^{99m}Tc-[Ac-CCEHdFRWCKPV-NH_2]

^{99m}Tc-CCMSH

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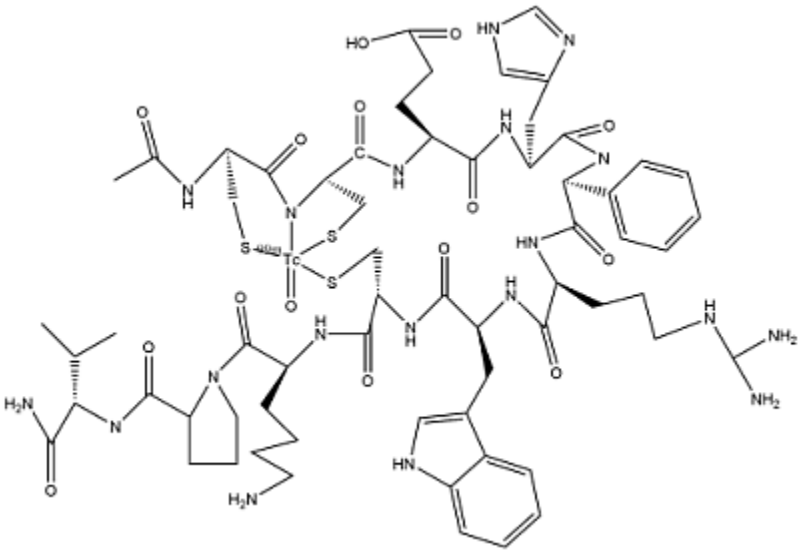
Chemical name:	^{99m}Tc-[Ac-CCEHdFRWCKPV-NH_2]
Abbreviated name:	^{99m}Tc-CCMSH
Synonym:	^{99m}Tc-cyclic α-MSH, ^{99m}Tc-labeled cyclic α-melanocyte –stimulating hormone peptides, ^{99m}Tc-[$Cys^{3,4,10}$,$_{D}$-Phe^{7}]α-MSH_{3-13}, ^{99m}Tc-[Acetyl-Cys-Cys-Glu-His-dPhe-Arg-Trp-Cys-Lys-Pro-Val-NH_2]
Agent Category:	Peptide
Target:	Melanocortin-1 (MC-1) receptor
Target Category:	Peptide-receptor binding
Method of detection:	Single-photon emission computed tomography (SPECT) or gamma planar imaging
Source of signal:	^{99m}Tc
Activation:	No



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Studies:	• *In vitro* • Rodents	^{99m}Tc-[Ac-CCEHdFRWCKPV-NH_2] structure. The ^{99m}Tc coordination configuration has not been confirmed by experiments. Click on sid:49693200 for additional information in PubChem.

Background

[PubMed]

^{99m}Tc-[Ac-CCEHdFRWCKPV-NH_2] (^{99m}Tc-CCMSH) is a radioligand that has been developed as a single-photon emission computed tomography (SPECT) imaging probe for primary and metastatic melanoma (1, 2). ^{99m}Tc-CCMSH is an α-melanocyte–stimulating hormone (MSH) peptide labeled with ^{99m}Tc, a gamma emitter with a physical half-life ($t_{½}$) of 6.02 h.

Malignant melanoma is the sixth most common cancer in the United States (3). Early diagnosis and prompt surgical removal make up the best approach for a possible cure (4). The melanocortin (MC) system is the best characterized neuropeptide network of the skin, and it is involved in pigmentation regulation, cortisol production, and many other physiological processes (5). Most cutaneous cell types express MC receptors, proopiomelanocortin (POMC), and prohormone convertases, and these cell types also release MCs. Five MC receptors (MC-1 to MC-5) have been cloned and characterized as receptors that belong to the G-protein–coupled receptor superfamily. MSHs (α-, β-, and γ-MSH) are derived from POMC by the proteolytic action of prohormone convertases. α-MSH (Ac-Ser^1-Tyr^2-Ser^3-Met^4-Glu^5-His^6-Phe^7-Arg^8-Trp^9-Gly^{10}-Lys^{11}-Pro^{12}-Val^{13}-NH_2) is a peptide composed of 13 amino acids and is the most potent, naturally occurring, melanotropic peptide (6). The biological effects of α-MSH are mediated *via* MC receptors.

Although positron emission tomography (PET) imaging with [^{18}F]fluoro-2-deoxy-2-D-glucose ([^{18}F]FDG) is effective in the detection of melanoma, it is not melanoma-specific and some melanoma cells do not take up [^{18}F]FDG (7, 8). Radiolabeled α-MSH peptide analogs have been shown to specifically bind to MC-1 receptors that are overexpressed on human and mouse melanoma cells (7, 9-12). Some studies have successfully used 1,4,7,10-tetraazacyclodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) coupled to the α-MSH peptide analogs for radiolabeling. These α-MSH derivatives (DOTA-α-MSH) can be labeled with a

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variety of radionuclides (7). To improve the *in vivo* pharmacokinetics of these radiolabeled peptides, a number of different α-MSH analogs have been designed (13, 14). Froidevaux et al. (13) showed that the kidney uptake of DOTA-NAPamide, a short linear DOTA–α-MSH analog, could be considerably reduced by neutralizing the charge of the Lys^{11} residue. Giblin et al. (2) used metal cyclization to design a new class of α-MSH peptide analogs that are resistant to chemical and proteolytic degradation *in vivo*, and their study showed that ^{99m}Tc-CCMSH complex had potential melanoma-imaging properties. Chen et al. (1) reported that ^{99m}Tc-CCMSH analogs, cyclized through site-specific coordination of ^{99m}TcO, showed excellent tumor uptake and retention properties in mice bearing B16/F1 murine melanomas.

Synthesis

[PubMed]

The CCMSH peptide was synthesized by using the standard fluorenylmethoxycarbonyl/2(1*H*-benzotriazol-1-yl)-1,1,3,3,tetramethyluronium hexafluorophosphate solid-phase peptide synthesis chemistry on amide resin (1, 2). A solid-phase peptide synthesizer was used. The peptide was acetylated by activating glacial acetic acid with 2(1*H*-benzotriazol-1-yl)-1,1,3,3,tetramethyluronium hexafluorophosphate after deprotecting the NH_2-terminal residue. The peptide was deprotected and cleaved from the resin by using a mixture of trifluoroacetic acid, thioanisol, ethanedithiol, and water at room temperature for 2 h. The peptide was then purified by high-performance liquid chromatography (HPLC), and the identity was confirmed by fast atom bombardment mass spectrometry. The molecular weight of CCMSH was determined to be 1,448 (calculated molecular weight = 1,447). The ^{99m}Tc radiolabeling was prepared *via* a glucoheptonate transchelation reaction (1). In this procedure, stannous chloride ($SnCl_2$) was used as a reducing agent and glucoheptonate was used as a transfer ligand. Briefly, 10 μg of the peptide were added to the mixture of $SnCl_2$, ^{99m}Tc-pertechnetate, and glucoheptonate after the pH of the mixture was adjusted to 9. The mixture was incubated at 75°C for 30 min. The final ^{99m}Tc-CCMSH product was purified by HPLC. The identity of ^{99m}Tc-CCMSH was confirmed by both mass spectrometry and HPLC analysis. The radiochemical stability was evaluated in phosphate-buffered saline (PBS; pH 7.4). Over a 24-h period of incubation at 25°C in PBS, only radiolabeled peptide and no detectable free radioactivity was detected by HPLC analysis (1). The specific activity and radiochemical purity were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Giblin et al. (2) performed *in vitro* quantitative receptor-binding assays of unlabeled CCMSH on B16/F1 murine melanoma cells. The inhibition constant (K_i) was determined to be $7.6 \times 10{-}^{9}$ M.

Chen et al. (1) performed receptor binding affinity, internalization, and cellular retention assays of ^{99m}Tc-CCMSH on B16/F1 murine melanoma cells. A B_{max} of 5.812 fmol/0.5 million cells (7,000 α-MSH receptors/cell) were determined. About 4% cell binding was found after 3 h of incubation. As early as 5 min after incubation, ~70% of the receptor-bound radioligand was internalized. This percentage of internalized radioactivity did not significantly change over the incubation period. When testing for cellular retention, ~36% of the internalized radioactivity was released from the cells during a 4-h incubation at 37°C. Analysis using a C-18 Sep-Pak column showed that the majority of the radioactivity released from the cells consisted of low molecular weight forms.

Animal Studies

Rodents

[PubMed]

Biodistribution and gamma imaging studies with ^{99m}Tc-CCMSH were conducted in normal mice and mice bearing the B16/F1 murine melanoma (2). Each mouse received 74–111 MBq (2–3 mCi) of ^{99m}Tc-CCMSH by i.v. administration. ^{99m}Tc-CCMSH radioactivity was rapidly cleared and excreted by the kidneys in the healthy mice. In melanoma-bearing mice, ^{99m}Tc-CCMSH radioactivity accumulated rapidly in the tumor. The radioactivity levels in the tumor (n = 5), shown as percentage injected dose per g (% ID/g) at various time points, were 10.74 ± 1.61 (0.5 h), 10.88 ± 0.54 (1 h), 9.51 ± 1.97 (4 h), and 1.38 ± 0.36 (24 h). The tumor/blood ratios were 3.12 (0.5 h), 6.80 (1 h), and 15.34 (4 h). The activity was cleared rapidly from the tumor-bearing animals with 74.84 ± 2.53% ID/g in the urine at 1 h. Other major organ radioactivity levels (% ID/g) at 1 h were 22.60 ± 2.70 (kidneys), 2.34 ± 2.19 (liver), 1.39 ± 0.20 (lung), 0.53 ± 0.17 (heart), 1.07 ± 0.26 (spleen), and 0.90 ± 0.10 (muscle). The radioactivity in the stomach (% ID/g) was 0.47 ± 0.10 at 1 h. Coinjection of 30 mg lysine appeared to reduce kidney radioactivity uptake by 48%, 55%, and 70% at 0.5, 1, and 4 h after injection, respectively (1). With injection of 1 mg of NDP ([NIe^4,D-Phe^7] αMSH), a high-affinity unlabeled α-MSH analog, 30 min before ^{99m}Tc-CCMSH injection, the tumor radioactivity was reduced from an average of 8.5% ID to 0.82% ID at 30 min (2). A tumor imaging study with a dose of 925 MBq (25 mCi) in a mouse bearing a 400-mg melanoma showed tumor visualization and significant tumor radioactivity accumulation at 30 min. The kidneys and bladder also showed radioactivity uptake. Another imaging study with a dose of 3.7 MBq (0.1 mCi) showed that the tumor was visualized after 1 h, and the tumor activity was extremely high at 8 h (1). The bladder activity was very high at 1 h, and the background in the normal tissues was low after the bladder contents were voided at 8 h.

The same group of researchers performed biodistribution studies of ^{99m}Tc-CCMSH in SCID mice bearing TXM-13JQ human melanomas (1). The TXM-13JQ human melanoma had a B_{max} value of 5.812 fmol/0.5 million cells (7,000 α-MSH receptors/cell). Each mouse received 0.925 MBq (0.025 mCi) ^{99m}Tc-CCMSH for the studies. The biodistribution pattern of ^{99m}Tc-CCMSH radioactivity in normal tissues was similar to

those of mice bearing B16/F1 murine melanomas. The radioactivity levels (% ID/g) in the tumor (n = 5) were 3.08 ± 0.58 (0.5 h), 3.26 ± 0.77 (1 h), 2.22 ± 0.25 (4 h), and 0.48 ± 0.12 (24 h). The tumor/blood ratios were 1.15 (0.5 h), 2.03 (1 h), 5.82 (4 h), and 3.31 (24 h). The tumor/muscle ratios were 7.89 (0.5 h), 11.55 (1 h), 38.71 (4 h), and 27.50 (24 h). Coinjection of 30 mg lysine decreased both the kidney and blood radioactivity levels. As a result, the tumor/blood ratios were improved to 1.44 (0.5 h), 4.11 (1 h), 11.52 (4 h), and 4.56 (24 h). The tumor/muscle ratios were improved to 6.17 (0.5 h), 19.14 (1 h), 50.90 (4 h), and 37.14 (24 h).

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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