Molecular imaging of macrophages in atherosclerotic plaques using bimodal PEG-micelles

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Introduction

Identification of high risk atherosclerotic plaques can be done non invasively with magnetic resonance molecular imaging (MMRI) and may lead to improved diagnosis and prognosis[1]. MMRI of macrophages may be of great use in predicting the severity of the disease, since macrophage rich plaques are believed to be unstable[2]. To that end, we developed a novel micellar contrast agent which contained both paramagnetic and fluorescent properties and was targeted to the macrophages by covalently linking a macrophage scavenger receptor (MSR) specific antibody. We applied this contrast agent to a mouse model with atherosclerosis and visualized macrophages in the abdominal aorta with MRI in vivo and confirmed our findings with optical techniques ex vivo.

Material and Methods

Pegylated micelles, composed of Gd-DTPA-BSA, PEG-DSPE, Mal-PEG-DSPE, and either containing an amphiphilic fluorophore in the lipidic layer or a quantum dot in the core[3], were prepared by lipid film hydration, followed by heating to 65 °C and vigorous stirring.

The monoclonal rat anti mouse CD204 (Serotec, Raleigh,NC) antibody against the macrophage scavenger receptor was coupled to micelles by a sulfhydryl-maleimide coupling method as described previously[4]. The antibody (5 mg/ml) was modified with N-succinimidyl S-acetylthioacetate by incubating for 45 minutes on a roller-bench at room temperature. The SATA-derivatized antibody was deacetylated by incubation with a hydroxylamine solution for 1 hour at room temperature. The activated antibody was added to the micelles. This preparation was stored at 4 °C under N2 overnight. Uncoupled antibody was separated from immunomicelles by washing twice. The size of this contrast agent was below 20 nm as determined with dynamic light scattering.

Twelve thirteen-month-old apoE-KO mice were used for this study. The apoE-KO animals were fed a Western diet for 20 weeks. MSR-targeted micelles or untargeted micelles were administered at a dose of 0.075 mmol Gd/kg via intravenous injection into the tail vein. High resolution T1-weighted images (pixel size: 0.109×0.109×0.5 mm^3, TR: 800 ms and TE 9ms) of the abdominal aorta were acquired before, and 24 hours post injection at a Bruker 9.4 T, 89 mm-bore system. Images were analyzed using Mathematica (Wolfram Research, Inc.). The contrast to noise was defined as CNR = (I wall – I muscle)/I noise, which is a measure for how well the vessel wall can be discriminated from the surrounding tissue. Immediately after the imaging at 24 hours post micelle injection, the animals were sacrificed. The presence of MSR-targeted micelles containing rhodamine was compared to the location of macrophages studied by immunohistochemistry (anti-CD68 antibody) on fluorescence microscopy of aortic cross-sections corresponding to MRI slices.

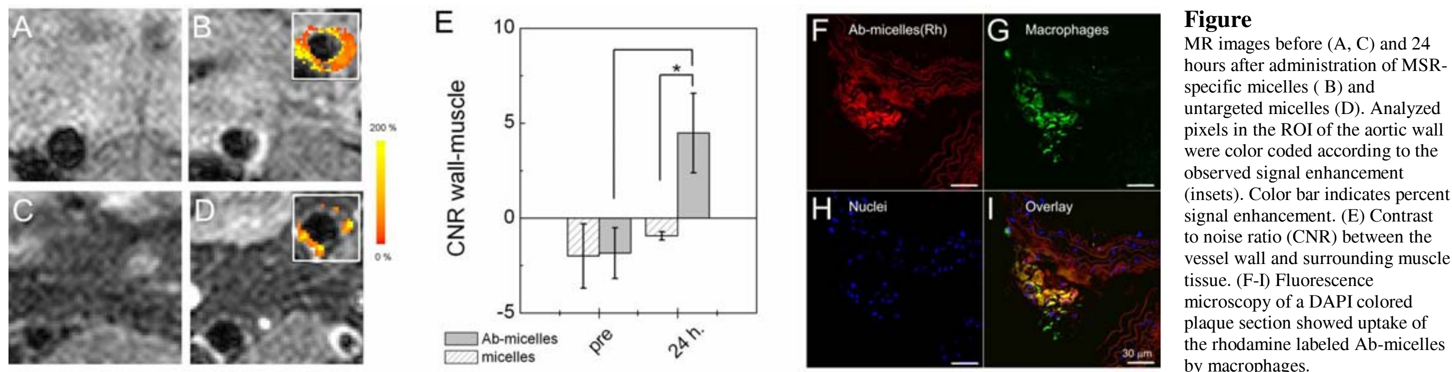

Figure
MR images before (A, C) and 24 hours after administration of MSR-specific micelles (B) and untargeted micelles (D). Analyzed pixels in the ROI of the aortic wall were color coded according to the observed signal enhancement (insets). Color bar indicates percent signal enhancement. (E) Contrast to noise ratio (CNR) between the vessel wall and surrounding muscle tissue. (F-I) Fluorescence microscopy of a DAPI colored plaque section showed uptake of the rhodamine labeled Ab-micelles by macrophages.

Results and Discussion

Typical images of the abdominal aorta of atherosclerotic apoE-KO mice that were imaged with T1-weighted high resolution MRI before and 24 hours after intravenous administration of the MSR-targeted micelles (Figure A, B) and untargeted micelles (C, D) are depicted in the Figure above. Pronounced signal enhancement (up to 200%) was observed for the vessel wall of apoE-KO mice that were injected with MSR-targeted micelles, while the aortic vessel wall of mice injected with non-targeted micelles showed little signal enhancement (Figure E). Fluorescence microscopy of aortic sections revealed the MRS-targeted rhodamine micelles (Figure F) to be associated with macrophages (G, I). In addition we used MSR-targeted micelles that were labeled with a quantum dot for optical investigation, which allowed us to visualize macrophage rich regions in the intact aorta (data not shown).

Conclusions

We have shown that by using this MSR-targeted contrast agent macrophages in apoE-KO mice can be effectively and specifically detected by molecular MRI and may help for the identification of the vulnerable plaque. Moreover, the size, specificity, and the bimodal character of the micellar contrast agent allowed MR and optical detection of extravascular targets and may be employed for detection of other markers of atherosclerosis, but also for extravascular targets in other disorders.

References

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