Recombinant HDL-Like Nanoparticles: A Specific Contrast Agent for MRI of Atherosclerotic Plaques

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Atherosclerosis remains a major health problem in the United States, with significant morbidity and mortality. Although advanced lesions can grow sufficiently large to impede blood flow, the most important clinical complication is an acute occlusion due to plaque rupture and then formation of an overlying thrombus, resulting in myocardial infarction or stroke.[1,2] The early detection of atherosclerosis may direct therapies to prevent its complications.

The ability to image the presence or biological activity of specific molecules ("molecular imaging") in atherosclerotic plaques in vivo would be of considerable interest. Assessment of molecular information in vivo requires high-affinity, target-specific contrast agents, with marked signal amplification, and high-resolution imaging modalities, such as magnetic resonance. Most of the available paramagnetic magnetic resonance (MR) contrast agent constructs, however, are not capable of delivering a large amount of gadolinium (Gd^{3+}) ions to induce a large MR signal. Moreover, some of the MR contrast agents may be too large to have free access to biochemical epitopes within the vascular subenthothelium of atherosclerotic plaques.[3]

Here, we present a high-density lipoprotein (HDL)-like nanoparticle contrast agent that selectively targets atherosclerotic plaques. High-density lipoproteins present in the plasma play a key role in reverse cholesterol transport by removing excess cellular cholesterol from the peripheral tissues.[4−6] These HDL-like particles have several advantages: a small size (7−12 nm diameter); protein components that are endogenous, biodegradable, and do not trigger immunoreactions; and the particles are not recognized by the reticuloendothelial system (RES). Furthermore, HDL-like particles are easily reconstituted[7] and can carry a considerable contrast agent payload.

The techniques for preparation of this contrast agent involve isolation and delipidation of normal human HDL by standard methods to obtain apo-HDL proteins, mainly apolipoprotein (apo) A-I. Using standard methods,[8,9] the apolipoproteins are extracted and reconstituted with phospholipids, with or without unesterified cholesterol, using detergent dialysis. For our purposes, we also include a phospholipid-based contrast agent, Gd-DTPA-DMPE[10] (Scheme 1) that becomes incorporated into the reconstituted particle ("recombinant HDL", or rHDL) of approximately 9 nm diameter and contains between 15 and 20 molecules of Gd-DTPA-DMPE, as measured by gradient gel electrophoresis and ICP-MS. Also, for confocal fluorescence microscopy studies, a fluorescent phospholipid with a green emission, NBD-DPPE[11] ($\lambda_{exc} = 460$ nm, $\lambda_{em} = 534$ nm), was added to the formulation (Scheme 1).[12,13] Reconstituted high-density lipoproteins can be obtained in large scale, suitable for therapeutic use.[14,15]

Scheme 1. Phospholipid-Based Contrast Agent (Gd-DTPA-DMPE) and NBD-DPPE-Labeled Phospholipid for Fluorescence Confocal Microscopy Used for the Reconstruction of HDL; (Bottom) Different Components of the Recombinant HDL-Like MRI Contrast Agent
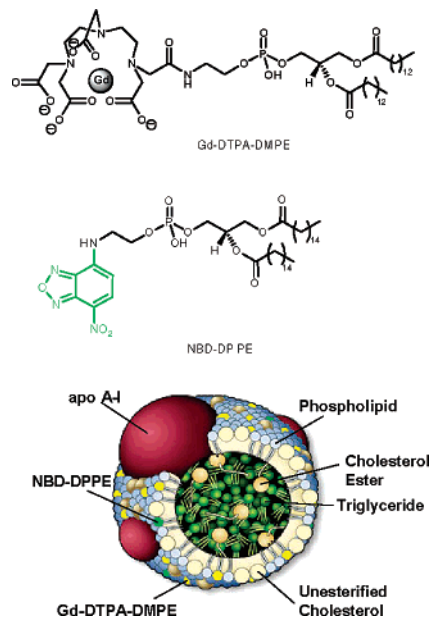

The relaxivity of the recombinant HDL-like contrast agent (rHDL) measured at 65 MHz and 25 °C in water at pH 7.4 provided a value of $r_1 = 10.4$ mM^{-1} s^{-1}. The relaxivity was found to be independent of gadolinium concentration. The emission and excitation spectra for the NBD-fluorescent-labeled recombinant HDL-like particle ($\lambda_{exc} = 470$ nm, $\lambda_{em} = 530$ nm), was recorded and used for matching the results obtained by in vivo magnetic resonance imaging (MRI) with those obtained by post-mortem confocal fluorescent microscopy (Supporting Information, S1). Genetically engineered hyperlipidemic mice (apoE-knockout) were used as models of human atherosclerosis and were imaged by in vivo MRI.[16] The rHDL contrast agent was injected via a tail vein catheter. The content of gadolinium in the rHDL was assessed by ICP-MS measurements. MRI aortic images of the matched (pre- and postcontrast) slices were used for analysis.

Sequential MRI showed that the contrast agent localized predominantly at the atherosclerotic plaque by 24 h after injection (post). The mean normalized enhancement ratio NER (NER = $(SI_{wall−post}/SI_{muscle−post})/(SI_{wall−pre}/SI_{muscle−pre})$) was determined from the signal intensities (SI) of the wall and muscle with standard region-of-interest (ROI) measurements on the corresponding MR images and provided a value of 35%. Furthermore, by 48 h after injection, the intensity of the plaque decreased to a value similar

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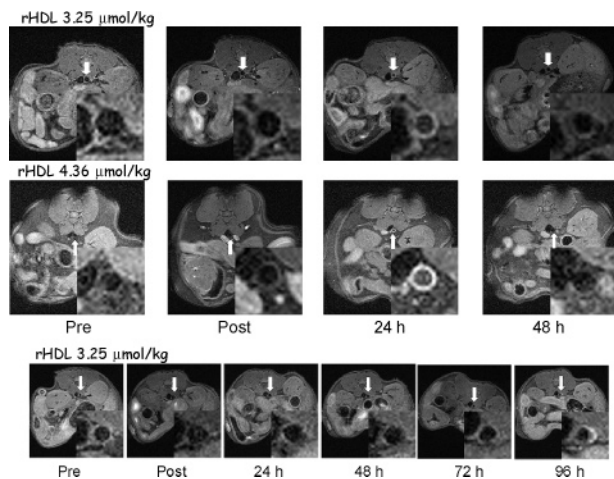
Figure 1. In vivo MRI at different time points (pre- and postinjection of contrast agent at 24, 48, 72, and 96 h) and dosages of Gd as determined by ICP-MS measurements. White arrows point to the abdominal aorta; the insets denote a magnification of the aorta region.

to that observed for the plaque immediately after injection (NER = 10%). Importantly, the enhancement was also related to plaque composition: the more cellular the content was (as judged by histology, data not shown), the more intense the signal was (Figure 1). A control experiment was performed by injecting Gd-DTPA-DMPE into mice (Supporting Information, S2). The images showed that the phospholipid-based contrast agent, without apo-HDL, accumulated and was retained in the plaque for >48 h. In contrast, the signal with the rHDL-Gd particles was substantially reduced by 48 h. Also, the injection of the rHDL-like contrast agent or Gd-DTPA-DMPE into wild-type animals resulted in no enhancement at any time point (Supporting Information, S3).

At 24 h postinjection, when MRI of the rHDL-like contrast agent showed its maximum intensity in plaques, aortas were removed and imaged by confocal fluorescence microscopy. The fluorescence was located mainly in the intimal layer, where the lipids accumulate.[17] Also, it can be seen that a small number of cells appeared to have internalized and retained the fluorescence. To characterize these cells, nuclei were stained with DAPI (blue) and with an RPE-labeled antibody selective for macrophages (anti-CD68:RPE; red fluorescence). Figure 2 shows that the rHDL contrast agent (green) is localized primarily within macrophages (red; co-localization is shown by yellow in the merged image).

In summary, we have demonstrated that Gd-loaded HDL-like nanoparticles localize to atherosclerotic plaques in vivo and substantially enhance the MRI image. Owing to the flexibility of the rHDL platform, targeting molecules can be easily incorporated into this contrast agent. Targeting molecules may increase the delivery and the retention of our rHDL contrast agent into specific regions or types of plaques, based on the extensive knowledge of specific molecules that are expressed in plaques at different stages of development. Our current technology, with or without future enhancements, may provide a way to achieve noninvasive in vivo molecular detection and characterization of atherosclerosis using MRI.
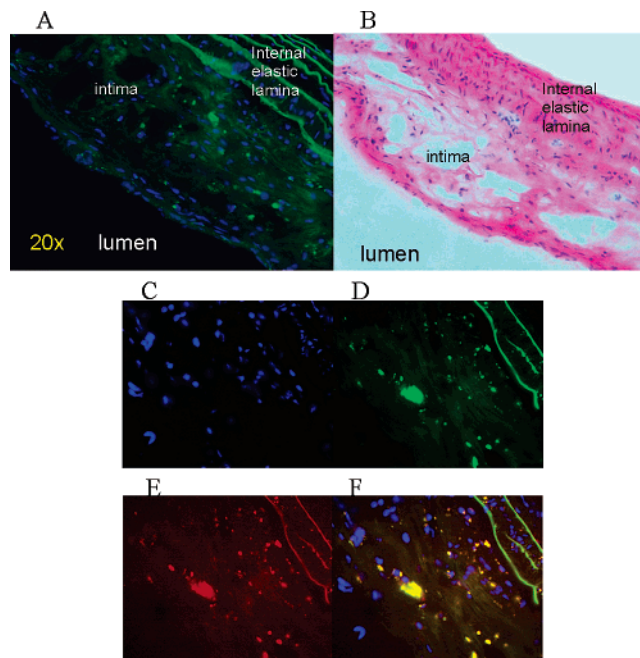
Figure 2. (A) confocal fluorescence microscopy of an atherosclerotic plaque. Blue denotes nuclei (DAPI staining), and green denotes rHDL NBD labeled. (B) Histopathological section stained with hematoxylin and eosin (H&E). (C) DAPI staining of an atherosclerotic plaque. (D) rHDL-NBD staining. (E) Antibody selective for macrophages, CD68:RPE. (F) Merged images (yellow indicates colocalization).

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