Anti-malondialdehyde-modified low-density lipoprotein MDA2 monoclonal antibody–labeled lipid-coated superparamagnetic iron oxide nanoparticles

MDA2 LSPIOs

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Created: May 15, 2012; Updated: August 2, 2012.

Chemical name:	Anti-malondialdehyde-modified low-density lipoprotein MDA2 monoclonal antibody–labeled lipid-coated superparamagnetic iron oxide nanoparticles	
Abbreviated name:	MDA2 LSPIOs	
Synonym:		
Agent category:	Antibody	
Target:	Malondialdehyde-modified low-density lipoprotein (MDA-LDL)	
Target category:	Antigen	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Iron oxide	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about apolipoprotein B.

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NLM Citation: Leung K. Anti-malondialdehyde-modified low-density lipoprotein MDA2 monoclonal antibody–labeled lipid-coated superparamagnetic iron oxide nanoparticles. 2012 May 15 [Updated 2012 Aug 2]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

Background

[PubMed]

Magnetic resonance imaging (MRI) maps information about tissues spatially and functionally. Protons (hydrogen nuclei) are widely used in imaging because of their abundance in water molecules. Water comprises ~80% of most soft tissue. The contrast of proton MRI depends primarily on the density of the nucleus (proton spins), the relaxation times of the nuclear magnetization (T1, longitudinal; T2, transverse), the magnetic environment of the tissues, and the blood flow to the tissues. However, insufficient contrast between normal and diseased tissues requires the development of contrast agents. Most contrast agents affect the T1 and T2 relaxation times of the surrounding nuclei, mainly the protons of water. T2* is the spin–spin relaxation time composed of variations from molecular interactions and intrinsic magnetic heterogeneities of tissues in the magnetic field (1). Cross-linked iron oxide nanoparticles and other iron oxide formulations affect T2 primarily and lead to decreased signals. On the other hand, paramagnetic T1 agents, such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}), accelerate T1 relaxation and lead to brighter contrast images.

Apolipoprotein E (apoE) is essential for the normal catabolism of triglyceride-rich lipoprotein chylomicrons (lipoprotein particles) (2). Oxidation of low-density lipoprotein (LDL) generates a number of highly reactive short chain-length aldehydic fragments of oxidized fatty acids capable of conjugating with lysine residues of apoliprotein B and other proteins. Oxidized LDL is present in atherosclerotic lesions and is essential for formation of foam cells in atherosclerotic plaques. During atherogenic conditions, depositions of lipids and extracellular matrix proteins on the endothelial cell surfaces of the aorta and cells lead to the development of atherosclerotic plaques (3), which may erode and rupture. MDA2 is a murine monoclonal antibody to malondialdehyde-lysine epitopes of MDA-LDL and other oxidatively modified proteins but not to normal LDL (4). Briley-Saebo et al. (5) showed accumulation of MDA2 micelles containing Gd (MDA2-Gd micelles) in macrophages of atherosclerotic lesions in apoE-deficient ($apoE^{-/-}$) mice using MRI. However, Gd may lead to renal toxicity in patients. In another study, Briley-Saebo et al. (6) conjugated MDA2 antibody to lipid-coated superparamagnetic iron oxide nanoparticles (LSPIOs) for *in vivo* detection of atherosclerotic lesions in $apoE^{-/-}$ mice using MRI.

Related Resource Links:

- Chapters in MICAD (MDA)
- Gene information in NCBI (apolipoprotein E)
- Articles in Online Mendelian Inheritance in Man (OMIM) (apolipoprotein E)

Synthesis

[PubMed]

LSPIOs were prepared by incubation of oleic acid–coated iron oxide nanoparticles (magnetite) with 1,2-distearoyl-sn-glycer-3-phosphoethanolamine-n-methoxy(polyethylene glycol-2000 (PEG-DSPE) and PEG-malamide-DSPE (17.3 mg:1.3 mg per mg Fe) in a chloroform solution, with rhodamine added as a fluorescent label (6). The solution was added stepwise to boiling HEPES buffer (pH 7) under vigorous stirring until a clear brown solution was formed. LSPIOs were isolated with ultracentrifugation. MDA2 (2.3 nmol/mg Fe) was modified with *S*-acetylthioglycolic acid *N*-hydroxysuccinimide ester and then covalently linked to the surface of LSPIOs. MDA2 LSPIOs have a hydrated diameter (HD) of 38 ± 4 nm with an r1 value of 11 $nM^{-1}s^{-1}$ and an r2 value of 117 $nM^{-1}s^{-1}$ at 60 MHz and 40ºC. Untargeted LSPIOs have a HD of 35 ± 5 nm with an r1 value of 12 $nM^{-1}s^{-1}$ and an r2 value of 103 $nM^{-1}s^{-1}$ at 60 MHz and 40ºC. Only one in every sixteen LSPIOs contained an MDA2 antibody. MDA2 LSPIOs exhibited <5% variation in size and/or r2/r1 values at 4°C for 30 d.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Confocal microscopy analysis showed accumulation of MDA2 LSPIOs (1 mM Fe in the extracellular solution) in MDA-LDL–pretreated J7774A.1 macrophages, whereas no accumulation (non-detectable) was observed with untargeted LSPIOs (1 mM Fe concentration) (6). The Fe content was 0.4 pg/cell for MDA2 LSPIOs and non-detectable for untargeted LSPIOs. The accumulation of dextran-coated Feridex (positive untargeted control, HD = 40 nm) by the macrophages was 1.9 pg Fe/cell; thus, the lipid-coating of LSPIOs limited their accumulation by the macrophages. If every MDA2-LSPIO contained MDA2 antibody, but only 1 in 16 did, a theoretical Fe accumulation of 5–6 pg Fe/cell was expected.

Animal Studies

Rodents

[PubMed]

Briley-Saebo et al. (6) performed *in vivo* T2* MRI (9.4 T) studies of MDA2 LSPIOs in 10-month-old $apoE^{-/-}$ knockout mice that were fed a high-fat, high-cholesterol diet at 6 weeks of age and 10-month-old wild-type normal mice on normal diet at 0 h and 24 h after intravenous injection. Animals were injected with either 3.9 mg Fe/kg MDA2 LSPIOs (n = 9 $apoE^{-/-}$ mice or 5 wild-type mice) or LSPIOs (n = 5 $apoE^{-/-}$ mice or 5 wild-type mice). There was a small loss (~10%) of R2* value in the $apoE^{-/-}$ with MDA2 LSPIOs and little loss of R2* signal with untargeted LSPIOs in the atherosclerotic lesions. The liver accumulations were 37% and 35% injected dose for MDA2 LSPIOs and LSPIOs, respectively. The blood half-lives were 7.28 h and 1.02 h for MDA2 LSPIOs and LSPIOs in $apoE^{-/-}$ mice, respectively. On the other hand, the blood half-lives (1.12 h *versus* 1.01 h) were similar in the wild-type mice. A similar loss (10%–15%) of R2* signal was also

observed with a human single-chain Fv antibody fragment IK17 targeted to MDA-like epitopes (IK17-LSPIOs) and a natural IgM autoantibody (E06) cloned from apo$E^{-/-}$ mice that binds to the phosphocholine head group of oxidized phospholipids (E06-LSPIOs). The investigators suggested that the LSPIOs are too large to allow for optimal diffusion into the atherosclerotic lesions. On the other hand, the use of lipid-coated ultra-small superparamagnetic iron oxide nanoparticles conjugated with MDA2 (MDA2 LUSPIOs, HD = 14 ± 3 nm) showed a significant ($P < 0.003$) loss of R2* signal in the atherosclerotic lesions in apo$E^{-/-}$ mice. Co-injection of 20 nmol MDA2 antibody inhibited the loss of R2* value by 75% ($P < 0.0002$).

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.

NIH Support

R01 EB009638, R01 EB009638-07, R01 HL071021-07, R01 HL078667-04S1, R01 HL71021, R01 HL78667, R21 HL091399-01A2, R21 HL091399-02

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