^{111}In-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-HHEAYGWMDF-NH_2 peptide

^{111}In-DOTA-H2-Met

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Chemical name:	^{111}In-Tetraazacyclododecane-*N*,*N*',*N*",*N*"'-tetraacetic acid-HHEAYGWMDF-NH_2 peptide	
Abbreviated name:	^{111}In-DOTA-H2-Met	
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/contrast:	^{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-HHEAYGWMDF-NH_2 peptide (^{111}In-DOTA-H2-Met) is a radiolabeled gastrin analog that can be used for single-photon emission computed tomography (SPECT) imaging of tumors that express

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the gastrin/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 on a multitude of tumor types (5). CCK-2 receptors have been found most frequently in medullary thyroid carcinoma, small-cell lung cancers, astrocytomas, and stromal ovarian cancers (2). CCK-1 receptors have been identified in gastroenteropancreatic tumors, meningiomas, and neuroblastoma.

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 non-sulfated 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 AR42J rat pancreatic tumor cells. von Guggenberg et al. (8) reported the synthesis of ^{99m}Tc-hydrazinonicotinic acid (HYNIC)-minigastrin complexes and showed 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 diethylenetriamine pentaacetic acid (DTPA) conjugation. Although their ultimate goal was to identify an analog that could be used for radiotherapeutic applications, ^{111}In-DOTA-H2-Met appeared to have the best pharmacokinetic properties with the highest tumor/kidney ratio and low uptake by nontarget tissues.

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-Met. The identity and purity were confirmed by matrix-assisted laser desorption/ionization mass spectroscopy and reverse-phase high-performance liquid chromatography (HPLC). Radiolabeling was performed by mixing ^{111}In-chloride in ammonium acetate and 0.04-M monothioglycerol (MTG), an antioxidant, with DOTA-H2-Met 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 efficiency was >90%. The radiochemical purity was not reported, but HPLC chromatography of MTG-added samples showed a single peak with a small peak of 5.9% identified as the oxidized peptide. The specific activity was not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

In the *in vitro* plasma stability study, ^{111}In-DOTA-H2-Met was incubated with fresh human blood at 37°C (1). HPLC analysis showed that >95% of intact peptide remained after 4 h incubation, but only 62% remained after 24 h. In comparison, 98% of the radioligand remained intact in phosphate-buffered saline after 24 h. An *in vitro* receptor affinity assay was performed by using 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}) were 3.9 nM and 5.7 nM, respectively.

Animal Studies

Rodents

[PubMed]

Biodistribution studies of ^{111}In-DOTA-H2-Met were performed in nude mice bearing AR42J rat pancreatic tumors (1), HT29 human colorectal tumors, or CA20948 rat pancreatic tumors. Both AR42J and CA20948 tumors expressed different levels of gastrin receptors, and HT29 was a cell line transfected with a relatively low level of gastrin receptors. Each mouse received 0.2 μg of ^{111}In-DOTA-H2-Met by i.v. injection. The tumor radioactivity levels at 4 h (n = 3–4), represented as percent injected dose per g (% ID/g), were 0.87 ± 0.21, 0.59 ± 0.10, and 0.19 ± 0.05 for AR42J, CA20948, and HT29 tumors, respectively. The radioactivity in the AR42J tumor reached ~2% ID/g at 1 h (Figure 1), and then decreased to ~0.5% ID/g in 72 h. With the coadministration of 100 μg of unlabeled peptide, the radioactivity level in the AR42J tumor at 4 h was reduced to ~0.1–0.2% ID/g. The blood clearance was extremely rapid, and there was a gradual washout from all tissues. The blood radioactivity levels at 4 h were 0.04 ± 0.01, 0.07 ± 0.02, and 0.08 ± 0.01 for AR42J, CA20948, and HT29 tumors, respectively. The kidney levels at 4 h were 1.87 ± 0.60, 2.41 ± 0.91, and 2.44 ± 0.47 for AR42J, CA20948, and HT29 tumors, respectively. The tumor/kidney ratios were 0.47 (n = 15), 0.24 (n = 7), and 0.08 (n = 6) for

AR42J, CA20948, and HT29 tumors, respectively. A dose-ranging study showed that the peptide dose >1 μg appeared to reduce the tumor uptake.

Gamma imaging of ^{111}In-DOTA-H2-Met in mice bearing AR42J tumors showed an uptake pattern that was similar to that of the biodistribution studies (1). There was a modest level of tumor uptake with heterogeneous distribution within the tumor mass. There was little uptake in the central organs.

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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