Passive Targeting of Atherosclerosis with Paramagnetic Lipid Nanoparticles in a Mouse Model of Vulnerable and Stable Plaques

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Introduction: Contrast-enhanced imaging of atherosclerotic plaques using Gd^{3+}-containing T_1 lowering contrast agents is developing into a powerful tool to detect and characterize atherosclerotic lesions. Targeted agents could be used to detect specific plaque components, especially those that play an important role in plaque destabilization are of interst. Recently, a mouse model has become available in which both stable and vulnerable plaque phenotypes are induced by the placement of a tapered cast around the right carotid artery of $apoE^{-/-}$ mice on a high cholesterol diet[1] (figure 1). The strength of this model is that it provides an excellent opportunity to study differentiation of vulnerable and stable plaque phenotypes with targeted contrast agents within the same scan period.

Aim: To evaluate the contrast generated in the atherosclerotic plaques by intravenous injection of Gd^{3+}-liposomes and Gd^{3+}-micelles[2] to obtain insight in their permeation into the stable and vulnerable lesions. This provides essential information for future studies with targeted agents.

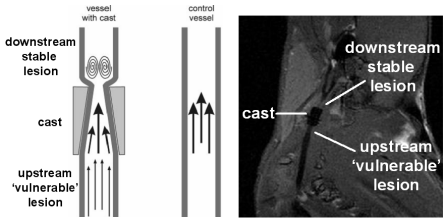

Figure 1: Schematic representation of the cast (figure adapted from [1]) and a sagittal image of the right carotid artery of a mouse with cast.

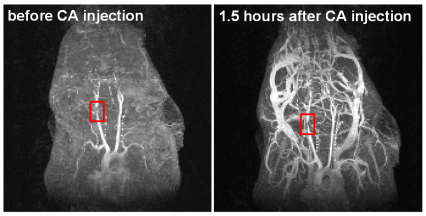

Figure 2: Angiogram (MIP) before (left) and 1.5 hours after (right) contrast agent injection. A signal void is observed at the position of the cast (red box). Arterial and venous enhancement is observed after contrast agent injection.

Materials and Methods:

Contrast agents: Liposomes (size~100 nm), containing Gd-DTPA-bis(stearylamide), DSPC, cholesterol, PEG(2000)-DSPE and Mal-PEG(2000)-DSPE and Rhodamine-PE were produced by lipid film hydration and extrusion[3]. Paramagnetic micelles (size~15 nm) were prepared from PEG(2000)-DSPE, Gd-DTPA-bis(stearylamide) and Rhodamine-PE.

Mouse model: 12 ApoE-/- mice were put on a high cholesterol diet. After 3 weeks a cast was surgically placed around the right carotid artery to induce both vulnerable (upstream) and stabilized (downstream) plaque[1]. For imaging the mice were separated into 4 groups: 6 weeks after surgery receiving liposomes (n=2) or micelles (n=4), 9 weeks after surgery receiving liposomes (n=2) or micelles (n=4).

MRI: T_1-weighted spin-echo images (TR/TE/NEX/slice thickness/FOV/matrix: 800ms/10.2ms/8/0.5mm/2.56x2.56cm^2/256x256) were acquired before, 15 min. after, 60 min. after and 24 hrs. after injection of contrast agent (1.25 µmol Gd) in all groups. Angiography was performed with a 3D-FLASH sequence (TR/TE/α/FOV/matrix: 15ms/2.5ms/30°/3.5x3.5x4cm^3/256x192x192) before and 1.5 hours injection of the contrast agent.

Data analysis: Signal intensities were calculated from circular regions of interest of the vessel wall and were normalized to muscle signal. One-way ANOVA was performed to test whether signal enhancement over time was significant.

Histology: Histological stainings were performed to evaluate plaque phenotype: Hematoxylin & Eosin, Oil Red O to stain the plaque lipids, Picrosirius Red for collagen and CD68 immunohistochemistry to visualize plaque macrophages.

Results: A sagittal view of the entire right carotid artery shows the signal void in the region of the cast and narrowing of the lumen due to plaque formation on both sides of the cast (figure 1). Angiography (figure 2) before contrast agent injection shows bright signal of the large arteries and the carotids, which confirms that blood flow through the cast was preserved. A signal void was observed at the position of the cast due to partial volume effects. After injection of the contrast agent the larger venous vessels and smaller arteries also became visible. This contrast was still visible after 24 hrs., indicating a long circulation time of the contrast agent.

Very little or no signal enhancement was observed for mice injected with liposomes. For the mice injected with micelles, the 6 weeks animals showed an increase in signal intensity after 24 hrs., especially in the upstream vulnerable lesion, whereas for the 9 weeks group the increase of signal intensity was near equal downstream and upstream (figures 3 and 4). Signal enhancement over time was statistically significant (one-way ANOVA, $p<0.05$) only in the upstream plaque for mice injected with micelles, both 6 weeks and 9 weeks after surgery. Rhodamine fluorescence from the micelles was observed diffuse throughout the plaques (figure 5). Plaque phenotype was confirmed by immunohistological staining of vessel wall sections. In the upstream vulnerable plaque less collagen and more lipids and macrophages were found as compared to the downstream stable plaque.

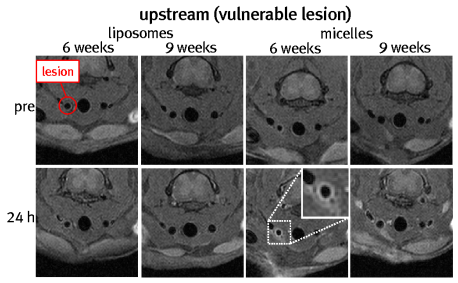

Figure 3: T_1-weighted images of the upstream (vulnerable) lesion, before and 24 hours after contrast agent injection

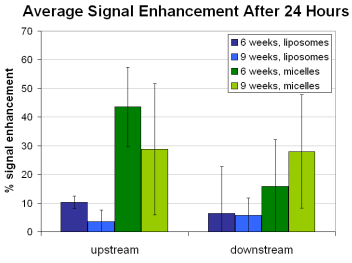

Figure 4: Average signal enhancement (±SD) 24 hours after contrast agent injection

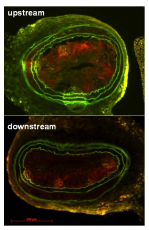

Figure 5: Fluorescence microscopy (6 weeks after surgery injected with micelles) Green: elastin Red: rhodamine-PE

Discussion and Conclusions: While both stable and vulnerable plaques seem to be non-permeable for liposomes, accumulation of the smaller micelles was observed in both lesion types, with the highest accumulation in the vulnerable plaque 6 weeks after surgery. Therefore, liposomes are a good candidate contrast agent for targeting endothelial markers. Micelles do enter both the lesions, and therefore may also be suitable for targeting factors inside the atherosclerotic plaque.

References: ***[1]*** Cheng C. et al. *Circulation*; 113(23):2744-2753, 2006 ***[2]*** Mulder W.J.M. et al. *NMR Biomed.*; 19:142-164, 2006
[3] Mulder W.J.M. et al. *Bioconjugate Chem*; 15(4):799-806, 2004