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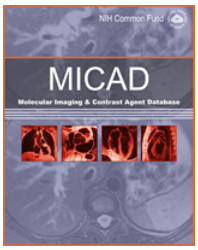

[^{74}As]-Labeled monoclonal antibody against anionic phospholipids

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Chemical name:	[^{74}As]-Labeled monoclonal antibody against anionic phospholipids	
Abbreviated name:	[^{74}As]Bavituximab	
Synonym:	Monoclonal antibody 3G4	
Agent Category:	Monoclonal antibody	
Target:	Anionic phospholipids	
Target Category:	Antibody-ligand binding	
Method of detection:	Positron emission tomography (PET)	
Source of Signal/Contrast:	^{74}As	
Activation:	No	
Studies:	• *In vitro* • Rodents	Structure not available.

Background

[PubMed]

Phospholipids such as phosphatidylserine (PS), phosphatidylinositol, and phosphatidic acid are the most abundant anionic molecules found on the internal surface of the cellular plasma membrane, including that of the vascular tissue (1). Maintenance of the membrane phospholipid topology is an energy-dependent process that involves the ATP-dependent activity of enzymes such as the aminophospholipid translocases, floppases, and scramblases (2, 3). The activity of these enzymes is altered under a variety of pathological conditions and results in the redistribution of membrane phospholipids because PS is exposed to the outer surface of the cell membrane (4). A variety of stress conditions (such as acidity, hypoxia, etc.) and molecules (cytokines, thrombin, oxygen free radicals, etc.), including Ca^{2+} fluxes, have been shown to induce PS exposure on the outer surface of the vascular endothelial cells under *in vitro* conditions, suggesting that the microenvironment in tumors may be responsible for the altered phospholipid distribution in these lesions (5, 6). Using a monoclonal antibody (mAb) directed toward anionic phospholipids, Ran et al. showed that these charged lipids were also exposed in the solid tumor blood vessel endothelium of mice and suggested that PS could be a specific marker for tumor vasculature

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(5, 7). Ran et al. subsequently developed another mAb, designated 3G4 or bavituximab, against anionic phospholipids that binds to the target in the presence of serum or the β2-glycoprotein I (8). Bavituximab was shown to destroy tumor blood vessels and slow the growth of tumors in mice.

Advancements in nuclear medicine have made it possible to label antibodies with radionuclides for use as tracers to detect clinically relevant targets, particularly for the treatment of cancers. The major limitation of using labeled antibodies for the detection of targets is that these macromolecules have a prolonged half-life ($t_{1/2}$; i.e. they stay in blood circulation for a long time) and may take a long time to reach optimal concentrations at the target for imaging. Positron emission tomography (PET) is among the several techniques used in nuclear medicine for imaging with nuclides such as copper (^{64}Cu), yttrium (^{86}Y), or iodine (^{124}I), but these radionuclides either have a very short half-life (^{64}Cu: $t_{1/2}$ = 12.7 h; ^{86}Y; $t_{1/2}$ = 17.8 h) or undergo metabolic dehalogenation (^{124}I: $t_{1/2}$ = 4.18 d) from the antibodies; therefore, it is not always suitable to use these nuclides for PET imaging. Although an isotope of arsenic (^{74}As: $t_{1/2}$ = 17.8 d) was used for PET imaging of the brain approximately four decades ago (9), the difficulty of isotope production and isolation of the nuclide, along with the lack of a process for the derivatization of the isotope, were major limitations for the popularization of this radioisotope as a PET agent. Advancement in the isolation and use of arsenic isotopes for the labeling of biomolecules has renewed the interest of investigators with regard to the use of this agent for PET imaging (10).

Jennewein et al. labeled bavituximab with ^{74}As and evaluated its use for the imaging of solid tumors in rats (10). The United States Food and Drug Administration has approved bavituximab for use in clinical trials to treat advanced tumor malignancies and hepatitis C.

Synthesis

[PubMed]

Ran et al. described the production of bavituximab (8). Briefly, BALB/c mice were immunized with bEnd.3 endothelial cells treated with hydrogen peroxide (H_2O_2) (2 h at 37°C). The H_2O_2 treatment causes a translocation of the anionic phospholipids to the outer surface of the cell membrane. The mice were injected intraperitoneally with cells five times at three weekly intervals, and hybridomas were obtained by fusing splenocytes from the immunized mice with myeloma partner P3 X 63AG.653 cells. The hybridoma growth medium supernatants were screened for reactivity with PS and other anionic phospholipids immobilized on plastic plates. Bavituximab binds PS through exogenously supplemented β2-glycoprotein I, which acts as a co-factor for the binding. The investigators did not provide any information regarding purification of the mAb before labeling or its use for imaging (8, 10).

The antibodies were modified with *N*-succinimidyl *S*-acetylthioacetate (SATA), and the sulfhydryl groups on the mAb were deprotected with hydroxylamine before labeling (10). The thiolated mAb in saline was combined with [^{74}As]arsenic chloride ([^{74}As]Cl_3) solution at 37°C for 30 min, and the quality of the labeled antibody was checked with high-performance liquid chromatography (HPLC) on a Bio-select Sec 250-5 column. Stability of radioarsenic-labeled mAb was investigated by incubating the labeled mAb in undiluted fetal bovine serum and analyzing the mixture with HPLC at several time points up to 72 h.

An average of 3.5 thiol groups per molecule of the antibody was added during the SATA modification. The specific activity of the labeled mAb was reported to be >100 GBq/μmol (>2.7 Ci/μmol), and the incubation in serum up to 72 h did not lead to any loss of the isotope from the labeled antibody or degradation of the mAb (10). The radiochemical purity and yield of the labeled mAb was not reported by the investigators (10).

For some work, the isotype-matched control mAb [^{74}As]retuximab was used, but the procedure used to prepare the labeled retuximab and its specific activity, radiochemical purity, and radiochemical yield were not reported in the publication (10).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

No publications are currently available.

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{74}As]bavituximab was studied in rats bearing Dunning prostate R3327-AT1 tumors in the left thigh (10). Animals (n = 3 for each agent) were injected intravenously through the tail vein either with [^{74}As]bavituximab or [^{74}As]rituximab (as a control), and the levels of accumulated radioactivity in the various organs were determined 48 and 72 h after administration. The tumor/normal tissue and the tumor/liver ratios at 72 h were 470 and 22, respectively. The tumor's accumulated bavituximab/rituximab ratios were 28 and 52 at 48 and 72 h, respectively. On imaging at 24 h after administration of the labeled mAb, the tumors were not clearly distinguishable because of the high body background, but by 72 h the tumors were clearly apparent because the labeled mAb had cleared from blood circulation. Small animal PET showed that [^{74}As]bavituximab was localized mainly on the tumor periphery at 48 h with a heterogeneous localization in the central region.

Frozen sections from the tumors and normal tissue were stained to detect human immunoglobulins bound to the cells (10), and the sections were also counterstained with anti-rat CD31 antibodies to detect the vascular endothelium. Images obtained from the two stainings were merged, and a coincidence was observed between the bavituximab and the CD31 antibody. The specificity of bavituximab was confirmed because tumors in rats injected with rituximab did not show any staining in the endothelium. Also, vascular endothelium staining was not observed in the animals' normal tissues, such as the heart, lungs, liver, pancreas, spleen, brain, and testis. From these studies the investigators concluded that [^{74}As]bavituximab was a promising agent for the imaging and detection of tumors.

Other Non-Primate Mammals

[PubMed]

No publications are currently available.

Non-Human Primates

[PubMed]

No publications are currently available.

Human Studies

[PubMed]

No publications are currently available.

Supplemental Information

[Disclaimers]

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