^{111}In-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-dihistidine-norleucine peptide analog

^{111}In-DOTA-H2-Nle

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Chemical name:	^{111}In-Tetraazacyclododecane-*N*,*N*',*N*",*N*"'-tetraacetic acid-dihistidine-norleucine peptide analog	
Abbreviated name:	^{111}In-DOTA-H2-Nle	
Synonym:	Radiolabeled minigastrin	
Agent Category:	Peptide	
Target:	Gastrin/cholecystokinin-2 (CCK-2, CCK-B) receptor	
Target Category:	Receptor binding	
Method of detection:	Single-photon emission computed tomography (SPECT), planar gamma imaging	
Source of signal:	^{111}In	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about.the CCK-2 receptor.

Background

[PubMed]

^{111}In-Tetraazacyclododecane-*N*,*N*',*N*",*N*"'-tetraacetic acid-dihistidine-norleucine peptide (^{111}In-DOTA-H2-Nle) is a radiolabeled gastrin analog that can be used for single-photon emission computed tomography (SPECT) imaging of tumors that express the gastrin/

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cholecystokinin-2 (CCK-2) receptor (1). ^{111}In is a gamma emitter with a physical half-life ($t_{½}$) of 2.8 days.

The gastrointestinal peptides gastrin and CCK have various regulatory functions in the brain and gastrointestinal tract (2). Gastrin and CCK have the same COOH-terminal pentapeptide amide sequence, which is the biologically active site (3). Human gastrin is a peptide composed of 34 amino acids and also exists in several C-terminal–truncated forms (1). These C-terminal–truncated forms include minigastrin, which is a 13-residue peptide with the sequence of LEEEEEAYGWMDF-NH_2. CCKs exist in a variety of biologically active molecular forms that are derived from a precursor molecule comprising 115 amino acids (4). These forms range from 4 to 58 amino acids in length and include sulphated and unsulphated CCK-8, which has the structure DYMGWMDF-NH_2. They bind to and act through transmembrane G-protein–coupled receptors (5). Two different CCK receptor subtypes have been identified in normal tissues. CCK-1 (CCK-A, alimentary) receptors have low affinity for gastrin, and CCK-2 (CCK-B, brain) receptors have high affinity for gastrin (4). They also differ in terms of molecular structure, distribution, and affinity for CCK. These receptors have also been found to be expressed or overexpressed in a multitude of tumor types (5). CCK-2 receptors have been found most frequently in medullary thyroid carcinomas, small-cell lung cancers, astrocytomas, and stromal ovarian cancers (2). CCK-1 receptors have been identified in gastroenteropancreatic tumors, meningiomas, and neuroblastomas.

Reubi et al. (6) designed a series of radiolabeled CCK-8 peptides that showed high specificity for potential *in vivo* imaging of tumors expressing CCK-2 receptors. de Jong et al. (7) developed an ^{111}In-labeled unsulphated CCK8 analog that used 1,4,7,10-tetraazacyclododecane-*N,N′,N″,N‴*-tetraacetic acid (DOTA) as a bifunctional chelating agent. The radioligand showed high specific internalization rates in receptor-positive rat pancreatic AR42J tumor cells. von Guggenberg et al. (8) reported the synthesis of ^{99m}Tc-hydrazinonicotinic acid (HYNIC)-minigastrin complexes and high tumor uptake in nude mice bearing AR42J tumors. Nock et al. (9) prepared ^{99m}Tc-labeled minigastrin analogs and found that they displayed high specific localization in nude mice bearing AR42J tumors. Mather et al. (1) synthesized a library of different peptide sequences based on the C-terminal sequences of CCK-8 or minigastrin. These peptides were labeled with ^{111}In by DOTA or diethylene triamine pentaacetic acid (DTPA) conjugation. The dihistidine analog ^{111}In-DOTA-H2-Nle was evaluated to study the effect of substituting a norleucine (Nle) residue for the methionine (Met) residue near the C-terminus. The study appeared to show that this substitution could result in a reduction in tumor uptake of the radiopeptide analog.

Synthesis

[PubMed]

The peptide sequence HHEAYGWMDF-amide was obtained by solid-phase peptide synthesis under standard conditions from commercial sources (1). The N-terminus was

capped with a DOTA chelating group to produce DOTA-H2-Nle. The identity and purity were confirmed by matrix-assisted laser desorption/ionization mass spectroscopy and reverse-phase high-performance liquid chromatography. Radiolabeling was performed by mixing ^{111}In-chloride in ammonium acetate and 0.04 M monothioglycerol (MTG), an antioxidant, with DOTA-H2-Nle in 0.01 M phosphate-buffered saline (pH 7.2). The mixture was heated at 98°C for 15 min, and 0.1 M ethylenediamine tetraacetic acid (EDTA) was then added to quench the reaction. The labeling yield was >90%. The radiochemical purity and specific activity were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

An *in vitro* receptor affinity assay was performed with AGS human gastric tumor cells transfected with the CCK-2 receptor (AGS-CCK2R) and ^{125}I-G17 as the radioligand. The inhibition constant (K_i) and half of the maximum binding fraction (EC_{50}) of unlabeled DOTA-H2-Nle were 16.7 nM and 11.3 nM, respectively. In comparison, the K_i and EC_{50} of DOTA-H2-Met were 5.7 nM and 3.9 nM, respectively.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{111}In-DOTA-H2-Nle were performed in nude mice bearing rat pancreatic AR42J tumors or rat pancreatic CA20948 tumors (1). Both AR42J and CA20948 tumors expressed gastrin receptors. Each mouse received 0.2 μg of ^{111}In-DOTA-H2-Nle by i.v. injection. The tumor radioactivity levels at 4 h (n = 3–4), represented as the percentage of injected dose per gram (% ID/g), were 0.73 ± 0.14 and 0.53 ± 0.08 for AR42J and CA20948, respectively. The blood radioactivity levels (% ID/g) at 4 h were 0.03 ± 0.00 and 0.03 ± 0.00 for AR42J and CA20948 tumors, respectively. The kidney radioactivity levels (% ID/g) at 4 h were 2.40 ± 2.13 and 1.73 ± 0.31 for AR42J and CA20948 tumors, respectively. No blocking study was performed. In comparison, the tumor radioactivity levels (% ID/g) of ^{111}In-DOTA-H2-Met at 4 h were 0.87 ± 0.21 and 0.59 ± 0.10 for AR42J and CA20948 tumors, respectively.

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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