^{124}I-Annexin V

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Chemical name:	^{124}I-Annexin V	
Abbreviated name:	^{124}I-Anx5	
Synonym:	^{124}I-MBP-Annexin V, ^{124}I-Annexin A5, ^{124}I-Anx A5, [^{124}I]*m*-IBA-Anx5, [^{124}I]4IB-Anx5, [^{124}I]SIB-Anx5.	
Agent Category:	Protein	
Target:	Phosphatidylserine (PS)	
Target Category:	Receptor binding	
Method of detection:	Positron emission tomography (PET)	
Source of signal:	^{124}I	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about Annexin V

Background

[PubMed]

^{124}I-Annexin V (^{124}I-Anx5) is a radioiodinated protein molecule developed for positron emission tomography (PET) imaging of programmed cell death (apoptosis) (1, 2). ^{124}I is a positron emitter with a relatively long physical $t_{½}$ of 4.2 d.

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, and ATP-requiring process (5). The apoptotic process can be triggered either by a decrease in factors required to maintain the

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cell in good health or by an increase in factors that causes 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 annexin are based on its high affinity for negatively charged phospholipids in the presence of physiological 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 (2, 7, 8). It is also possible that Anx5 may bind to PS exposed on the cell surface in pathological conditions associated with necrosis and vascular damage.

Anx5 has been labeled with various radionuclides for single photon emission computed tomography imaging and PET imaging of apoptosis (7, 9). The long physical $t_{½}$ of ^{124}I allows for the possibilities for monitoring and quantification of long-term biological processes involving Anx5 (7). The detection and quantification of cell death *in vivo* have potential clinical value for diagnosis and assessment of therapeutic efficacy in transplanted organ rejections, autoimmune deficiency syndrome, 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 (10). Alternatively, Anx5 has also been radioiodinated indirectly by use of prelabeled reagents (1). Because of their structural similarity to thyroxine, the directly iodinated proteins are more likely subject to *in vivo* deiodination than the indirectly iodinated proteins (11).

Synthesis

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Glaser et al. (12) compared two ^{124}I radioiodination methods to label annexin V (wild-type and polyhistidine-labeled recombinant Anx5). Anx5 was radioiodinated directly by the chloramine-T (CAT) method and indirectly by *N*-succinimidyl-3-[^{124}I]iodobenzoate ([^{124}I]*m*-SIB). [^{124}I]Iodide was produced by the ^{124}Te(p,n)^{124}I reaction using 12.5 MeV proton irradiation. The specific activity of sodium [^{124}I]iodide obtained was 27.6 GBq/μmol (0.75 Ci/μmol). In the CAT method, sodium [^{124}I]iodide was mixed with 1 M hydrochloric acid and sodium phosphate buffer. Anx5 (2.78 x 10^{-10} mol) and CAT (5 μg) in sodium phosphate buffer were added and incubated for 5 min at pH 6.5 at room temperature. A solution of *m*-tyrosine solution was then added for another 5 min of incubation. Labeling efficiency was nearly 80%. After gel filtration purification, the radiochemical yield of ^{124}I-Anx5 was 22.3 ± 2.6% (n = 3) with a radiochemical purity of 97.7 ± 1.0%. The specific activity was reported to be 14.5 GBq/μmol (0.39 Ci/μmol). In the indirect method, *N*-acylation of Anx5 lysine amino acid residues was used to produce a

[^{124}I]*m*-iodobenzoate conjugation product. [^{124}I]*m*-SIB was first prepared from *N*-succinimidyl-3-(tri-methylstannyl) benzoate and then radiolabeled by IodoGen with a radiochemical yield of 39.3 ± 8.4% (n = 3) and a radiochemical purity of 98.7 ± 0.4% (13). Anx5 was incubated with [^{124}I]*m*-SIB in borate buffer for 1 h at pH 8.5 at 0 °C (14). [^{124}I]*m*-IBA-Anx5 was then purified by fast protein liquid chromatography or gel filtration with a radiochemical yield of 14% or 25%, respectively. The radiochemical purity was 96.7 ± 2.1% (n = 3), and the specific radioactivity was 1.61 GBq/μmol (0.044 Ci/μmol).

Keen et al. (15) and Dekker et al. (1) reported the direct radioiodination of placental Anx5 and recombinant maltose-binding protein/Anx5 chimera (MBP-Anx5) by 1,3,4,6-tetrachloro-3α,6α-diphenylglycouril (IodoGen) coated on microfuge tubes. ^{124}I was produced by the ^{126}Te(p,3n)^{124}I reaction with a radionuclide purity of 95-98%. Briefly, Anx5 and [^{124}I]NaI in binding buffer were dispensed into the IodoGen-coated microfuge tubes and incubated at room temperature for 20 min. The final product of ^{124}I-Anx5 was purified by gel filtration. The overall labeling efficiency was 20-70%. The authors suggested that the wide variations in efficiency were due to the presence of iodate in the total trapped ^{124}I activity. Dekker et al. (16) also conducted indirect iodination of Anx5 by using [^{124}I]*N*-hydroxysuccinimidyl-4-iodobenzoate. This prelabeled reagent was prepared by iododestannylation of *N*-hydroxysuccinimidyl-4-(tributylstannyl)benzoate. [^{124}I]*N*-hydroxysuccinimidyl-4-iodobenzoate was produced from radiolabeling *N*-hydroxysuccinimidyl-4-(tributylstannyl)benzoate with ^{124}I in the presence of N-chlorosuccinimide and 5% acetic acid. Finally, Anx5 in borate buffer (pH 8.5) was incubated with this ^{124}I-labeled reagent on ice for 2 h or at room temperature for 15 min to produce the [^{124}I]41B-Anx5. The radiochemical yield was 20-30% after gel filtration purification.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The *in vitro* storage stability of ^{124}I-Anx5 (CAT labeling) and [^{124}I]*m*-IBA-Anx5 ([^{124}I]*m*-SIB labeling) was studied (12). Both preparations of <5 MBq (0.14 mCi) radioactivity appeared to be stable at 4 °C for about 4 d without any noticeable deiodination. The *in vitro* biological activity of these two preparations was studied in human leukemia cells HL60. These cells were treated with the topoisomerase inhibitor camptothecin (CPT; 5 μM in dimethyl sulfoxide (DMSO)), to induce apoptosis. [^{124}I]*m*-IBA-Anx5 showed 21% (P<0.05) higher binding with treated cells than with untreated cells. These bindings were decreased by 68% and 60% when pre-incubated with a 100-fold excess of unlabeled Anx5 for treated and untreated cells, respectively. ^{124}I-Anx5 showed only 17% (P<0.05 in only one out of three studies) higher binding in treated cells because of possible reduced biological activity. When HL60 cells were heated at 50°C for 1 h to force the PS exposure, [^{124}I]*m*-IBA-Anx5 had a 56% increase in binding to heated cells (14). Lahorte et al. (7) suggested that the DMSO solvent used for dissolving CPT was capable of inducing apoptosis in control cells. These preparations were also studied in

drug-induced apoptosis in tumors by using RIF-1 fibrosarcoma cells treated with 5-fluorouracil (5-FU). In these clonogenic survival studies, ^{125}I was used instead of ^{124}I. Both ^{124}I-Anx5 and [^{124}I]*m*-IBA-Anx5 showed higher binding in treated cells compared to untreated cells (17). There was also a large decrease in binding after pretreatment with unlabeled Anx5.

Dekker et al. (16) tested the *in vitro* biological activity of ^{124}I-Anx5 (IodoGen) and [^{124}I]41B-Anx5 ([^{124}I]*N*-hydroxysuccinimidyl-4-iodobenzoate labeling) in CPT-treated Jurkat cells. [^{124}I]4IB-Anx5 exhibited approximately four times greater binding to treated cells than that of untreated cells. Keen et al. (15) reported that ^{124}I-Anx5 showed 7.5 times greater binding in treated cells. In another study, flow cytometry confirmed that the CPT-treated cells contained a large apoptotic population. Dekker et al. (1) also found significantly greater binding to treated Jurkat cells for [^{124}I]-MBP-Anx5 (recombinant maltose-binding protein/annexin V chimera, IodoGen), there was an eight-fold increase in binding to treated cells compared to untreated cells (18).

Dekker et al. (16) used PS-enzyme-linked immunosorbent assay (ELISA) to study the *in vitro* activity of radioiodinated annexin V. Good linear correlations were found for both [^{124}I]41B-Anx5and [^{124}I]-Anx5 (IodoGen) with the gradient of [^{124}I]4IB-Anx5 being steeper indicating that its PS-binding rate was faster. Dekker et al. (1) used ^{125}I labeling to show that both radioiodinated (IodoGen) MBP-Anx5 and placental Anx5 bound to PS in the ELISA.

Animal Studies

Rodents

[PubMed]

Collingridge et al. (17) used [^{125}I]*m*-IBA-Anx5 (polyhistidine-labeled recombinant Anx5) for 2–120 min distribution studies in RIF-1–bearing mice. The tissue radioactivity uptake was rapid and the washout was slower, with the highest radioactivity levels in all tissues found at 2 min. The highest radioactivity was observed in kidneys and urine. For tumor uptake, the 60-min tumor/blood ratios of [^{125}I]*m*-IBA-Anx5 radioactivity were 0.84 ± 0.16 (n = 3-10), 0.36 ± 0.05, and 0 for 5–FU–treated (165 mg/kg), untreated, and unlabeled Anx5 pretreatment, respectively. In comparison, the 60-min tumor/blood uptake ratio values for ^{125}I-Anx5(CAT) were 0.99 ± 0.05, 0.94 ± 0.17, and 0.55 ± 0.03, respectively. PET imaging with [^{124}I]m-IBA-Anx5 (370 kBq (10 μCi)) and ^{124}I-Anx5 (1480 kBq (40 μCi)) at 30–60 min after injection showed high kidney and bladder radioactivity in 5–FU–treated mice. There was thyroid (unblocked) activity with the ^{124}I-Anx5 mouse. The tumors were not visualized in either case.

Keen et al. (15) and Dekker et al. (16) studied the biodistribution of ^{124}I-Anx5 (IodoGen) and [^{124}I]4IB-Anx5 ([^{124}I]*N*-hydroxysuccinimidyl-4-iodobenzoate labeling) in a mouse model of anti-Fas antibody–induced hepatic apoptosis. Each mouse received approximately 0.1 MBq (2.7 μCi) of the radiolabeled protein by i.v. injection and was

euthanized 2 h after injection. The major organ radioactivity levels (percent injected dose/g (% ID/g); $n = 4$) for ^{124}I-Anx5 in anti-Fas–treated mice were 57 ± 9 (liver), 40 ± 26 (urine), 11 ± 5.3 (stomach content), 6.7 ± 1.2 (blocked thyroid), 4.5 ± 0.2 (kidney), and 5.7 ± 1.0 (blood). The radioactivity of ^{124}I-Anx5 was rapidly cleared from the blood with a $t_{½}$<12 min. High-performance liquid chromatography (HPLC) of urine radioactivity at 2 h showed that it was all in the form of unbound ^{124}I–. In the liver, at 2 h after administration 70% of the radioactivity was ^{124}I-Anx5, 15% was a high molecular weight metabolite, and 12% was radioactive iodide at 2 h after administration. In PET imaging of the treated mouse, there was a very high liver radioactivity which was 17 times greater than that in the untreated mouse. There was a strong correlation between the apoptotic density (AD) in the liver determined by histology and the biodistribution/PET imaging data. For [^{124}I]4IB-Anx5, the organ radioactivity levels (%ID/g) were 25 ± 7.7 (liver), 127 ± 118 (urine), 2.0 ± 2.8 (stomach content), 7.6 ± 2.3 (blocked thyroid), 20 ± 10 (kidney), and 4.1 ± 2.6 (blood) (16). There was a significantly higher hepatic accumulation of both preparations in treated mice with hepatic apoptosis than seen in untreated mice. The liver radioactivity levels in untreated mice were 2.1 ± 1.0% ID/g and 4.1 ± 3.0% ID/g for ^{124}I-Anx5 (IodoGen) and [^{124}I]4IB-Anx5, respectively. These uptake values correlated well with *ex vivo* histology studies of AD in the liver. PET imaging of the treated mice injected with [^{124}I]4IB-Anx5 revealed high radioactivity in the liver. HPLC and PS–binding assays of liver samples from the imaging animals positively identified [^{124}I]4IB-Anx5. HPLC analysis of kidney and urine samples indicated that the predominant form of radioactivity was a low molecular weight metabolite.

Dekker et al. (1) reported the biodistribution (0.1–1 MBq (2.7–27 μCi)) and PET imaging (2–4 MBq (54–108 μCi)) studies of ^{124}I-MBP-Anx5 (maltose-binding protein/annexin V chimera; IodoGen) in anti-FAS–treated mice. In untreated mice, there was negligible uptake in all tissues except the thyroid (blocked), urine, and stomach content. Liver radioactivity ($n = 4$) of ^{125}I-MBP-Anx5 was nine times greater in treated mice (20.4 ± 5.5% ID/g) than in untreated mice (2.3 ± 0.3% ID/g). This was confirmed by ^{124}I-MBP-Anx5 PET imaging. Immunohistochemistry showed good correlation of AD and radioactivity distribution in the liver.

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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