

Contrast-enhanced MRI of atherosclerosis with collagen targeted CNA35-micelles

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Introduction

Collagen plays an important role in the stabilization of atherosclerotic plaques¹. Therefore, collagen imaging of the vessel wall would be a valuable method to characterize atherosclerotic plaques and possibly enable the differentiation between stable and vulnerable plaques.

Aim: The goal of this study was to develop and characterize an MRI contrast agent based on paramagnetic micelles conjugated with the collagen binding CNA35 protein and to test this contrast agent in a mouse model of stable and vulnerable plaque.

Materials and methods

Contrast agent: Paramagnetic micelles (figure 1) were prepared from DSPE-PEG(2000), Gd-DTPA-bis(stearylamide) and Lissamine-Rhodamine-PE². CNA35, or the non-binding mutant-CNA35, was conjugated to the distal end of maleimide functionalized DSPE-PEG(2000) after SATA-modification of its primary amines.

Micelle characterization: The hydrodynamic diameter of the micelles was determined with Dynamic Light Scattering. NMRD-profiles were measured for both unconjugated and conjugated micelles in HBS (pH7.4) and in bloodserum. The critical micelle concentration was determined by ¹H₂O relaxivity measurements of a dilution series³. For determination of the specificity of binding in vitro, a 96-wells plate coated with rat-tail collagen I was used. The wells were incubated for 2 hours with CNA35-micelles (n=3), mutant-CNA35-micelles (n=3) and unconjugated micelles (n=3). After incubation, the wells were washed three times with HBS (pH7.4) and the fluorescence was measured with a plate reader.

In vivo imaging: Plaque imaging was performed on ApoE^{-/-} mice (n=7), fed a cholesterol-rich diet for 12 weeks. A tapered polymeric cast⁴ was placed around the right carotid artery to induce stable (collagen-rich) plaques downstream and vulnerable plaques upstream of the cast. No atherosclerotic plaques are expected in the left carotid artery, which was used as a control. CNA35-micelles (n=4) or mutant-CNA35-micelles (n=3) containing 1.25 μmol Gd were injected intravenously. For imaging a T₁-weighted spin-echo sequence on a 6.3T Bruker scanner (TE=10.2 ms, TR=800 ms, pixel dimension=100x100 μm², NEX=8, slice thickness= 0.5 mm, scan time=30 min.) was used. The mice were scanned before and at 15 min., 1 hr. and 24 hrs. after contrast agent injection (CI), and sacrificed for histology. For analysis vessel wall contrast (signal wall / signal muscle) was calculated in the MR slices directly above and below the cast, and a one-way ANOVA statistical test (p<0.05) was performed. Histological sections were studied with fluorescence microscopy and stained with H&E, ORO (lipids), Picrosirius Red (collagen), CD68 (macrophages) and VSMC (smooth muscle cells) stainings.

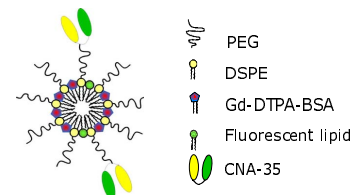


Figure 1 CNA35-micelle

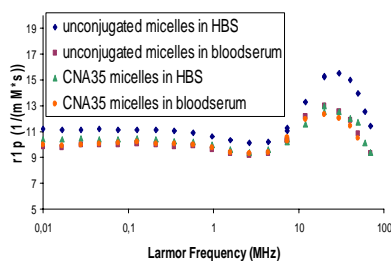


Figure 2 NMRD profiles

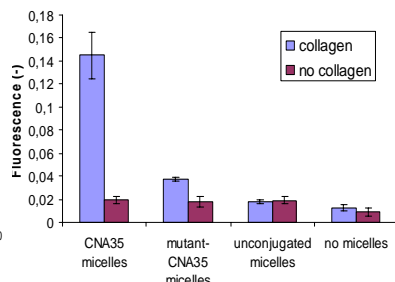


Figure 3 In vitro binding (mean ± SD)

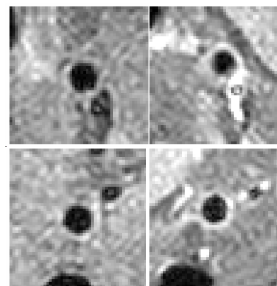


Figure 4. MR Images of right carotid artery. Upper images= downstream, lower images= upstream, left= pre, right= 24h post CI

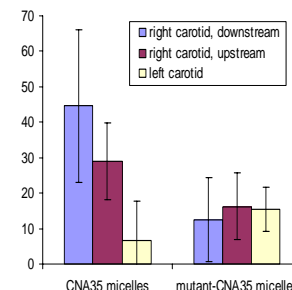


Figure 5. Percentage signal enhancement of the vessel wall (mean ± SD) at 24h post CI.

Results and discussion

Micelle characterization: The conjugation of proteins increased the diameter of the micelles from 16 ± 2 nm for unconjugated to 27 ± 4 nm for CNA35- and mutant-CNA35-micelles. In HBS (pH7.4), the NMRD-profile showed a higher relaxivity for the unconjugated micelles (figure 2), while in bloodserum the relaxivity was the same as for conjugated micelles. For the conjugated micelles, the NMRD-profiles were similar in HBS and bloodserum, suggesting that these micelles were still intact and interaction with the serum proteins was negligible. The critical micelle concentration was 0.09 ± 0.03 mM. The in vitro binding experiment showed a strong binding of the CNA35-micelles to collagen (figure 3), while minor binding occurred for mutant-CNA35 and unconjugated micelles.

In vivo imaging: MR images revealed contrast enhancement of the vessel wall 24 hrs. after contrast injection (figure 4). The percentage contrast enhancement of the vessel wall at 24 hours is shown in figure 5. The largest contrast enhancement was seen for the CNA35-micelles downstream of the cast, where stable plaques with large amounts of collagen are indeed expected. Statistics showed a significant signal enhancement of the right carotid artery for the CNA35-micelles, both upstream and downstream of the cast. Although the signal enhancement was less, the mutant-CNA35-micelles also showed a significant signal enhancement for the right carotid artery upstream of the cast and for the left carotid artery, which might be caused by a-specific uptake of the micelles. Fluorescence microscopy of the histological sections showed a diffuse rhodamine signal, widely distributed through the plaque (not shown). Histological stainings confirmed a stable plaque downstream and a vulnerable plaque upstream of the cast.

Conclusions

Because of their size, high relaxivity, stability in bloodserum and strong binding to collagen, Gd-containing CNA35-micelles can be suitable as MRI contrast agent for collagen imaging, for example in atherosclerotic plaques. Initial use of the contrast agent in a mouse model of stable and vulnerable plaque indeed has shown that the region where stable (collagen-rich) plaques are expected showed the strongest contrast enhancement. Experiments are ongoing to optimize the timing for post-contrast imaging in order to achieve the highest target to background ratio.

References: 1. Reikhter, *Cardiovascular Research* 1999, 41, 376-384 2. Mulder W.J.M. et al. *NMR Biomed.* 2006, 19, 142-164 3. Galle et al. *J Biol Inorg Chem* 2002, 7, 757-769 4. Cheng et al. *Circulation* 2006, 113, 2744-2753