptake was 7.49 ± 1.62%ID/g for [^{177}Lu]Lu-DOTATATE, 31.14 ± 7.06%ID/g for [^{177}Lu]Lu-GluAB-DOTATATE, and 28.82 ± 13.82%ID/g for [^{177}Lu]Lu-AspAB-DOTATATE at 1 h and decreased thereafter.
Conclusion: The addition of albumin binder motifs to [^{177}Lu]Lu-DOTATATE enhanced mean residence time in blood. Increased tumour uptake was observed for [^{177}Lu]Lu-AspAB-DOTATATE compared to [^{177}Lu]Lu-DOTATATE at later time points, but its higher kidney uptake diminished the therapeutic index.

1. Introduction

Gastroenteropancreatic neuroendocrine tumours (GEP-NETs), initially described as carcinoids ("karzinoide") by Oberndofer in 1907, are a heterogeneous group of rare tumours originating from tissues derived from APUD cells and that are characterized by high rates of metastatic disease on initial diagnosis and sometimes florid clinical presentations secondary to their secretion of neurotransmitters and hormones [1–4]. NETs are characterized by the overexpression of somatostatin receptors (SSTRs). The advent of somatostatin analogues coupled with radiometal chelators has enabled the delivery of beta-emitting radionuclides to SSTR2 expressing cells in a targeted manner with peptide receptor radionuclide therapy (PRRT) [5–7]. For agonists, there is internalization of the compound and since ^{177}Lu is a residualizing radionuclide, it remains trapped within the cell [8]. Advantages of the technique include rarity of severe toxicity (0.6% hepatic insufficiency, 0.8% myelodysplastic syndrome, 0.4% renal insufficiency) for patients and good control of disease (24%–46% overall response rate), especially for [^{177}Lu]Lu-DOTATATE [2,5,7].

In the case of ^{177}Lu-labeled somatostatin analogues the dose-limiting organ is the kidney, followed by the bone marrow [6]. The ratio of tumour dose to kidney dose can be defined as the therapeutic index and a higher index is advantageous because the dose that can be delivered to the tumour is then higher for the same kidney toxicity. The uptake mechanism is related to expression of somatostatin

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receptors 1–5 in normal kidneys, but high uptake is also attributable to reabsorption by proximal tubules of the peptides filtered by the glomeruli. This reabsorption pathway can be partially blocked by intravenous infusion of cationic amino acid, as previously reported [9–13]. Even though healthy and tumour tissue express the receptor, differences in expression levels and in tissue-specific pharmacokinetics can allow for a useful therapeutic index.

For patients with somatostatin-expressing tumours, PRRT has become a useful treatment modality that can target disease not amenable to surgical excision. Although this technique has shown good results in clinical trials, we hypothesize that the pharmacokinetics of DOTATATE, most notably its rapid blood clearance (<10%ID in blood at 3 h), can be enhanced to facilitate higher tumour binding [14]. Albumin binding is a strategy that has been employed to increase blood residence time of a variety of compounds including long-lived insulin derivatives like Levemir and chemotherapeutic drugs like Abraxane [15]. Several techniques have been explored to exploit albumin binding including in vitro/in vivo covalent conjugation and in vitro/in vivo non-covalent human serum albumin binding. For example, Müller et al. reported the use of albumin binders to develop radiopharmaceutical agents targeting folate receptor for cancer imaging and therapy [16]. Herein, we investigated the use of two albumin binders to increase bioavailability of [^{177}Lu]Lu-DOTATATE derivatives, to reduce renal accumulation, and to deliver higher radiation dose to tumour.

2. Materials and methods

2.1. Reagents and equipment

All reagents and solvents were purchased from commercial sources and used without further purification. Peptides were synthesized on an AAPPTec (Louisville, KY) Endeavor 90 peptide synthesizer. High performance liquid chromatography (HPLC) was performed on an Agilent (Santa Clara, CA) 1260 infinity system equipped with a model 1200 quaternary pump, a model 1200 UV absorbance detector (set at 220 nm), and a Bioscan (Washington, DC) NaI scintillation detector. The HPLC columns used were a semi-preparative column (Luna C18, 5 μ, 250 × 10 mm) and an analytical column (Luna C18, 5 μ, 250 × 4.6 mm) both purchased from Phenomenex (Torrance, CA). Mass analyses were performed using an AB SCIEX (Framingham, MA) 4000 QTRAP mass spectrometer system with an ESI ion source. [^{177}Lu]$LuCl_3$ was purchased from ITG (Munich, Germany). Activity of ^{177}Lu-labeled peptides was measured using a Capintec (Ramsey, NJ) CRC®-25R/W dose calibrator, and the activity of mouse tissues collected from biodistribution studies were counted using a Perkin Elmer (Waltham, MA) Wizard2 2480 automatic gamma counter.

2.2. Synthesis of precursor and standards

The peptide octreotate (TATE) was synthesized according to literature procedure using standard Fmoc-based solid-phase peptide synthesis [17]. TATE-coupled resin was then treated with 20% piperidine (15 mL × 2) in DMF to remove the N^{α}-Fmoc protecting group. Three equivalents of Fmoc-Lys(IvDde)-OH, Fmoc-Glu(^{t}Bu)-OH (for GluAB-DOTATATE) or Fmoc-Asp(^{t}Bu)-OH (for AspAB-DOTATATE), and 4-(*p*-Iodophenyl)butyric acid, pre-activated with HBTU/HOBT/DIEA in a ratio of 3/3/6 were subsequently coupled to the sequence. N^{ε}-(ivDde) protecting group was removed by incubating with 1% hydrazine monohydrate in DMF. Three equivalents of tri-tert-butyl 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA-Tris(*t*-Bu ester)) pre-activated with HBTU/DIEA (3/20) were then coupled to the lysine N^{ε}-side chain. The desired albumin binder and DOTA conjugated TATE peptides were cleaved from the resin and simultaneously deprotected by incubating with TFA/TIS/DI water/thioanisole/phenol (82.5/2.5/5/5/5) cocktail solution. The purification of both peptides was performed on HPLC with the semi-preparative column under a flow rate of 4.5 mL/min. The HPLC conditions are summarized in Table 1.

Lutetium chelated non-radioactive standards of GluAB-DOTATATE and AspAB-DOTATATE were prepared by incubating the corresponding peptide with 5 equivalents of $LuCl_3$ in 0.5 mL 0.1 M NaOAc buffer (pH 4.5) at 100 °C for 15 min. At the end of the incubation period, the reaction mixtures were purified by HPLC using the semi-preparative column under a flow rate of 4.5 mL/min. The HPLC conditions are summarized in Table 1.

2.3. Radiolabeling

For radiolabeling, 15–30 μL of [^{177}Lu]$LuCl_3$ containing activity between 388.5 MBq to 654.9 MBq were added into a solution of 25 μg of the corresponding albumin binder and DOTA conjugated TATE peptide in NaOAc buffer (0.5 mL, 0.1 M, pH 4.5). The reaction mixture was incubated at 100 °C for 15 min. Following incubation, the reaction mixtures were injected to HPLC for purification (to separate the radiotracer from free [^{177}Lu]$LuCl_3$ and unlabeled precursor) with the semi-preparative column under a flow rate of 4.5 mL/min. Quality control, including determination of molar activity, was performed on HPLC using the analytical column under a flow rate of 2 mL/min. The HPLC conditions and radiochemistry data are summarized in Table 2.

2.4. Binding affinity

The binding affinities of Lu-DOTATATE, Lu-GluAB-DOTATATE, and Lu-AspAB-DOTATATE to SSTR2 were determined using a membrane-based competition binding assay per published procedures [17]. Purified CHO-K1 membranes (25 μg/well) overexpressing human SSTR2 (Perkin Elmer, Waltham, MA) were incubated with [^{125}I]I-Tyr^{11}-somatostatin-14 (0.05 nM, Perkin Elmer) and competing non-radioactive ligands (10 μM to 1 pM) in a 96-well, 1.2 μm glass fibre filter plate (EMD Millipore, Darmstadt, Germany). The plates were pre-incubated with 0.1% polyethylenimine for 1 h at room temperature. The membrane, radioligand and competing peptides were diluted in assay buffer (25 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% BSA) and incubated for 1 h at 27 °C with moderate shaking. Following the incubation period, the mixture was aspirated through the filters, followed by 6 washes with 200 μL ice-cold wash buffer (50 mM Tris–HCl pH 7.4, 0.2% BSA). Each filter was removed and counted on a gamma counter. Each assay plate contained triplicates and assays were repeated three times for each radiotracer. The inhibition constants (K_i) were calculated by fitting the data to a one-site Fit-Ki curve in GraphPad Prism 7 (GraphPad Software, Inc., La Jolla, CA). The values are reported as mean ± standard deviation, with the reported standard deviation values representing inter-assay variations.

Table 1
HPLC purification conditions, chemical yield and mass-spec analysis of precursors and standards.

Peptide	HPLC solvent	Retention time (min)	Yield %	Molecular formula	M^+ calculated	M^+ found
GluAB-DOTATATE	31% MeCN (0.1% TFA), 69% water (0.1% TFA)	20.4	18.8	$C_{86}H_{118}IN_{17}O_{24}S_2$	1963.7	$[M + 2H]^{2+}$ 983.0
AspAB-DOTATATE	32% MeCN (0.1% TFA), 68% water (0.1% TFA)	15.3	14.3	$C_{85}H_{116}IN_{17}O_{24}S_2$	1949.7	$[M + 2H]^{2+}$ 975.8
Lu-GluAB-DOTATATE	31% MeCN (0.1% TFA), 69% water (0.1% TFA)	24.3	86.5	$C_{86}H_{115}ILuN_{17}O_{24}S_2$	2136.6	$[M + H + Na]^{2+}$ 1080.4
Lu-AspAB-DOTATATE	32% MeCN (0.1% TFA), 68% water (0.1% TFA)	19.5	50.0	$C_{85}H_{113}ILuN_{17}O_{24}S_2$	2121.6	$[M + 2H]^{2+}$ 1061.8