^{123}I-Annexin V

^{123}I-Anx5

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Chemical name:	^{123}I-Annexin V	
Abbreviated name:	^{123}I-Anx5	
Synonym:	^{123}I-rh-Anx5, ^{123}I-labeled human recombinant annexin V, ^{123}I-rh-annexin V, ^{125}I-Anx A5	
Agent Category:	Protein	
Target:	Phosphatidylserine (PS)	
Target Category:	Receptor binding	
Method of detection:	Single-Photon Emission Computed Tomography (SPECT), gamma planar imaging	
Source of signal:	^{123}I	
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]

^{123}I-Annexin V (^{123}I-Anx5) is a radioiodinated protein molecule developed for single-photon emission computed tomography (SPECT) imaging of programmed cell death (apoptosis) (1, 2). ^{123}I is a gamma emitter with a physical $t_{½}$ of 13.2 h.

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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 phospholipids-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.

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. Anx5 was originally obtained from human tissue. Currently, recombinant human Anx5 (rh-Anx5) can be produced in high yields with excellent purity by bioengineering techniques. Direct radioiodination of Anx5 proteins has been performed by means of electrophilic aromatic substitution of the molecule's tyrosine residues (1). Alternatively, Anx5 has also been radioiodinated indirectly using prelabeled reagents (11). Because of their structural similarity to thyroxine, the directly iodinated proteins are more likely to exhibit *in vivo* deiodination than the indirectly iodinated proteins (12).

Synthesis

[PubMed]

Tait et al. (13) prepared ^{123}I-Anx5 using Anx5 purified from human placenta with ≥99% purity. Radiolabeling was done by the direct radioiodination method using 1,3,4,6-tetrachloro-3,6-diphenyl-glycoluril (Iodo-Gen). Anx5 protein was combined with Na^{123}I in a Tris buffer (pH 8.0) in a glass tube coated with Iodo-Gen. After incubation for 15 min at room temperature, the reaction was quenched with sodium metasulfite, and excess Na^{123}I was removed by gel filtration. The specific activity was 56,500 cpm/ng, and the labeling efficiency was 99.2–99.9%. Using the same radioiodination method, ^{125}I-Anx5 (specific activity = 7480-14100 cpm/ng) fully retained its phospholipids- and human platelets-binding.

Lahorte et al. (1) reported the synthesis of ^{123}I-rh-Anx5 (recombinant human Anx5) by the Iodo-Gen method. Briefly, Iodo-Gen–coated Eppendorf vials were prepared by evaporation of an Iodo-Gen solution at a concentraton of 60 µg/200 µl. The best result was obtained with 5 µg rh-Anx5 in 40 µl potassium phosphate (KH_2PO_4) buffer (0.1 M, pH 8) at ambient temperature (18°C) for 10 min. The final product was purified by high-performance liquid chromatography. The radiochemical yield was 70% with >95% radiochemical purity. The specific activity was 7.4-92.5 MBq/µg.(0.2–2.5 mCi/µg).

To prepare clinical grade ^{123}I-rh-Anx5 (recombinant human Anx5), Lahorte et al. (2) used a modified IodoGen method. In this method, 250 µg/ml of freshly prepared IodoGen in 2 ml was evaporated under a gentle stream of nitrogen in a 10-ml reaction vial. The vial was washed twice with 2 ml 0.05 M phosphate-buffered saline (PBS) before use. Then 1 mg of rh-Anx5 in 0.05 M PBS (pH 7.4) was mixed with 370 MBq (10 mCi) ^{123}I in 0.5 M PBS (volume twice that of ^{123}I) and incubated with gentle stirring at room temperature for 20 min. The reaction was stopped by purification on a PD10(G25) Sephadex column using 0.25% human serum albumin PBS as eluent. The final product was filtered by a 0.22-µm sterile filter. The radiochemical yield was 87.0 ± 6.5% with >98% radiochemical purity. The specific activity of ^{123}I-rh-Anx5 was 13,400 MBq/µmol (362 mCi/µmol).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Lahorte et al. (1) used human platelets to determine whether ^{123}I-rh-Anx5 maintained its biological activity. Isolated human platelets (108 cells/ml) were incubated with ^{123}I-rh-Anx5 at ambient temperature for 30 min in the presence of various Ca^{2+} concentrations. The binding of ^{123}I-rh-Anx5 to human platelets showed a Ca^{2+} -dependent response that reached a plateau of 67% at 20 mM Ca^{2+}.

Animal Studies

Rodents

[PubMed]

Lahorte et al. (2) studied the acute toxicity of rh-Anx5 in mice. All mice showed few signs of adverse effects with 0.335, 1.342 or 2.685 mg/kg of decayed, clinical-grade ^{123}I-rh-Anx5 in a single i.v. dose.

The biodistribution of ^{123}I-rh-Anx5 was studied in mice (2). Each mouse received 74.0 ± 3.7 kBq (2 ± 0.1 µCi) of ^{123}I-rh-Anx5. The radiolabeled protein was rapidly cleared from the blood after a bi-exponential clearance characterized by a rapid phase ($t_{½\alpha}$ = 3.87 ± 0.90 min) and a slow phase ($t_{½\beta}$ = 4.13 ± 2.04 h). The major organ radioactivity [percent injected dose per gram (%ID/g)] levels (n = 3) at 60 min were 35.37 ± 13.70 (stomach), 21.29 ± 9.97 (kidneys), 12.62 ± 4.27 (bladder), 8.17 ± 2.93 (liver), 7.27 ± 3.66 (spleen), 6.18 ± 1.89 (lungs), 5.91 ± 1.00 (blood), and 0.38 ± 0.09 (brain), At 6 h, the

radioactivity levels (%ID/g) were 8.05 ± 0.69 (stomach), 7.71 ± 3.93 (kidneys), 12.62 ± 0.00 (bladder), 4.22 ± 2.01 (liver), 2.38 ± 0.95 (spleen), 2.28 ± 2.41 (lungs), 3.66 ± 3.79 (blood), and 0.34 ± 0.30 (brain), ^{123}I-rh-Anx5 was mainly cleared by the kidneys with 86.6% ID of total radioactivity excreted at 48 h.

Lahorte et al. (14) found significantly increased ^{123}I-Anx5 radioactivity localization in myocardial apoptosis induced in a rat model of septic shock. Cornelissen et al. (15) also reported that ^{123}I-Anx5 could be used to monitor apoptosis induced by farnesyltransferase inhibitors in athymic mice bearing LoVo- human colon adenocarcinoma bearing athymic mice.

Other Non-Primate Mammals

[PubMed]

Tait et al. (13) evaluated ^{123}I-Anx5 (obtained from human placenta) as a platelet-directed thrombus targeting agent in a swine model. Fully occluded arterial thrombi were induced in Yorkshire swine. Each swine received 48.1–506.9 MBq (1.3–13.7 mCi) ^{123}I-Anx5 (50–500 μg) by i.v. administration. The blood clearance followed a biexponential clearance pattern with an initial rapid phase of $t_{½}$ = 6.4 min and a slow phase with a $t_{½}$ = 71 min. The combined (60 and 120 min, n = 9) showed that the radioactivity levels (%ID/g) in major organs were 0.1652 ± 0.0511 (kidney), 0.0403 ± 0.0734 (unblocked thyroid), 0.0389 ± 0.0286 (spleen), 0.0169 ± 0.0030 (lung), 0.0072 ± 0.0020 (liver), and 0.0071 ± 0.0088 (carotid artery). The radioactivity levels in the carotid thrombus and femoral thrombus were 0.0482 ± 0.0362 and 0.0466 ± 0.0432, respectively. In comparison, nonspecifically radiolabeled globular protein of comparable size (ovalbumin) had a thrombus radioactivity level of only 0.0059 ± 0.0017% ID/g.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

Lahorte et al. (2) used SPECT imaging to study the biodistribution and dosimetry of ^{123}I-rh-Anx5 in six volunteers. Each volunteer was injected with an i.v. dose of 345 ± 38 MBq (9.3 ± 1.03 mCi) ^{123}I-rh-Anx5 (940 μg).The 1-6 h whole-body images showed that most of the radioactivity was distributed in the kidneys, bladder, liver, thyroid (blocked), and stomach. The pattern was consistent with urinary excretion and hepatic uptake of the radiolabeled protein and trapping of free ^{123}I– by the stomach. Radioactivity levels in the lungs and brain were relatively low. Using the MIRDOSE program, non-decay-corrected geometric mean time-activity curves of major organs were generated for radiation-absorbed dose estimates. The highest radiation-absorbed dose estimate was found in the

blocked thyroid [0.1157 ± 0.0621 mGy/MBq (0.31 ± 0.17 mrad/mCi)] despite pretreatment with lugol. The dose (mGy/MBq) to the kidney was 0.03027 ± 0.00797 (0.082 ± 0.022 mrad/mCi), and the dose to the stomach was 0.00779 ± 0.00207 (0.021 ± 0.0056 mrad/mCi;). The estimated mean effective dose for an adult was 0.0201 ± 0.0100 mSv/MBq (0.057 ± 0.027 mrem/mCi).

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