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^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide exhibits higher tumor accumulation and lower renal radioactivity than ^{111}In-DTPA-D-Phe^{1}-octreotide

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ABSTRACT

Introduction: ^{111}In-DTPA-D-Phe^{1}-octreotide scintigraphy is an important method of detecting neuroendocrine tumors. We previously reported that a new derivative of ^{111}In-DTPA-D-Phe^{1}-octreotide, ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide, accomplished the reduction of prolonged renal accumulation of radioactivity. The aim of this study was to evaluate the tumor accumulation of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide *in vitro* and *in vivo* by comparing it with ^{111}In-DTPA-D-Phe^{1}-octreotide.

Methods: The tumor accumulation of this octreotide derivative was determined by measuring its uptake using cultured AR42J cells *in vitro* and biodistribution studies *in vivo*. The distribution of the radiotracer and the extent of somatostatin receptor-specific uptake in the tumor were estimated by a counting method using AR42J tumor-bearing mice. The radioactive metabolite species in the tumor and kidney were identified by HPLC analyses at 3 and 24 h post-injection of the ^{111}In-DTPA-conjugated peptide.

Results: In both cases, *in vitro* and *in vivo*, the tumor radioactivity levels of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide were approximately 2–4 times higher than those of ^{111}In-DTPA-D-Phe^{1}-octreotide. On *in vitro* cellular uptake inhibition and radioreceptor assay, ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide exhibited a binding affinity to somatostatin receptor highly similar to that of ^{111}In-DTPA-D-Phe^{1}-octreotide. As the additional cellular uptake of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide was significantly lower at low temperature than at 37 °C, it was considered that a cellular uptake pathway is involved in energy-dependent endocytotic processes. In the radiometabolite analysis of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide, ^{111}In-DTPA-D-Phe-Asp-OH was a major metabolite in the tumor at 24 h post-injection.

Conclusion: ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide exhibited higher tumor accumulation and persistence of tumor radioactivity than ^{111}In-DTPA-D-Phe^{1}-octreotide. We reasoned that this higher tumor accumulation would not be based on the receptor affinity but on a receptor-mediated endocytotic process involved in temperature-dependent cellular uptake. The present study demonstrated the great potential of the pharmaceutical development of a new radiolabeled peptide with high tumor accumulation and low renal radioactivity by the chemical modification of ^{111}In-DTPA-D-Phe^{1}-octreotide.

1. Introduction

Somatostatin is a release-inhibitor of growth hormones and mediates its effects through binding to the somatostatin receptor [1]. Since the somatostatin receptor is overexpressed in neuroendocrine tumors such as carcinoids [2], gastrinomas [3], and endocrine pancreatic tumors [3–5], their visualization by somatostatin receptor-binding radiolabeled agents is influential in a clinical context [6]. ^{111}In-DTPA-D-Phe^{1}-octreotide (Fig. 1), a suitable radiolabeled analog of somatostatin, is currently used clinically to diagnose neuroendocrine tumors [7–10]. However, because the radiolabeled somatostatin analogs, including ^{111}In-DTPA-D-Phe^{1}-octreotide, show high and prolonged radioactivity in the kidneys [11,12], diagnostic inaccuracy and

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^{111}In-DTPA-D-Phe^{1}-octreotide :

^{111}In-DTPA-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)

^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide :

^{111}In-DTPA-D-Phe-Asp-D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr(ol)

Fig. 1. Chemical structure of ^{111}In-DTPA-D-Phe^{1}-octreotide and ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide.

nephrotoxicity are caused by undesirable radiation exposure [13,14]. Therefore, several attempts have been made to reduce the radioactivity levels in the kidneys [15,16].

We have investigated a strategy to reduce the renal radioactivity levels, by focusing on the interaction of the proximal tubule cells and radiolabeled octreotide. Our methodology is based on a chemical modification of the radioligand that introduces a negatively charged amino acid to induce electrostatic repulsion between the negatively charged renal cell surface and the radioligand. In fact, we reported on derivatives of ^{111}In-DTPA-D-Phe^{1}-octreotide in which the *N*-terminal D-Phe was replaced with an acidic amino acid such as L-aspartic acid (Asp) [17], glutamic acid [18], γ-carboxy-glutamic acid [18], or *p*-carboxy-phenylalanine [19] to impart a negative charge. The resulting derivatives appeared to be negatively charged at physiological pH and successfully exhibited reduced levels of renal radioactivity.

However, the receptor-binding affinity of ^{111}In-DTPA-Asp^{1}-octreotide was decreased by the replacement of *N*-terminal D-Phe, indicating that it has an important role in binding to the somatostatin receptor [20]. Subsequently, we designed, synthesized, and evaluated ^{111}In-DTPA-Asp^{0}-D-Phe^{1}-octreotide in which an Asp was incorporated between DTPA and the *N*-terminal D-Phe of ^{111}In-DTPA-D-Phe^{1}-octreotide. Although ^{111}In-DTPA-Asp^{0}-D-Phe^{1}-octreotide was successful at reducing renal radioactivity levels at early post-injection times without decreasing the inherent binding affinity, it exhibited higher radioactivity levels in the kidney than did ^{111}In-DTPA-D-Phe^{1}-octreotide at later post-injection times [20].

In our recent study [21], to improve this renal retention of radioactivity at later post-injection times, we reported the novel ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide (Fig. 1). This octreotide derivative has a structure in which an additional D-Phe is located between DTPA and the *N*-terminal Asp of ^{111}In-DTPA-Asp^{0}-D-Phe^{1}-octreotide. We designed this ^{111}In-DTPA-conjugated peptide by the consideration of radiometabolites and their lipophilicity generated from ^{111}In-DTPA-D-Phe^{1}-octreotide and ^{111}In-DTPA-Asp^{0}-D-Phe^{1}-octreotide, and achieved the desired objectives of both the suppression of renal uptake and the reduction in retention of the radiometabolite in the kidney of healthy mice. In this biodistribution study, the observation of high accumulation of radioactivity in the pancreas and spleen, in which somatostatin receptor is highly expressed physiologically in mouse, raised our expectations of a high affinity for somatostatin receptors in tumors [21].

The present study aimed to evaluate the *in vitro* and *in vivo* tumor accumulation of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide. We carried out cellular uptake studies and radioreceptor assays using somatostatin receptor-expressing cultured AR42J tumor cells and their prepared cell membrane. The biodistribution studies of ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide were performed in AR42J tumor-bearing nude mice.

2. Materials and methods

2.1. Reagents and chemicals

$^{111}InCl_3$ (74 MBq/mL in 0.02 N HCl) was purchased from Nihon Medi-Physics (Tokyo, Japan). Nonradioactive indium chloride was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) as $InCl_3 \cdot 4H_2O$. [^{125}I]Tyr^{11}-somatostatin-14 (1.85 MBq; specific activity 81.4 TBq/mmol) was purchased from PerkinElmer, Inc. (Waltham, MA, USA). Reverse-phase high-performance liquid chromatography (RP-HPLC) was performed on a Cosmosil 5C_{18}-AR-II column (4.6 × 250 mm and 10.0 × 250 mm; Nacalai Tesque, Kyoto, Japan). Radiometabolites of ^{111}In-DTPA-conjugated peptides in kidney and tumor were eluted with 5% methanol in 50 mM acetate buffer (pH 5.5) for the first 15 min. This was followed by a linear gradient of 5%–55% methanol in 50 mM acetate buffer (pH 5.5) over 10 min and the solvent composition was maintained for the next 25 min. Finally, the proportion of methanol was increased to 70% in 50 mM acetate buffer (pH 5.5) over 10 min. The effluent was monitored by the detection of its optical absorption at 254 nm and radioactivity output. Cellulose acetate electrophoresis (CAE; Joko Co. Ltd., Tokyo, Japan) was run in an electrostatic field of 1.0 mA/cm. To determine the radiochemical purity of ^{111}In-DTPA-D-Phe^{1}-octreotide and ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide, CAE was performed in veronal buffer (I=0.06, pH 8.6; Nacalai Tesque) for 40 min. Thin-layer chromatography (TLC) was performed using silica gel 60 F_{254} (Merck KGaA, Darmstadt, Germany) in a mixture of 10% aqueous ammonium chloride solution and methanol (1:1). Electrospray ionization low-resolution mass spectrometry (ESI-LRMS) was performed on JMS-T100LP (JEOL Ltd., Tokyo, Japan). Monoreactive DTPA (mDTPA) was synthesized as previously reported [22]. Unless otherwise stated, all chemicals were of reagent grade and used without further purification.

2.2. Synthesis of DTPA-D-Phe^{1}-octreotide and DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide

DTPA-D-Phe^{1}-octreotide and DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide were synthesized using mDTPA as described previously [21,23]. In brief, these precursors were synthesized by a solid-phase peptide synthesis method on 2-chlorotrityl resin using the Fmoc strategy. Coupling of Fmoc-protected amino acids was performed by the agitation of 2.5 mol equivalents with *N,N'*-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole monohydrate (HOBt·H_2O) in DMF for 1.5 h. Fmoc deprotection was performed by treatment with 20% *v/v* piperidine in DMF for 20 min. After the completion of peptide chain assembly, the peptide was cleaved from the resin with a mixture of trifluoroacetic acid (95% *v/v*) and thioanisole (5% *v/v*) and then purified by RP-HPLC.

2.3. Radiolabeling of DTPA-conjugated precursors and characterization of ^{111}In-DTPA-D-Phe^{1}-octreotide and ^{111}In-DTPA-D-Phe^{-1}-Asp^{0}-D-Phe^{1}-octreotide

Lyophilized kits (containing 10 μg of DTPA-conjugated peptide), with trisodium citrate (4.91 mg), citric acid (0.37 mg), inositol (10.0 mg), and gentisic acid (2.0 mg) were prepared using ultra-pure water. To the kits, 100–200 μL of a solution of $^{111}InCl_3$ (555 kBq–37 MBq) in 0.02 N HCl was added, and the resultant mixture was incubated at room temperature for 1 h. To determine the radiochemical purity of ^{111}In-DTPA-D-Phe^{1}-octreotide, CAE and TLC were carried out as previously described [21,23].

2.4. Cell culture

AR42J rat pancreatic tumor cells (ATCC Number CRL-1492) were purchased from DS Pharma Biomedical Co., Ltd. (Osaka, Japan). AR42J cells were cultured in Ham's F12K medium (Wako Pure Chemical Industries, Ltd.) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. Cultures were maintained in a humidified incubator at 37 °C and 5% CO_2. Subculturing was performed employing a 0.25% Trypsin–0.1% EDTA solution.