Gadolinium-reconstituted high-density liproprotein-[palmitoyl-WK(palmitoyl)G(LRKLRKRLLR)$_2$-NH$_2$] nanoparticles

rHDL-P2A2

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Chemical name:	Gadolinium- reconstituted high-density liproprotein-[palmitoyl-WK(palmitoyl)G(LRKLRKRLLR)$_2$-NH$_2$] nanoparticles	
Abbreviated name:	rHDL-P2A2	
Synonym:		
Agent category:	Peptide	
Target:	Low-density lipoprotein receptor (LDLR)	
Target category:	Receptor	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/ contrast:	Gadolinium, Gd	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click on protein, nucleotide (RefSeq), and gene for more information about apolipoprotein E.

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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, and 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).

Gadolinium (Gd), a lanthanide metal ion with seven unpaired electrons, has been shown to be very effective in enhancing proton relaxation because of its high magnetic moment and water coordination (2, 3). Gd-labeled diethylenetriaminepentaacetic acid (Gd-DTPA) was the first intravenous MRI contrast agent used clinically, and a number of similar Gd chelates have been developed in an effort to further improve clinical use. However, these low molecular weight Gd chelates have short blood and tissue retention times, which limit their use as imaging agents in the vasculature and cancer. Various macromolecular Gd complexes have demonstrated superior contrast enhancement for MRI of the vasculature and carcinomas (4-6); however, these Gd complexes cannot proceed into further clinical development because of high tissue accumulation and slow excretion of toxic Gd ions. Furthermore, they are largely nonspecific.

Apolipoprotein E (apoE) is essential for the normal catabolism of triglyceride-rich lipoprotein chylomicrons (lipoprotein particles) (7). Under atherogenic conditions, deposits of lipids and extracellular matrix proteins on the endothelial cell surfaces of aortic and inflammatory cells lead to the development of atherosclerotic plaques (8), which may erode and rupture. The apoE-derived peptide A2 ($(LRKLRKRLLR)_2$) is a tandem dimer derived from the low-density lipoprotein receptor (LDLR)–binding domain of apoE (9). A2 has been modified by the addition of two palmitoyl chains to form palmitoyl-WK(palmitoyl)G$(LRKLRKRLLR)_2$-NH_2 (P2A2), which can be incorporated into lipid-based nanoparticles (10). Gd-Reconstituted high-density lipoprotein nanoparticles (rHDL) have been developed for the detection of atherosclerotic plaques (11). Chen et al. (12) has incorporated a carboxyfluorescence-modified P2A2 (P2fA2) into rHDL nanoparticles (rHDL-P2A2) for imaging atherosclerotic plaques in apoE knockout mice.

Synthesis

[PubMed]

P2fA2, prepared with solid-phase synthesis, was incorporated into rHDL nanoparticles (12). 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), Gd-DPTA-BSA, and rhodamine-PE were dissolved in chloroform-ethanol and then dried to a lipid film overnight. The film was dissolved in sodium cholate buffer (pH 7.5) with the addition of apoA1 and P2fA2, and the mixture was incubated for 2 h at 0–5°C. The molar ratio of apoAI:DPPC:P2fA2:Gd-DPTA-BSA:rhodamine-PE:sodium cholate was1:113:10:25:2:200. rHDL-P2A2 nanoparticles were purified with dialysis. There were 22 and 19 Gd^{3+} ions per nanoparticle for rHDL and rHDL-P2A2, respectively. The longitudinal relaxivity (r_1) values, measured at 60 MHz, were 11.3 and 10.5 $mM^{-1}s^{-1}$ per Gd^{3+} ion for rHDL and rHDL-P2A2, respectively. The zeta potentials of rHDL and rHDL-P2A2 were -20.3 and +15.6mV, respectively. The nanoparticles had a mean diameter of 11.6 ± 3.7 nm in aqueous solution as determined with dynamic light scattering.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The accumulation of rHDL and rHDL-P2A2 in macrophage J774A.1 cells was measured with fluorescent intensities and the longitudinal relaxation rates (R_1) of the cell lysates after 24 h of incubation (12). Cell lysates from rHDL-P2A2 incubation exhibited two-fold higher fluorescent intensities than did rHDL at 0.05 nM Gd^{3+}. The lysates of cells incubated with rHDL nanoparticles had an R_1 value of 0.281 ± 0.008 s^{-1}, while the cells incubated with rHDL-P2A2 nanoparticles had an R_1 value of 0.412 ± 0.008 s^{-1}. Endocytosis of both rHDL and rHDL–P2A2 into J774A.1 macrophages was confirmed with confocal images. However, rHDL–P2A2 binds to its receptor on the surface of macrophages, followed by endocytosis.

Animal Studies

Rodents

[PubMed]

Chen et al. (12) performed *in vivo* MRI imaging (9.4 T) on 6-month-old $apoE^{-/-}$ mice that were fed a high-fat, high-cholesterol diet for 4.5 months. Animals were injected with either 50 mmol Gd/kg rHDL (n = 6 mice) or rHDL-P2A2 (n = 5 mice). Elimination of the nanoparticles was bi-exponential. The two time constants for rHDL were 125 min and 3.9 × 10^6 min, respectively. The two time constants for rHDL-P2A2 were 39 min and 723 min. Sequential T1-weighted MRI images showed that both the rHDL and rHDL-P2A2 caused increased atherosclerotic arterial vessel wall enhancement at 24 h after injection. The mean normalized enhancement ratio of rHDL-P2A2 at 24 h after injection was 90%, whereas that of rHDL was 53% at the same time point. The signal with the rHDL was substantially reduced to 24% by 48 h. On the other hand, the enhancement ratio of rHDL-P2A2 remained high at 66% at 48 h after injection. The contrast/noise ratio of rHDL-P2A2 was 14 ± 5, significantly higher than that of rHDL (6 ± 5) at 24 h after injection. In

contrast, no significant enhancement of the arterial vessel wall of wild-type mice was observed after injection of either rHDL or rHDL-P2A2. Confocal laser scanning microscopy revealed that rHDL-P2A2 co-localized primarily with intraplaque macrophages with CD68 staining. No blocking experiment was performed.

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

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