Nature-inspired nanoformulations for contrast-enhanced in vivo MR imaging of macrophages

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Introduction

In atherosclerosis, discrimination between stable and vulnerable plaques is of particular clinical importance. For imaging purposes, the intraplaque macrophage content can be used as a distinctive feature and a specific marker of vulnerable plaques. Thus, MRI of macrophages using contrast-enhancing agents can help to discriminate between these plaques.[1] Recently, synthetic particles that mimic human high density lipoproteins (HDL) were proposed as a delivery vehicle for Gd-based contrast agents (GBCA).[2,3] However, native HDL are not normally uptaken by macrophages, leading to the need for targeting moieties to image plaques.[4] To avoid the pitfalls and complications associated with these moieties, new approaches are needed.

In this study, a naturally occurring modification in the major HDL protein, apolipoprotein (apo) A-I, was exploited as a natural way to target GBCA-HDL to macrophages. We also tested if fully functional GBCA-HDL can be generated using synthetic apo A-I peptides in place of native apo A-I.

Methods

Self-assembled paramagnetic and fluorescent HDL containing either the oxidized or unmodified apo A-I protein were synthesized using the molar ratio of 1:65:25:38:2 for apo A-I:DMPC:DMPG:PE-DTPA-Gd:rho B-PE. Alternatively, a 1:1 mixture of either oxidized or unmodified 22-mer peptides that correspond to amphipathic apo A-I helixes 4 (H4) and 6 (H6) was used. The obtained particles of discoidal morphology with the mean size of 12 nm were characterized by a variety of biophysical methods, including electron microscopy. Fluorescence and MRI studies of in vitro macrophage uptake were carried out using J774 macrophages. The abdominal aortas of either apo E-deficient atherosclerotic mice kept on a Western diet or wild type mice kept on a normal chow diet were imaged using a 3.0T MRI system before and 24 h after injection of the synthesized contrast agents. In vivo MRI data were complemented by immunohistology and fluorescence microscopy.

Results

Specific oxidation of apo A-I or its peptides does not affect the size, composition, and relaxivity properties of GBCA-HDL but increases the in vitro macrophage uptake by 2-3 times and significantly improves atherosclerotic plaque detection in vivo (Fig. 1). At 24 h post-injection of 0.05 mmol Gd/kg GBCA-HDL containing oxidized apo A-I or its peptides, the atherosclerotic wall/muscle normalized enhancement ratios were 90% and 120%, respectively, while those of GBCA-HDL containing their unmodified counterparts were 35% and 45%, respectively. Immunohistology and confocal fluorescence microscopy confirm the accumulation of GBCA-HDL containing oxidized apo A-I or its peptides in intraplaque macrophages (Fig. 1).

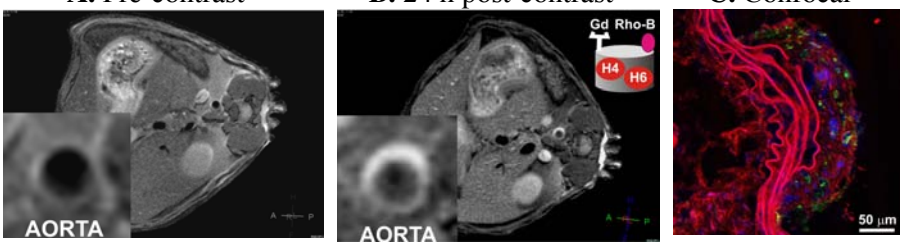

Fig. 1. Pre- and 24 h post-contrast images (**A,B**) of the aorta of an apo E-deficient mouse show the improved plaque detection using GBCA-HDL with oxidized apo A-I peptides. (**C**) The merged confocal image demonstrates uptake of these GBCA-HDL by intraplaque macrophages.

Conclusions

Specific oxidation of apo A-I targets GBCA-HDL to macrophages in vitro and in vivo. Synthetic peptides can functionally replace the native apo A-I protein in HDL, encouraging the further development of these agents with broad application in cardiology, oncology and other diseases, where macrophage imaging has important diagnostic and prognostic value.

References

[1] R.P. Choudhury, et al., Arterioscler Thromb Vasc Biol 2009, 29: 983.
[2] M. Rui, et al., Int J Nanomedicine 2012, 7: 3751.
[3] J.C. Frias, et al., Contrast Media Mol Imaging 2007, 2: 16.
[4] W. Chen, et al., Contrast Media Mol Imaging 2008, 3: 233.

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