Modified HDL as Specific Carrier for MR Imaging of Atherosclerotic Plaques

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Vessel wall imaging and the study of the progression and regression of atherosclerosis using in vivo MRI is actively been pursued. The ability to image the presence or biological activity of specific molecules in vivo (i.e., molecular imaging) in atherosclerotic plaques would be of considerable interest. Current non-contrast and contrast-enhanced methods do not interrogate specific biochemical processes. Distinctions among plaque components may be enhanced by the introduction of plaque-specific contrast agents that are related to molecular signatures involved in atherosclerosis. Microemulsions such as lipoproteins are good candidates for atherosclerotic plaque site-specific MR contrast agent delivery carriers. Being endogenous, lipoproteins are biodegradable, do not trigger immune reactions, and are not recognized by the reticuloendothelial system (RES).[3] High density lipoproteins (HDL) are easily reconstituted, can carry a considerable contrast agent (i.e., Gd) payload, and is sufficiently small to penetrate readily in the extracellular space and freely enter and exist plaques. Considering these properties, we reconstituted HDL by introducing a lipophilic gadolinium complex to create an MR contrast agent and determined its utility as a diagnostic marker for atherosclerotic disease.

Methods: ApoAI/POPC/GdDTPADMPE/sodium cholate rHDL was prepared by spontaneous association of lipid-free apoAI and small unilamellar vesicles of POPC (palmitoyloleoyl phosphatidylcholine), and GdDTPEDMPE (dimiristoyl phosphatidylethanolamine). POPC (2.4 mg) and GdDTPADMPE (0.4 mg) in chloroform were dried in a thin film under nitrogen. Sodium cholate (3.1 mg) dissolved in TBS (200 μL) was added to the lipid film to give a turbid suspension that clearified after incubation at 37°C for 1.5 hours. To the clear solution was added apoAI (1 mg) dissolved in 1 mL of TBS and the resulting mixture was allowed to incubate for one hour at 37°C.[4] After incubation the sample was exhaustively dialyzed to get rid off the excess of cholate. The rHDL-GdDTPA-DMPE contrast agent diameter was determined with a laser light-scattering submicron particle sizer. Thirteen-month-old atherosclerotic Apolipoprotein E knockout (KO) mice (n=4) on high fat diet and Wild Type (WT) (n=4) group underwent in vivo MR microscopy (MRM) of the abdominal aorta using a 9.4T MR system. Pre- and post- contrast enhanced (CE) (1 hr and 24 hrs post) MRM was performed using a T1W black blood sequence. Sixteen contiguous 500 μm thick slices with an in-plane resolution of 93 μm were acquired in 30 minutes. The rHDL contrast agent (47.2 nmol) was injected via the tail vein. MRM images of the matched (pre and post) slices were used for analysis.

Results: The diameter of the rHDL contrast agent was 47 nm. The in vivo MR images reveal that after 1 hour post-injection of rHDL-GdDTPA-DMPE a substantial enhancement in the plaque is observed (Fig. 1). The ratio of the post to pre signal intensity of wall normalized with respect to muscle was 1.21 (21% enhancement) in KO mice after 1 hour. This enhancement increased after 24 hours to a value ofy 1.41 (41% enhancement). There was no enhancement in the WT group.

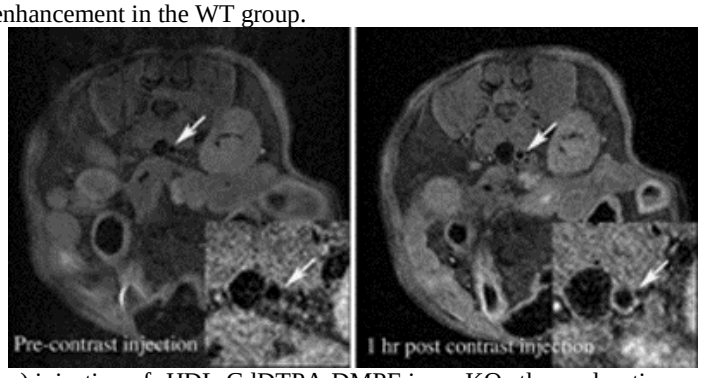

Figure 1. Pre and post contrast (1hour) injection of rHDL-GdDTPA-DMPE in an KO atherosclerotic mouse, showing the enhancement in the abdominal aorta (right; arrow).

Conclusions: We demonstrated in this in vivo MR study that Gd loaded reconstituted HDL localize and substantially enhance atherosclerotic plaques. Targeting molecules can be easily incorporated in the rHDL-GdDTPA-DMPE contrast agent. The targeting molecules will accomplish delivery and retention of our rHDL contrast agent into plaques, based on the fairly extensive knowledge of specific molecules that are present in plaques at different stages of development. This may provide a way to achieve noninvasive optimal sensitive and specific in vivo molecular detection of atherosclerosis using MR.

References:

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