N-[2-(*N*-(2-mercaptoethyl)) amino ethyl]-*N*-(2-mercaptoethyl)-3,5-dimethylacetamide amantadine-technetium

^{99m}Tc-NCAM

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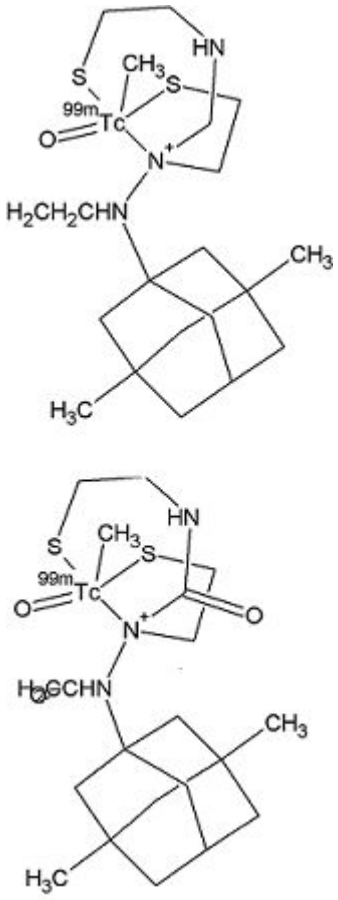
Chemical name:	*N*-[2-(*N*-(2-mercaptoethyl)) amino ethyl]-*N*-(2-mercaptoethyl)-3,5-dimethylacetamide amantadine-technetium
Abbreviated name:	^{99m}Tc-NCAM
Synonym:	
Agent Category:	Compounds
Target:	*N*-methyl-D-aspartate receptor (NMDAR)
Target Category:	Receptors
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging
Source of signal / contrast:	^{99m}Tc
Activation:	No
Studies:	• *In vitro* • Rodents



Structures of ^{99m}Tc-NHAM (upper) and ^{99m}Tc-NCAM (lower)

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Background

[PubMed]

N-[2-(*N*-(2-mercaptoethyl)) amino ethyl]-*N*-(2-mercaptoethyl)-3,5-dimethylacetamide amantadine-technetium (^{99m}Tc-NCAM) and 1-[*N*-[*N*-(2-mercaptoethyl)]-*N*-[2-[*N*-(2-mercaptoethyl) amino] ethyl] aminoethyl] amino-3,5-dimethyladmantane-technetium (^{99m}Tc-NHAM) are two memantine derivatives synthesized by Zhou et al. for imaging of *N*-methyl-D-aspartate receptors (NMDARs) (1).

NMDARs are oligomeric ligand-gated, voltage-dependent ion channels formed by the assembly of a NR1 subunit and various NR2 subunits. NR1 has eight subtypes and encodes the ion channel, while NR2 has four subtypes (NR2A, NR2B, NR2C and NR2D) and mediates the fast excitatory neurotransmission in combination with NR1 (2). Recently the NR3 subunit (two subtypes, NR3A and NR3B) has been shown to combine with NR1 and NR2 to functional heterotrimers (3). In terms of agonist requirement and channel operation, the three subunit families exhibit distinct properties; NR1 and NR3 require glycine as the agonist and have no binding site for glutamate, whereas NR2 is activated by glutamate. Opening of the ion channel is dependent on the voltage state of the cell membrane as well as the binding of dual ligands; glutamate binds to a site on the NR2 subunit, and glycine or D-serine binds to a modulatory site on the NR1 subunit (2, 4). NMDARs present in both intra- and extra-synaptic areas, with a higher density within the synapse. The extra-synaptic NMDARs have been proposed to mediate excitotoxicity, while intra-synaptic NMDARs appear to be neuroprotective (5).

NMDARs have been a drug target for >25 years for neurological and psychiatric indications (2). A large number of mediators have been developed by targeting different modulatory sites on the NMDARs. With better understanding of the NMDAR pathophysiology, the therapeutic concept with channel mediators has changed over the years. As reviewed by Koller and Urwyler, the most important recent strategies aiming for inhibition of NMDAR-mediated neurotransmission is to avoid full receptor blockade while allowing a low degree of normal receptor function for safety reasons (2). To this aim, approaches include blocking the channel with compounds of low affinity, antagonizing receptor activity with highly potent NR2B ligands, partial agonism at the glutamate or glycine binding site, and improvement of pharmacokinetic properties of well established, safe antagonists by deuteration (2, 6).

Molecular imaging with radiotracers has been used to map the changes in the density of NMDARs in specific regions of brain and to establish receptor occupancy of a drug that

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may facilitate drug development and dose optimization. Because of the considerable number of binding sites (especially the channel pore, the glycine site, and the NR2B subunit), the NMDAR complex offers opportunities to develop imaging probes by targeting different sites (1, 7, 8). In general, most probes either have unfavorable pharmacokinetics or exhibit less specific binding, failing to reflect the distribution and density of NMDARs in different regions of brain. Memantine is a partial NMDAR antagonist approved in the United States and Europe for the treatment of moderate to severe Alzheimer's disease. By acting at the channel pore, memantine preferentially blocks excessive NMDAR activity without disrupting its normal activity. Zhou et al. synthesized ^{99m}Tc-NCAM and ^{99m}Tc-NHAM with memantine as the lead compound (1). This chapter summarizes the data obtained with ^{99m}Tc-NCAM and ^{99m}Tc-NHAM.

Related Resource Links:

NMDAR-related agents in MICAD

Nucleotide and protein sequences of NMDAR

NMDAR-related articles in Online Mendelian Inheritance in Man

NMDAR-related clinical trials in ClinicalTrials.gov

Synthesis

[PubMed]

Zhou et al. described the synthesis of NCAM and NHAM in detail (1). ^{99m}Tc-Labeling was performed in a 1.5-ml centrifuge tube containing sodium glucoheptonate, ethylenediamine tetraacetic acid (disodium salt), and NHAM or NCAM, followed by adding [^{99m}Tc]$NaTcO_4$ (37 MBq (1 mCi)) and fresh SnF_2 solution. After the volume was adjusted to 1 ml with phosphate buffer, the mixture was heated for 45 min in boiling water and then cooled to room temperature. The radiochemical purity and entity of ^{99m}Tc-NCAM and ^{99m}Tc-NHAM were determined with high-performance liquid chromatography. The results showed that the radiochemical purity for both ^{99m}Tc-NCAM and ^{99m}Tc-NHAM was >95%, and >90% was retained after >6 h at room temperature. The retention times on chromatography were 11.27, 7.9, and 3.3 min for ^{99m}Tc-NCAM, ^{99m}Tc-NHAM, and [^{99m}Tc][TcO_4]$^-$, respectively. The radiochemical yield and specific activity of the probes were not reported.

Partition coefficients with 1-octanol and phosphate-buffered saline were 1.79 ± 0.02 for ^{99m}Tc-NCAM and 1.95 ± 0.05 for ^{99m}Tc-NHAM, indicating that both probes were lipophilic.

Plasma protein binding was determined after incubation of each probe with 200 μl plasma for 3 h at 37°C, showing that ^{99m}Tc-NCAM had slightly higher plasma protein binding than ^{99m}Tc-NHAM (Table 1).

NMDAR binding kinetics was analyzed with NMDAR proteins prepared from Sprague-Dawley rat brain tissue (Table 1). The equilibrium dissociation constant (K_d) was estimated with a saturation curve. The competitive binding of ^{99m}Tc-NCAM and ^{99m}Tc-NHAM with NMDAR was determined with two NMDAR-specific antagonists (ketamine and dizocilpine) and with a dopamine D_2 and $5HT_{1A}$ receptor partial agonist (aripiprazole). The results showed that the specific binding of both ^{99m}Tc-NCAM and ^{99m}Tc-NHAM to NMDARs was blocked by ketamine and dizocilpine but not by aripiprazole. The 50% inhibitory concentration (IC_{50}) was calculated according to the equation $K_i = IC_{50}/(1 + LT/K_d)$, where K_i is the inhibition constant of ketamine or dizocilpine and LT is ligand concentration. The IC_{50} and K_i values indicated that both ^{99m}Tc-NCAM and ^{99m}Tc-NHAM had a high affinity for NMDARs (Table 2).

Table 1: Plasma protein binding and NMDAR binding kinetics of probes.

Plasma protein binding	Dose	^{99m}Tc-NHAM (%)	^{99m}Tc-NCAM (%)
	3.7 MBq (0.1 mCi)	62.5	79.6
	0.74 MBq (0.02 mCi)	58.1	81.3
	0.148 MBq (0.004 mCi)	63.2	83.5
K_d (mmol/l)*	N/A	584.32 ± 87.27	701.21 ± 24.15
B_{max} (nmol/mg)*	N/A	267.05 ± 22.06	62.47 ± 10.23

*Measured with NMDAR proteins prepared from Sprague-Dawley rat brain tissue

Table 2: Binding affinities of ^{99m}Tc-NCAM and ^{99m}Tc-NHAM.

	Ketamine (6×10^{-1} to 6×10^{-11} mol/L)		Dizocilpine (6×10^{-1} to 6×10^{-11} mol/L)	
	K_i	IC_{50}	K_i	IC_{50}
^{99m}Tc-NCAM*	8.43×10^{-6}	1.22×10^{-5}	3.15×10^{-8}	5.08×10^{-8}
^{99m}Tc-NHAM*	9.28×10^{-7}	9.46×10^{-6}	2.43×10^{-8}	3.69×10^{-8}

*Measured with NMDAR proteins prepared from Sprague-Dawley rat brain tissue

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Cell uptake of ^{99m}Tc-NHAM and ^{99m}Tc-NCAM was measured after incubation of SH-SY5Y cell suspension (a human neuroblastoma cell line) (4.0×10^5 cells/ml) with 0.185 MBq (0.05 mCi) ^{99m}Tc-NHAM or ^{99m}Tc-NCAM for different times. The protective effect of the precursors (NCAM and NHAM) on glutamate (5 mmol/L)-induced SH-HY5Y cell injury was also evaluated (1).

The data showed that the SH-HY5Y cell uptake of both ^{99m}Tc-NHAM and ^{99m}Tc-NCAM was significantly higher than that of [^{99m}Tc]$NaTcO_4$, peaking at 2.7% and 3.8% of added radioactivity, respectively, at 60 min. The cell uptake of both complexes then decreased slowly with prolonged incubation. Uptake of [^{99m}Tc]$NaTcO_4$ by SH-HY5Y cells was only

1%, and this value did not change as incubation time was extended. The precursors (NCAM, NHAM, and memantine) exhibited a protective effect on glutamate (at high concentrations)-induced cell death, showing that the survival of cells treated with NHAM, NCAM, and memantine was 159%, 143%, and 151%, respectively, compared to that of cells treated with glutamate alone.

Animal Studies

Rodents

[PubMed]

Biodistribution of the ^{99m}Tc-NCAM and ^{99m}Tc-NHAM was studied in normal Kunming mice after tail vein injection of ~10 MBq (0.27 mCi) ^{99m}Tc-NCAM or ^{99m}Tc-NHAM (*n* = 5 mice/time point per agent) (1). Accumulation in organs and brain regions of interest was expressed as percentage of injected dose per gram tissue (% ID/g). The cortex (especially the parietal lobe) and the hippocampus were considered to be the target regions because of the highest density of NMDARs in these areas, and the cerebellum (CB) was considered to be the nontarget region because of the lowest density of NMDARs in CB.

Zhou et al. reported the detailed data of the probe uptake over time (see Figures 5 and 6 in reference (1)). Both ^{99m}Tc-NCAM and ^{99m}Tc-NHAM exhibited good penetration into the brain, reaching 1.23% and 1.47% ID/g, respectively, at 5 min after injection. For ^{99m}Tc-NCAM, the highest regional uptake was observed in the cortex and hippocampus, followed by the striatum, and the lowest uptake was found in the CB. The frontal lobe/CB and hippocampus/CB ratios peaked at ~2.98 and 2.52, respectively, at 60 min after injection. In contrast, ^{99m}Tc-NHAM showed random distribution throughout the brain without clear regional targeting. In the peripheral organs, high initial uptake was seen in the spleen and lungs for ^{99m}Tc-NCAM and in the liver and kidneys for ^{99m}Tc-NHAM, both of which then decreased quickly over time. No blocking studies were reported.

The blood kinetics of ^{99m}Tc-NCAM was analyzed after injection into six mice *via* tail vein (1). The pharmacokinetic equation was $C = 5.492e^{-0.3141t} + 0.275e^{-0.0042t}$. The absorption half-life of ^{99m}Tc-NCAM was 2.2 min.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

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