Comparison of synthetic HDL contrast agents for atherosclerosis imaging

D. P. Cormode¹, R. Chandrasekar², K. C. Briley-Saebo¹, A. Barazza¹, W. J. Mulder¹, E. A. Fisher³, and Z. A. Fayad¹

¹Department of Radiology, Mount Sinai School of Medicine, New York, New York, United States, ²The Cooper Union for the Advancement in Science and Art, New York, New York, United States, ³School Of Medicine, New York University, New York, New York, United States

Introduction

Due to the high worldwide level of mortality caused by heart disease, there is a strong motivation to non-invasively image the prime precursor of heart disease, atherosclerosis. One of the major markers of plaque progression and inflammation is the macrophage burden and thus MR imaging techniques that can non-invasively monitor this burden are of great interest. We have previously reported gadolinium-labeled HDL as a macrophage specific MRI contrast agent. That formulation may have safety issues for widespread use as the HDL was extracted from expired human plasma. Here we report a comparison study between two different types of synthetic, gadolinium and rhodamine labeled HDL formed from the apoA-I mimicking peptides 18A or 37pA (the number refers to the amino acid sequence length). These HDL-nanodiscs have been thoroughly characterized and their in vivo properties assessed with regards macrophage cholesterol efflux and macrophage uptake. The in vivo properties of this nanoparticle platform were assessed with respect to atherosclerotic plaque signal, and the biodistribution and pharmacokinetics. MRI findings were validated ex vivo with confocal microscopy of aortic sections.

Materials and Methods

Synthetic, paramagnetic HDL nanoparticles were synthesized via the formation of a mixed lipid film (Gd-DTPA-DMPE, DMPC and Rhodamine-PE) which was hydrated with a solution of either the 37pA or 18A amphiphatic, α-helical peptide followed by sonication. A schematic representation of these agents is shown in Figure 1. A control agent of the same lipids without a peptide was also synthesized. The particles were characterized with DLS, TEM, gel electrophoresis, FPLC, relaxometry, and peptide, phosphorous and gadolinium content analysis. Cholesterol efflux from macrophages was measured by incubating ³H-cholesterol fed macrophages with the synthetic HDL agents and performing gamma counting on the media. Native HDL and BSA were used as controls. Macrophage uptake was investigated via incubation with the agents and T1 determination of the resulting cell pellets. The peptide based agents were each injected, via the tail vein, into n=7 apoE KO and n=2 wild type mice and these mice were scanned on a 9.4 T small animal imaging system pre- and 24, 48, 72 and 96 hours post-injection. The control agent was applied to apoE KO mice (n=3) with the same imaging protocol. The mice used were an average of 11 months old and were fed a high cholesterol diet for 8 months. Confocal microscopy was performed on sections of the aorta stained with CD68 for macrophages and with DAPI for nuclei. The biodistribution of the agents at the 24 hour timepoint were determined via ICP-MS of the heart, lungs, liver, spleen and kidneys. The pharmacokinetics of the agents were determined via ICP-MS on blood drawn sequentially from the same mouse over a 48 hour period.

Results and Discussion

The particles formed from the two peptides have very similar physical properties. The 18A complex was found, by DLS, to have a mean diameter of 8.0 nm and a longitudinal relaxivity of 10.0 mM⁻¹s⁻¹; the corresponding values for the 37pA complex are 7.6 nm and 9.8 mM⁻¹s⁻¹, respectively. In vitro, the peptide particles showed higher cholesterol efflux than for HDL, demonstrating their therapeutic potential. Macrophage incubation experiments showed little difference between the rates of uptake of the agents. In vivo, both agents gave significant enhancements of the signal density in the aorta of apoE KO mice, as shown in Figure 2. We did not observe significant enhancement for the peptide agents in wild type mice or for the control agent in apoE KO mice. The 18A agent gave an average aortic wall enhancement of 91% ± 12% while the 37pA agent gave an enhancement of 94% ± 20%, confirmed to be not significantly different by t-testing. Confocal microscopy of the aorta showed co-localization of nanodiscs with macrophages (Figure 3).

Conclusions

The agents based on 37pA and 18A both accumulate in areas of high macrophage density in the plaque. This accumulation is detectable by MRI. There seems to be little difference in the efficacy of the two agents. From a practical point of view, 18A should be favored as the shorter amino acid sequence will make it cheaper to synthesize.

References