MR visualization of neovascularization in advanced atherosclerotic plaques using targeted paramagnetic and fluorescent micelles

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Introduction

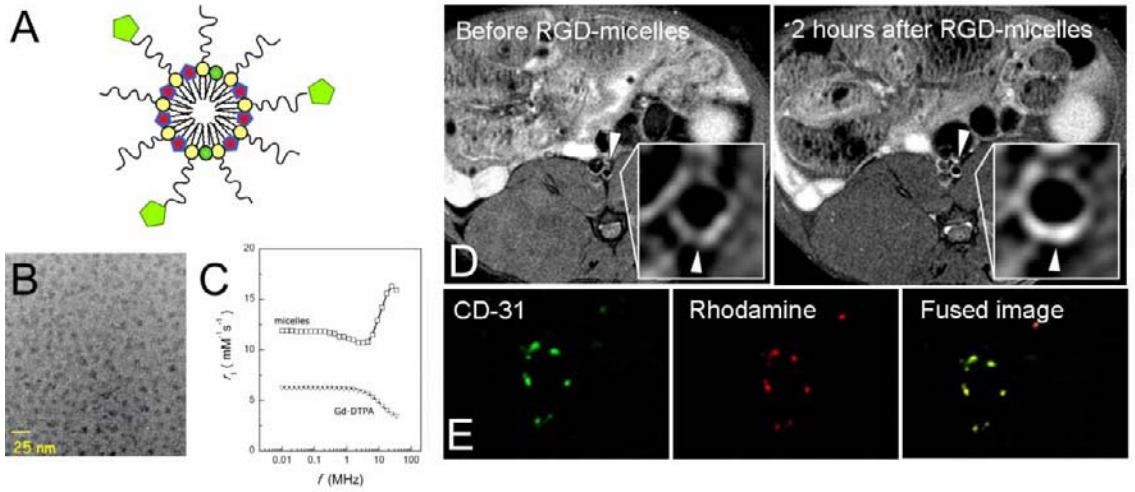
Neovascularization is involved in the growth and rupture of atherosclerotic plaques and is predominantly present in advanced lesions[1, 2]. The activated endothelium of such vessels expresses αvβ3-integrins, which can be used for target specific imaging. To that end, we developed a novel micellar contrast agent which contained both paramagnetic and fluorescent amphiphiles (Figure A) to allow a multimodal detection of neovessels. The contrast agent was fully characterized by transmission electron microscopy and NMRD measurements. Specificity for αvβ3-integrins was introduced by conjugating multiple cyclic RGD peptides[3]. We applied this contrast agent to a rabbit model with advanced plaques[4] and investigated neovascularization 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 an amphiphilic fluorophore, were prepared by lipid film hydration, followed by heating to 65 °C and vigorous stirring. The micelles were characterized with cryo-transmission electron microscopy (cryo TEM) and ^{1}H nuclear magnetic relaxation dispersion (NMRD) measurements. The cyclic 5mer RGD (c(RGDf(-S-acetylthioacetyl)K)) was conjugated to Mal-PEG-DSPE after de-protecting the peptide with a hydroxylamine-HCl HEPES buffer (pH 7.0).

Atherosclerotic plaques were induced in the aorta of 6 New-Zealand White rabbits by repeated balloon injury (4 weeks apart) and 4 months of hypercholesterolemic diet. Two non-injured rabbits fed a chow diet were used as controls. T1-weighted MRI of the aortic region was acquired before and 2 hours after intravenous injection of 3 µM Gd/kg of RGD-micelles. Two weeks later, a competition study was performed in 2 rabbits. The same imaging protocol was repeated 15 minutes after the pre-injection of RGD-micelles without gadolinium. Contrast to noise ratio (CNR) was calculated by dividing the signal to noise ratio of atherosclerotic plaques by the signal intensity of muscle. Enhancement of atherosclerotic plaques was measured as follows: Enhancement = [(CNR after contrast agent / CNR before contrast agent) - 1] x100. Immediately after the imaging, the rabbits were sacrificed. The presence of RGD-micelles containing rhodamine was compared to the location of neovessels studied by immunohistochemistry (anti-CD31 antibody) on fluorescence microscopy of aortic cross-sections corresponding to MRI slices.

Figure



(A) Schematic representation of the micellar contrast agent conjugated with RGD-peptides (), and containing paramagnetic amphiphiles (), and an amphiphilic fluoro-phore (). (B) TEM of a micelle suspension. (C) NMRD profiles of the paramagnetic PEG-micelles and Gd-DTPA. (D) A T1-weighted MR image of the aorta of an atherosclerotic rabbit before and 2 hours post injection. (E) Fluorescence microscopy of endothelial cells (CD-31) and RGD-micelles (Rhodamine) showed good co-localization (Fused image) on histological section.

Results and Discussion

Cryo-TEM revealed the size of the micelles to be 10 to 15 nm (Figure B). The NMRD profile of the micellar contrast agent shows a typical peak at clinical field strengths, in agreement with the increase of the rotational correlation times as compared to the low molecular weight compound Gd-DTPA (Figure C).

Increased signal intensities (Figure D) were detected 2 hours after RGD-micelles injection on axial MRI views in the aortic wall of all (6/6) atherosclerotic rabbits (mean increase of 15.7 ± 5 %), but not in control rabbits (2/2) (not shown). Pre-injection of RGD-micelles without gadolinium, followed by injection of Gd-micelles, led to a 37 % decrease of the enhancement in atherosclerotic plaques. The highest MR signal intensities after injection of RGD-micelles were found predominantly in atherosclerotic plaques rich in neovessels on corresponding aortic cross-sections. On fluorescence microscopy, we confirmed that RGD-micelles colocalized with neovessels in the intima of atherosclerotic plaques (Figure E), whereas no fluorescence was detected in the aortic wall of control rabbits (not shown).

Conclusions

We designed a relatively small nanoparticulate agent, with a high ionic relaxivity, and optical properties incorporated as well. Neovessels were detected with MRI in advanced atherosclerotic plaques of rabbits using this contrast agent targeted to αvβ3-integrins. Fluorescence microscopy confirmed the specific binding of RGD-micelles to the endothelium of neovessels on histological sections.

References

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