VCAM-1 targeted MRI for imaging of inflammation in mouse atherosclerosis using paramagnetic and superparamagnetic lipid-based contrast agents

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Introduction: The recruitment of leukocytes into the vessel wall intima plays a significant role in the onset and progression of atherosclerotic lesions. Imaging of VCAM-1 expression in atherosclerotic plaques may serve as a surrogate marker of inflammatory cell recruitment into plaques. The purpose of this study was to image VCAM-1 expression in carotid artery lesions of apoE-/- mice, to compare the efficacy of a lipid-based paramagnetic and a lipid-based superparamagnetic contrast agent, and to relate contrast changes to plaque location.

Methods: Paramagnetic liposomes and superparamagnetic micellar iron oxides were prepared as previously described [1] and antibodies were conjugated to the distal ends of the PEG chains. Particles were either non-conjugated, conjugated to a non-binding control IgG, or an anti mouse VCAM-1 antibody. The contrast agents were incubated on mouse endothelioma cells over-expressing VCAM-1 upon TNFα stimulation, to verify their binding specificity. T_1 and T_2 values of cell pellets were measured at 6.3T. A tapered cast was placed around the right carotid artery of apoE-/- mice on 'western' type diet 9 weeks before the MRI experiment to induce a more vulnerable lesion upstream and a stabilized lesion downstream of the cast [2]. In vivo T_1w and T_2w spin echo imaging with fat saturation was performed at 6.3T before and 24 hours after injection of contrast agent. Mice were injected with control IgG coupled liposomes (n=4), anti-VCAM-1 liposomes (n=4), control IgG coupled micellar iron oxides (n=3) or anti VCAM-1 micellar iron oxides (n=3). The injected dose was 100 µmol Gd / kg or 10 mg Fe / kg body weight. Parameters for T_1w imaging were: TR=800 ms, TE=10.3 ms, FOV=2.56^2 cm^2, matrix=256^2, slice thickness=0.5 mm, NA=8, scan time=27 min, and for T_2w imaging: TR=2000 ms, TE=20 ms, NA=4, scan time=34 min, and otherwise identical parameters.

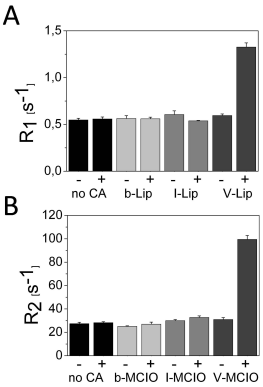

Figure 1: A) R_1 values of liposome incubated cell pellets. **B)** R_2 values of micellar iron oxide incubated cell pellets. (+) are TNFα stimulated cells and (-) are non-TNFα stimulated cells

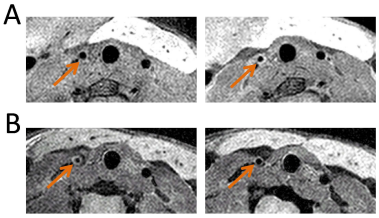

Figure 2: MR images of mice injected with **A)** anti-VCAM-1 liposomes or **B)** anti-VCAM-1 micellar iron oxides before (left) and 24 hours after (right) i.v. administration. The lesioned artery is indicated by the arrow.

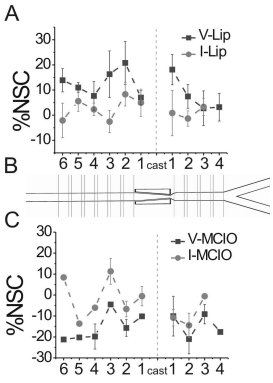

Figure 3: Percentage of normalized signal change (%NSC) per slice position for **A)** liposomes and **C)** micellar iron oxides. **B)** shows an overview of the slice position relative to the cast.

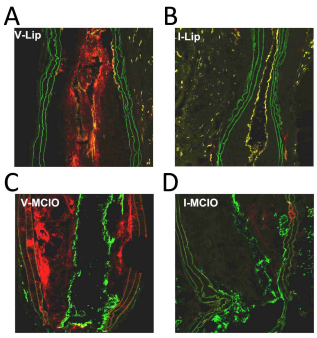

Figure 4: CLSM images of longitudinal sectioned right carotid arteries of mice injected with **A)** Anti-VCAM-1 liposomes, **B)** control IgG-liposomes, **C)** anti-VCAM-1 micellar iron oxides and **D)** control IgG-micellar iron oxides. **Red:** contrast agent; **Green:** elastin and CD31 in C and D; **Yellow:** CD31 in A and B.

Results: Coupling of antibodies to the nanoparticles led to an increase in diameter (dynamic light scattering). For liposomes the diameter increased from ~125 nm to 150-175 nm, and for micellar iron oxides from ~40 nm to 50-55 nm. A significant decrease in blood circulation half-life was found for the anti-VCAM-1 conjugated particles. For liposomes the half-life decreased from 7 hrs to 35 min, and for micellar iron oxides from 2.5 hrs to 45 min. In vitro experiments showed high and specific uptake of VCAM-1 targeted particles by VCAM-1 over-expressing cells. Figure 1 shows R_1 and R_2 values for liposome and micellar iron oxide incubated cell pellets, respectively. Both show a strong increase in relaxation rate only after incubation with VCAM-1 targeted particles on VCAM-1 expressing cells (indicated by +) and not with the controls. Contrast changes were found with in vivo MRI of the right carotid artery. Figure 2 shows images before and after administration of anti-VCAM-1 liposomes (fig 2A, T1w) and anti-VCAM-1 micellar iron oxides (fig 2b, T2w). Significant vessel wall intensity increase and decrease was found for liposomes and micellar iron oxides, respectively. Figure 3 shows the average signal change per slice position. Maximum signal change was found at the shoulders of the plaque, at the sites which are most active in inflammatory cell recruitment and less at the positions where the plaques are largest. This aspect as well as quantitative differences between para- and superparamagnetic agents need further statistical analysis. Figure 4 shows fluorescence microscopy images of ex vivo tissue sections. This confirmed that only VCAM-1 targeted agents significantly accumulated in the plaques. Both contrast agents co-localized with endothelial cells, while further accumulation differed. Liposomes tended to stay closer to the vessel lumen, while micellar iron oxides penetrated deeper into the plaques. This might be caused by differences in their size.

Conclusions: Paramagnetic and superparamagnetic lipid-based contrast agents targeted to VCAM-1 were prepared. Both contrast agents showed strong and specific accumulation in VCAM-1 over-expressing cells in vitro. In vivo targeting to mouse carotid artery atherosclerotic plaques was demonstrated, where location of contrast enhancement indicated a higher association with the plaque shoulders, which are known to be most active in inflammatory cell recruitment

References: [1] van Tilborg, G.A. et al. Bioconjug Chem, 2006, 17: 741-749; [2] Cheng, C. et al. Circulation, 2006, 113: 2744-2753.