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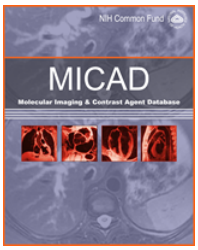


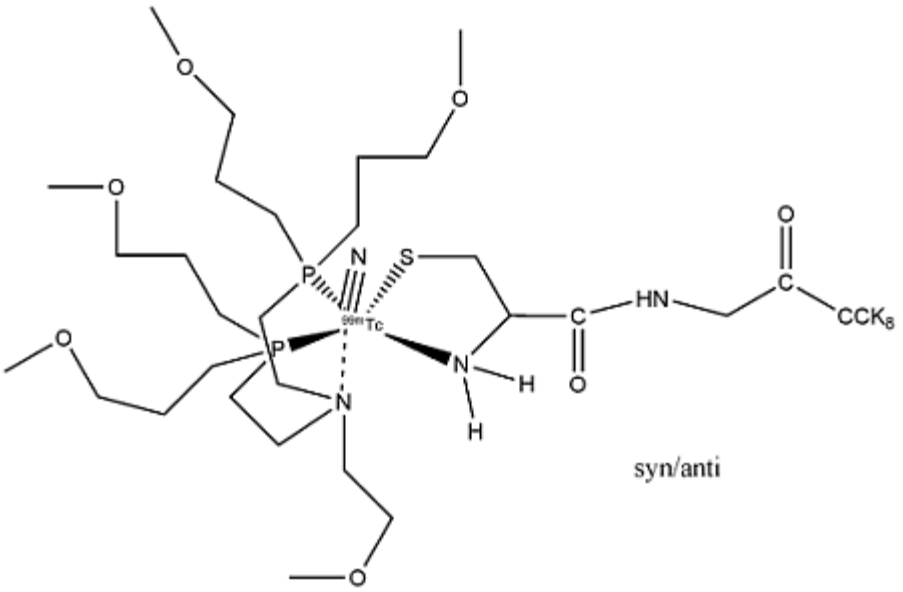
[^{99m}Tc(N)(NS-Cys-Gly-CCK-8)(PNP3)]$^{+}$

^{99m}Tc(N)(PNP3)-CCK-8

The MICAD Research Team

Created: April 30, 2007; Updated: May 30, 2007.

Chemical name:	[^{99m}Tc(N)(NS-Cys-Gly-CCK-8)(PNP3)]$^{+}$
Abbreviated name:	^{99m}Tc(N)(PNP3)-CCK-8
Synonym:	^{99m}Tc-CCK-8
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:	^{99m}Tc
Activation:	No
Studies:	• *In vitro* • Rodents



^{99m}Tc(N)(PNP3)-CCK-8

Background

[PubMed]

[^{99m}Tc-(N)(NS-Cys-Gly-CCK-8)(PNP3)]$^{+}$ (^{99m}Tc(N)(PNP3)-CCK-8) is a radiolabeled peptide developed for single-photon emission computed tomography (SPECT) imaging of tumors that express the gastrin/cholecystokinin-2 (CCK-2) receptor (1). ^{99m}Tc is a gamma emitter with a physical half-life ($t_{½}$) of 6.01 h.

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 (4). 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 (5). 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. These forms bind to and act through transmembrane G-protein–coupled receptors (6). 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 (5). 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 (6). 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. (7) designed a series of radiolabeled CCK-8 peptides that showed high specificity for potential *in vivo* imaging of CCK-2 receptor-expressing tumors. de Jong et al (8) developed a ^{111}In-labeled nonsulfated CCK-8 analog using DOTA as a bifunctional chelating agent. The radioligand showed high specific internalization rates in the receptor-positive AR42J rat pancreatic tumor cells. von Guggenberg et al (9) reported the synthesis of ^{99m}Tc-HYNIC-minigastrin complexes and showed high tumor uptake in nude mice bearing AR42J tumors. Nock et al. (10) prepared 99mTc-labeled minigastrin analogs and found that they displayed high specific localization in nude mice bearing AR42J tumors. Mather et al. (4) synthesized a library of different peptide sequences based on the C-terminal sequences of CCK-8 or minigastrin. Tc mixed-ligand complexes that display characteristic substitution-inert metal fragments $[Tc(CO_3)]^+$ and $[Tc(N)(PNP)]^{2+}$can be used as platforms in the design of potential ^{99m}Tc radiopharmaceuticals (11-14). The $[Tc(N)(PNP)]^{2+}$ technology can produce well-defined complexes with very high yields (>90%) depending on the co-ligand (15). Agostini et al (1). applied this technology to the labeling of a nonsulfated CCK-8 peptide analog. PNP3 (*N*,*N*-bis(dimethyoxypropylphosphino-ethyl)methoxyethylamine) was selected as the aminodiphosphine ligand. *In vivo* evaluation of ^{99m}Tc(N)(PNP3)-CCK-8 showed rapid and specific targeting to CCK-2 receptors.

Synthesis

[PubMed]

Agostini et al. (1) described the synthesis of ^{99m}Tc(N)(PNP3)-CCK-8. The NS-Cys^{24}-Gly^{25}-Asp^{26}Tyr^{27}Met^{28}Gly^{29}Trp^{30}Met^{31}Asp^{32}Phe^{33}NH_2 (NS-Cys-Gly-CCK-8 peptide was prepared by the standard solid-phase peptide synthesis with the use of an automated peptide synthesizer based on the fluorenylmethoxycarbonyl (Fmoc) strategy. The peptide was combined through the terminal carboxylic group of the cysteine residue to produce a COO-functionalized NH_2, S-cysteine ligand. A glycine residue was introduced as the spacer group. The peptide identity was confirmed by mass spectra with a M_W of 1220. This peptide contained cysteine which could bind the $[Tc(N)(PNP)]^{2+}$ moiety either through the [NH_2, S–] pair or the {O–,S–] pair of donor atoms to yield the corresponding mixed compound with high specific activity. The PNP3 ligand was obtained commercially. Radiolabeling with ^{99m}Tc used the “metal fragment” approach by reacting the bifunctional ligand with the metal fragment to produce the monopositive asymmertrical complex.

Two methods of radiolabeling were tested (1). In the two-step procedure, sodium ^{99m}Tc pertechnetate ($Na^{99m}TcO_4$) was added to a mixture of succinic dehydrate (SDH, nitride nitrogen atom donor), stannous chloride (reducing agent), and ethanol. The mixture was incubated at room temperature for 30 min. This produced a mixture of ^{99m}Tc-nitrido precursors that contained the $[Tc{\equiv}N]^{2+}$ core. Then the PNP3 ligand and the NS-Cys-Gly-CCK-8 peptide were added simultaneously to the mixture (pH 7) and heated at 80°C for 60 min to produce the final ^{99m}Tc(N)(PNP3)-CCK-8. The radiochemical yield (RCY) of this approach was 90.0%. In the one-step (one-pot) procedure, $Na^{99m}TcO_4$ was added to a mixture of succinic dehydrate, stannous chloride, ethanol, the peptide in phosphate buffer (pH 7.4), and PNP3. The RCY of this procedure was 90.9%. The final monocationic complexes from both procedures were further purified by high-performance liquid chromatography (HPLC) which yielded a radiochemical purity of >95%. The authors suggested that the similar high yield of the one-pot method demonstrated that the PNP ligand stabilized the $[^{99m}Tc^{V}(N)]^{2+}$ core and

promoted the reactivity of the intermediate $[^{99m}Tc(N)(PNP)]^{2+}$ moiety for a rapid coordination of PNP3 to form the metal fragment. Thin–layer chromatography revealed that the final asymmetrical ^{99m}Tc(N)(PNP3)-CCK-8 consisted of two isomeric forms in the ratio 60:40 (syn/anti) dependent on the orientation of the COO-substituted cysteine pendant group with respect to the central Tc≡N-terminal core. Resolution of this mixture was not possible by HPLC. No specific activity was reported. In a study of the influence of peptide concentration on RCY, the highest RCY (86.6 ± 1.09% to 91.0 ± 3.4%) appeared to be achieved with an amount of peptide in the range of 50–200 µg (4.1×10^{-5} to 1.6×10^{-4} mmol). In this concentration range (1.13×10^{-4} to 2.81×10^{-5} M), no significant change in RCY was found with reaction temperature increased to 100°C. Both shorter reaction time (15 min) and lower temperature (<50°C) decreased the RCY.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* stability of ^{99m}Tc(N)(PNP3)-CCK-8 was evaluated by incubation in saline, human serum, or mouse serum at 37°C for 24 h (1). At different time points, samples were taken for HPLC analysis. The ^{99m}Tc-labeled complex was stable in all conditions within the first 3 h. After 24 h, ~80% and 50% of the peptide remained intact in human serum and mouse serum, respectively. The labeled complex was stable in saline and phosphate buffer (pH 7.4) for 24 h and was highly inert to transchelation challenges with excess cysteine or glutathione. Human epidermoid carcinoma A431 cells that overexpress CCK-2 receptors were used in cell binding studies (16). The compound showed high specific binding at 4°C with an apparent dissociation constant (K_d) of 19.0 ± 4.6 nmol/liter and the number of binding sites (B_{max}) was 7.7×10^5/cell.

Animal Studies

Rodents

[PubMed]

Agostini et al. (1) conducted the biodistribution studies in nude mice bearing both the A431 s.c. tumor and receptor-negative tumor in the opposite flank. Each mouse received ~1.85 MBq (50 µCi) ^{99m}Tc(N)(PNP3)-CCK-8 and was euthanized at 1 h. The radioactivity appeared to clear rapidly from blood and normal tissues (lungs, spleen and muscle). Rapid radioactivity localization was observed in the tumors with the radioactivity level ~0.8% injected dose/g (% ID/g) at 1 h (extrapolated from Figure 4). This represented a four-fold higher level than that of receptor-negative tumors. There were relatively high radioactivity levels in the liver (~0.9% ID/g) and kidneys (~1.8% ID/g). At 1 h, the highest radioactivity level was found in the intestinal tract (~4.3% ID/g). The authors suggested that the clearance of the unbound radioactivity was through the hepatobiliary system. The high intestinal uptake could be minimized by increasing the hydrophilic properties of the labeled peptide.

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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