^{64}Cu-DOTA-Tyr^{3}-c(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-Lys(cypate)-NH_2

^{64}Cu-LS172

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Chemical name:	^{64}Cu-DOTA-Tyr^{3}-c(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-Lys(cypate)-NH_2	
Abbreviated name:	^{64}Cu-LS172	
Synonym:	^{64}Cu-DOTA-Tyr^{3}-octreotate-Lys-cypate, ^{64}Cu-DOTA-Y3-TATE-Lys-cypate	
Agent category:	Peptide	
Target:	Somatostatin receptor subtype-2 (SSTR-2)	
Target category:	Receptor	
Method of detection:	Positron emission tomography (PET), near-infrared (NIR) fluorescent imaging, optical fluorescent imaging	
Source of signal/contrast:	^{64}Cu, cypate	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Somatostatin (SST) (somatotropin release-inhibiting hormone, somatotropin release-inhibiting factor) is a cyclic disulphide-containing peptide hormone of 14 amino acids (1). SST inhibits hormone secretion, cell proliferation, and promotes apoptosis through binding to specific cell-surface somatostatin receptors (SSTRs) (2). Five SSTR subtypes are identified in the central nervous system (CNS), gastrointestinal tract, and a variety of

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benign and malignant tumors (2). All subtypes of SSTRs belong to the family of G protein–coupled receptors, and exhibit high affinity to SST by recognizing the β-turn in the peptide sequence of SST including Phe7, Trp8, Lys9, and Thr10 (2). The structural difference between SSTRs generates specific pharmacological and physiological properties for each subtype, which allows targeting of SSTRs with subtype-specific SST analogs (1). For example, octreotide, a SST analog with a peptide sequence of eight amino acids (D-Phe-c(Cys-Tyr-D-Trp-Lys-Thr-Cys)-Thr(ol)), possesses a much higher affinity (~2 nM) binding to SSTR-2, -3, and -5 than binding to SSTR-1 and -4 (>1,000 nM). In addition to symptomatic treatment of tumors in clinic, these analogs are also labeled with a variety of probes for imaging (1).

^{64}Cu-DOTA-Tyr^{3}-c(Cys-Tyr-Trp-Lys-Thr-Cys)-Thr-Lys(cypate)-NH_2 (^{64}Cu-LS172) is a monomolecular multimodal imaging agent (MOMIA) for positron emission tomography (PET) and optical imaging (3). ^{64}Cu-LS172 consists of three components: the STT analog Tyr3-octreotate (Y3-TATE) with an additional lysine at the N-terminal as a spacer linker, a complex of ^{64}Cu-1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (^{64}Cu-DOTA) attached at the C-terminal of Y3-TATE, and a cypate attached at the ε-amine group of the spacer lysine at the N-terminal of Y3-TATE. As a STT analog, TATE is produced by replacing the C-terminal threoninol in the octreotide with threonine, which substantially increases the binding affinity for SSTR-2 (1). ^{64}Cu is a radionuclide belonged to IIB group with a half-life of 12.7 h. ^{64}Cu has been a promising radionuclide in radiotherapy for effective short-range cell killing in tumors through β^- emission (578 keV; 38%). ^{64}Cu is also a β^+ emitter (653 keV) with modest positron yield (18%), which provides an adequate flux of annihilation photons for imaging its biodistribution by PET (4). Cypate (mw 625.34) is a carbocyanine derivative withstructure and optical properties very similar to indocyanine green dye (ICG). Cypate exhibits a high extinction coefficient (224,000 $M^{-1}cm^{-1}$) in the near-infrared (NIR) region (700–900 nm) (3, 6). The low light scattering and absorption of endogenous photoactive biomolecules in this region permit photons to penetrate several centimeters into tissues (7, 8). Two carboxyl groups in cypate can be conjugated with amines to generate peptide-based optical imaging agents (9).

Synthesis

[PubMed]

The synthesis of ^{64}Cu-LS172 was conducted in several steps (3). Y3-TATE was synthesized with a peptide synthesizer by standard automated Fmoc protocols. Rink amide resin was initially loaded with Fmoc amino acids, followed by automatic activation of the carboxyl group with a mixture of 1-hydroxybenzotriazole (HOBt) and *o*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and subsequent coupling with Fmoc-protected amino acid. LS172 was produced by attaching DOTA and cypate to Y3-TATE *in situ*. The carboxylic acid of tri-t-butyl-DOTA was coupled to the amine of the N-terminal phenylalanine, and the carboxylic acid of cypate was coupled to the ε-amino group of the C-terminal lysine. The overall yield of LS172 was found to be 96%. The radiolabling of LS172 was accomplished by mixing $^{64}CuCl_2$ with

LS172 in a buffer (pH 5.5) and incubated at 95°C for 60 min. The produced ^{64}Cu-LS172 had a radiochemical purity of ~80% and a final specific activity of 152 Ci/μg (332 Ci/mmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The spectral and binding properties of LS172 were examined *in vitro* (3). Similar to cypate, LS172 exhibited broad excitation and emission bands with a narrow Stokes shift (23 nm). The relative quantum yield of LS172 (0.12) was the same as that of ICG. Complexation of LS172 with Cu^{2+} induced negligible reduction in the quantum yield. Because of the modifications in both C- and N-terminal, the affinity of LS172 binding to SSTR-2 increased ~10- and ~5-fold relative to cypate-Y3-TATE and Y3-TATE, respectively. But complexation with Cu^{2+} appeared not to affect the resultant affinity (11.5 nM for Cu-LS172 *versus* 2.03 nM for LS172). The internalization of LS172 and Cu-LS172 was evaluated with NIR fluorescence microscopy in A427-7 membranes (SSTR-2–positive) and HEK293 cells (STTR-2–negative). LS172 was weakly internalized at 25 nM. The internalization was inhibited by 5 μM Y3-TATE with an incubation time <60 min. Cu-LS172 appeared not to be internalized to any significant extent in cells. In comparison, cypate-Y3-TATE was strongly internalized in a time-dependent manner, and the internalization was completely blocked by 5 μM Y3-TATE.

Animal Studies

Rodents

[PubMed]

The biochemical distribution of LS172 was examined in AR42J tumors in rats (n = 5) (3). ^{64}Cu-LS172 (4 Ci/12 mmol (27 mg/12 mmol)) was injected intravenously in rats at 10–14 days after tumor implantation in the legs or after the tumors had grown ~1.5–2.0 g. In another group of tumor-implanted rats (n = 5), Y3-TATE (100μg/95 nmol) was also included as a competitive dose in addition to the radiolabeled LS172. The pharmacokinetic profile ^{64}Cu-LS172 exhibited a rapid blood clearance of 0.228 ± 0.047% injected dose per gram (ID/g) (0.151 ± 0.027% ID/g) at 1 h after injection. Clearance of ^{64}Cu-LS172 was predominately hepatic (16.824 ± 1.520% ID/g, 95.8 ± 7.12% ID/liver, 1 h) with relatively low kidney accumulation (1–2%). Accumulation of activity for ^{64}Cu-LS172 in the SSTR-2–positive tissues of adrenal, pituitary, pancreas, and tumor were relatively low (≤1% ID/g). ^{64}Cu-LS172 accumulation in tumors decreased in the presence of Y3-TATE, but the observed decrease in other SSTR-2–positive organs was not significant.

The NIR fluorescence imaging of LS172 was performed in tumor-bearing NCR nu/nu mice (n = 6) (3). After inoculation with lung adenocarcinoma cell line A427-7 in the right and left flanks of each mouse, tumors were allowed to grow to 5–10 mm maximum diameter before imaging. Three mice were injected with 2 nmol LS172, and two other

mice were injected with 2 nmol LS172 and a competitive dose of 95 nmol Y3-TATE. NIR fluorescence images were collected at 1, 4, 8, and 24 h after injection. No significant accumulation of LS172 was found in tumors compared to normal tissue at different time points. The competitive inhibition with Y3-TATE did not significantly alter the tumor/muscle uptake ratio. After 24 h, the mice were euthanized, and tissues were harvested for *ex vivo* fluorescence biodistribution imaging. In addition to the liver and kidneys, LS172 accumulated in the spleen and accounted for 35% of the fluorescence intensity relative to that in the liver. LS172 accumulation in mouse kidney was somewhat higher than that of the radiolabeled analogs in rat kidneys, possibly indicating the reduction of overall charge on the DOTA upon chelation with a metal ion. The uptake and clearance profiles of LS172 in tumor-bearing A427-7 mice were similar to those of ^{64}Cu-LS172 in tumor-bearing AR427 rats.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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