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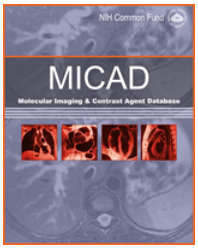

^{111}In-Diethylenetriaminepentaacetic acid-polyethylene glycol-annexin V

^{111}In-DTPA-PEG-Anx5

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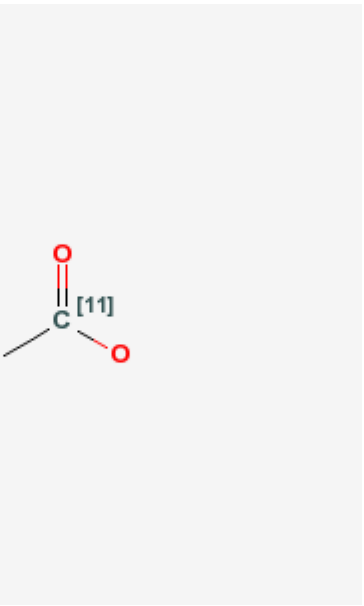
Chemical name:	^{111}In-Diethylenetriaminepentaacetic acid-polyethylene glycol -annexin V
Abbreviated name:	^{111}In-DTPA-PEG-Anx5
Synonym:	^{111}In-PEGylated annexin V, ^{111}In-DTP-Anx5
Agent Category:	Protein
Target:	Phosphatidylserine (PS)
Target Category:	Specific binding
Method of detection:	SPECT (Single Photon Emission Computed Tomography), gamma planar imaging
Source of signal:	^{111}In
Activation:	No
Studies:	• *In vitro* • Rodents • Non-primate non-rodent mammals • Humans



Click on protein, nucleotide (RefSeq), and gene for more information about Annexin V

Background

[PubMed]

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^{111}In-Diethylenetriaminepentaacetic acid-polyethylene glycol-annexin V (^{111}In-DTPA-PEG-Anx5) is a radiolabeled protein developed for single-photon emission computed tomography (SPECT) imaging of programmed cell death (apoptosis) (1, 2). ^{111}In is a gamma emitter with a physical $t_{½}$ of 2.8 days.

Apoptosis is an essential biological process that maintains homeostasis of tissues and organs in concert with proliferation, growth, and differentiation (3, 4). Cell death can occur by the process of necrosis or by the process of apoptosis. Apoptosis is a highly regulated, genetically controlled, noninflammatory process requiring ATP (5). The apoptotic process can be triggered either by a decrease in factors required to maintain the cell in good health or by an increase in factors that cause cells to die (6). The two known mechanisms of apoptosis are the death receptor (extrinsic) and the mitochondrial (intrinsic) pathways (7). Annexin V (Anx5) is one of the numerous members of the calcium- and phospholipid-binding superfamily of annexin proteins. The mature Anx5 molecule consists of 319 amino acids with a total molecular weight of 35.8 kDa. Most of the biological functions of Anx5 are based on its high affinity for negatively charged phospholipids in the presence of physiologic concentrations of calcium. Anx5 binds to membrane-bound phophatidylserine (PS) which is normally restricted to the inner leaflet of the plasma membrane lipid bilayer (6). PS is exposed on the surfaces of cells as they undergo apoptosis. This change in the membrane can be detected by the binding of Anx5 to the external PS (7-9). It is also possible that Anx5 binds to PS exposed on the cell surface in pathologic conditions associated with necrosis and vascular damage. As a result, PS-targeting is not entirely specific to apoptotic cell death. *In vivo* imaging using radiolabeled Anx5 does not discriminate between apoptotic and necrotic cell death (7).

Anx5 has been labeled with various radionuclides for SPECT and positron emission tomography imaging of apoptosis (7, 10). The detection of cell death *in vivo* has potential clinical value for possible diagnosis and assessment of therapeutic efficacy in transplanted organ rejections, AIDS, septic shock, cardiovascular diseases, neurodegenerative disorders, and cancer. Ke et al. (11) hypothesized that radiolabeled Anx5 with sufficiently long $t_{½}$ would allow tumor imaging with deeper penetration into the tumor mass and would capture a more complete picture of the dynamic process of the apoptotic cell generation and removal (7). Based on the ^{111}In-DTPA-PEG (^{111}In-diethylenetriaminepentaacetic acid-polyethylene glycol) conjugation method developed by Wen et al. (12), Ke et al. (11) and Wen et al. (13). Anx5 radiolabeled with ^{111}In for imaging of tumor apoptosis. The introduction of an uncharged, amphiphilic linear polymer (PEG) to protein molecules appeared to improve their biocompatibility, increase circulation $t_{½}$, decrease immunogenicity, increase resistance to proteolysis, and enhance solubility and stability (14). PEG modification also reportedly interfered with the recognition of foreign particles and proteins by the reticuloendothelial system and reduced the liver uptake.

Synthesis

[PubMed]

Wen et al. (14) discussed the preparation of a heterfunctional PEG with one end attached to the radiometal chelator, DTPA , and the other end attached to the protected thiol group, S-acetylthioacetate (S-ATA). This allowed the simultaneous introduction of both a metal-chelating agent and the PEG molecule to the protein molecule and avoided excessive modification of the protein molecule. Briefly, DTPA-PEG-NH_2 was prepared by adding t-Boc-NH-PEG-NH_2 to a stirred suspension of DTPA-dianhydride and triethylamine (TEA) in chloroform at room temperature for 2 h. The t-Boc protecting group was then removed by adding trifluoroacetic acid at room temperature for 4 h. DTPA-PEG-NH_2 was then reacted with S-ATA in chloroform at room temperature for 1 h to produce DTPA-PEG-ATA. DTPA-PEG-ATA can conjugate with any maleimide-activated proteins. Wen et al. (13) further improved this technique by introducing an NH_2-reactive isothiocyanate (SCN–) functional group in place of the ATA. This eliminated the required maleimide-activation of the protein molecules. In this improved method, *p*-NO_2-benzoyl-PEG-DTPA was produced by reacting DTPA-PEG-NH2 with p-nitrobenzoyl chloride and TEA in chloroform for 4 h with a yield of 90%. The resulting *p*-NO_2-benzoyl-PEG-DTPA was then mixed with 10% Pd/C in water under 35 psi H_2 overnight to produce *p*-NH_2-benzoyl-

PEG-DTPA. This compound was reacted with thiophosgene in chloroform at room temperature for 2 h to give *p*-SCN-benzoyl-PEG-DTPA with a yield of 95%. The molecular weight of *p*-SCN-benzoyl-PEG-DTPA was estimated to be 81-114 kDa and each PEGylated Anx molecule contained approximately 10-16 PEG molecules.

The radiosynthesis of ^{111}In-PEG-Anx5 involved adding 0.09 μmol *p*-SCN-benzoyl-PEG-DTPA to 0.006 μmol Anx5 (15:1 ratio of DTPA-PEG to Anx5) in 0.1M sodium phosphate buffer and stirred at 4°C overnight (13). After purification by anionic ion exchange column, DTPA-PEG-Anx5 was incubated with $^{111}InCl_3$ in 20 mM Tris buffer for 15 min. ^{111}In-Anx5 was purified by gel filtration chromatography to remove unreacted ^{111}In. Specific activity was 296-370 kBq (8-10 μCi)/0.028 nmole (based on 35.8 kDa molecular weight) and the radiochemical yield was 91%. Successful preparations of ^{111}In-PEG-Anx5 in the 30:1 and 60:1 ratios of DTPA-PEG to Anx5 were also achieved.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Wen et al. (13) reported that ^{111}In-PEG-Anx5 was relatively stable in 20% fetal bovine serum at 37°C. Using high-performance liquid chromatography analysis, the percentages of intact conjugate were 94%, 69%, and 61% at 1 day, 2 days, and 5 days after incubation. Cell binding assays were carried out in human leukemia HL60 cells and human B-cell lymphoma Raji cells. These cells were treated with Ara-C at 1.0 μM for 22 h to induce apoptosis. Flow cytometry analysis using fluorescent Anx5-FITC as a probe showed a good correlation between cell-associated radioactivity of the 30:1 ^{111}In-PEG-Anx5 preparation and the percentage of induced apoptotic cells.

Animal Studies

Rodents

[PubMed]

Wen et al (13) studied the pharmacokinetics and biodistribution of ^{111}In-PEG-Anx5 (15:1 preparation) in nude mice. Each mouse received a dose of 2.59 MBq (70 μC i) per 7 μg. The activity-time profile of ^{111}In-PEG-Anx5 fitted well into a two-compartment model. The rate of clearance and volume of distribution were 0.01 ml/h and 0.2 ml, respectively. The $t_{½\alpha}$ and $t_{½\beta}$ values were 4.90 h and 26.3 h, respectively. In comparison, the ^{111}In-DTPA-unPEGylated Anx5 had the clearance rate, volume of distribution, and $t_{½\alpha}$ and $t_{½\beta}$ values of 0.4 ml/h, 6.1 ml, 0.07 h, and 17.4 h, respectively. Biodistribution studies at 120 h after the injection showed that ^{111}In-PEG-Anx5 had significantly reduced localization in the kidney and increased activity in the spleen compared with ^{111}In-DTPA-unPEGylated Anx5. The percentages of injected dose per gram of tissue (%ID/g, $n = 3$) for blood, liver, kidney, spleen, and muscle were 0.36 ± 0.05%, 8.37 ± 2.76%, 22.35 ± 4.74%, 6.75 ± 0.44%, and 0.75 ± 0.04%, respectively.

Ke et al. (11) evaluated ^{111}In-PEG-Anx5 for tumor imaging in a tumor model of poly(L-glutamic acid)-paclitaxel (a chemotherapeutic agent) and anti-EGR C-225 monoclonal antibody (MAb)-induced apoptosis in mammary MDA-MB-468 tumor-bearing nude mice. Each mouse received a dose of 1.48 MBq (40 μCi) per 4 μg. The radioactivity levels (%ID/g, $n = 3$) at 48 h after injection in paclitaxel-treated mice (treatment of 4 days) were 4.56 ± 0.64 (blood), 49.24 ± 7.70 (liver), 27.22 ± 4.36 (kidney), 1.21 ± 0.25 (muscle), and 15.99 ± 4.27 (tumor). The tumor radioactivity was 8.13 ± 0.28% ID/g in untreated mice. In comparison, the tumor radioactivity levels of ^{111}In-DTPA-unPEGylated Anx5 were 0.62 ± 0.14, 1.23 ± 0.67, for untreated and treated mice, respectively. Histologic analysis of tumor tissues showed a significant correlation ($r = 0.87$ $P = 0.02$) between the tumor radioactivity level of ^{111}In-PEG-Anx5 and the apoptotic index (percentage of apoptotic nuclei induced by drug treatment). Autoradiograms of tumor tissues showed that the radioactivity of ^{111}In-PEG-Anx5 was localized in

the central zone as well as in the periphery. In comparison, the radioactivity of ^{111}In-DTPA-unPEGylated Anx5 was mainly confined to the periphery. Intense TUNEL (DNA fragmentation) staining corresponded to hot spots from ^{111}In-PEG-Anx5. Gamma imaging was able to clearly visualize the changes in tumor radioactivity levels of ^{111}In-PEG-Anx5 in treated mice. ^{111}In-DTPA-unPEGylated Anx5 did not reveal the tumors in either treated or untreated mice.

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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