In vivo MR imaging of endothelial VCAM-1 expression in a mouse model of both stable and vulnerable atherosclerotic plaque

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Introduction:

VCAM-1 expression on the vascular endothelium plays an important role in the onset of atherosclerotic lesions and remains upregulated during plaque progression. Recently, a mouse model has been developed in which both advanced stable and vulnerable lesions are induced, by the placement of a tapered cast around the right carotid artery of $apoE^{-/-}$ mice[1]. Although VCAM-1 specific imaging with MRI by itself is not new[2], our approach is. In this work, *in vivo* MR-imaging of endothelial VCAM-1 expression was studied in both types of atherosclerotic lesions using VCAM-1 targeted paramagnetic and fluorescent liposomes[3]. Liposomes do not enter these atherosclerotic plaques easily due to their size. This is required for a low background signal by nonspecific accumulation of contrast agent in plaque and to discriminate between endothelial and smooth muscle cell VCAM-1 expression. However, this approach is challenging, because the contrast agent location will be restricted to one layer of cells, which pushes the limits of MRI to detect these sparse molecular epitopes.

Materials and Methods:

Contrast agent: 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].

In vitro: To investigate the specificity of targeted liposomes for VCAM-1, mouse endothelioma cells, H5V, were activated for 16h with TNFα (250ng/mL) to upregulate VCAM-1 and subsequently incubated for 1h at 37°C with either control liposomes (no ligand) or liposomes conjugated with a nonspecific antibody (IgG) or anti-mouse VCAM-1 antibody. Cell pellets were made for determination of the T_1 and quantification of Gd^{3+} and rhodamine-PE content. Furthermore, rhodamine-PE was visualized with confocal laser scanning microscopy.

In vivo: Contrast enhancement by intravenous injection of control (n=2), control IgG (n=3) or anti-VCAM-1 (n=5) liposomes was studied in $apoE^{-/-}$ mice on a high cholesterol diet, 9w after placement of a cast around the right carotid artery. Multi slice *in vivo* T1w MR-images (TR/TE/NEX/FOV/matrix/slice thickness=800ms/10.2ms/8/2.56*2.56cm^2/256*256/0.5mm) were acquired before and 15min, 60min and 24h after injection of liposomes. Plaque characterization and liposome localization was performed *ex vivo* by fluorescence microscopy (FM) on histological sections.

Results:

The cellular relaxation rate (R_1) was significantly increased by incubation of activated H5V cells with anti-VCAM-1 liposomes compared to nonactivated cells incubated with anti-VCAM-1 liposomes or (non)activated cells incubated without contrast agent or with control (IgG) liposomes (figure 1). The high level of association of anti-VCAM-1 liposomes with VCAM-1 expressing cells was confirmed quantitatively by determining gadolinium and rhodamine-PE content with ICP-MS and fluorimetry, respectively (not shown).

Minor signal enhancement (SE) was found in all mice in both vulnerable (upstream of cast) and stable (downstream) plaques at all time points after injection of control (IgG) liposomes, indicating low permeability of plaques to liposomes and low nonspecific binding to the endothelium. SE was only found in one mouse upstream of the cast 15min and 60min after injection of anti-VCAM-1 liposomes (figure 2) and downstream in four mice, although the latter effect did not reach significance (one-way ANOVA, p=0.08). The presence of anti-VCAM-1 liposomes on the endothelium in the vulnerable plaque was confirmed by FM (figure 3) and was specific, since IgG conjugated liposomes were not detected.

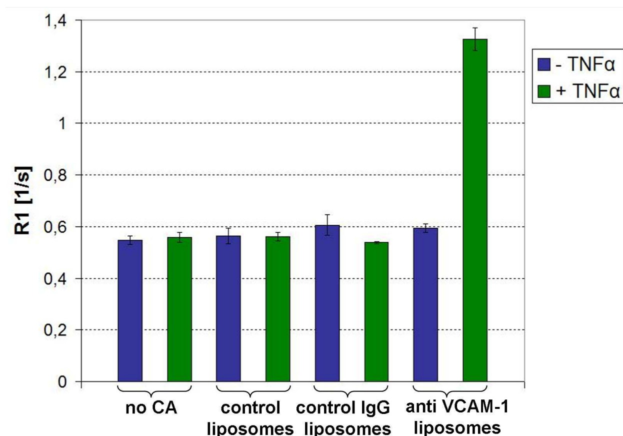

Figure 1: Longitudinal relaxation rate of pellet of H5V cells at 6.3T (mean±sd; n=2 or 3).

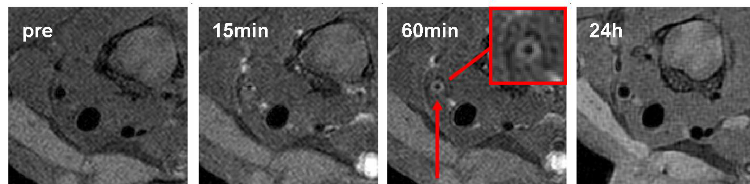

Figure 2: *In vivo* T1w MRI upstream of cast before and after injection of anti-VCAM-1 liposomes. Contrast enhancement was found 15min and especially 60min (arrow) after injection, and was still visible after 24h. Right carotid artery is shown left.

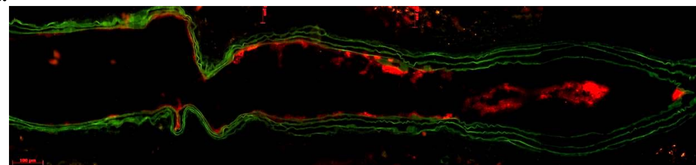

Figure 3: FM of longitudinal cross section of right carotid artery 24h after injection of anti-VCAM-1 liposomes; from left to right: upstream of cast to beginning cast. Image was reconstructed from 5 separate images (red=rhodamine-PE)

Discussion:

In this study the possibilities of MR-imaging to *in vivo* visualize the expression of VCAM-1 on the endothelium covering atherosclerotic plaques were explored. Anti-VCAM-1 liposomes were designed that showed a high specificity for VCAM-1 *in vitro*. Fluorescence microscopy showed that *in vivo* targeting to vascular endothelium occurred. MRI of VCAM-1 expression with these liposomes showed variable signal enhancements. The MRI contrast may have been affected by several factors such as the interaction mechanism of targeted liposomes with endothelial receptors or blood flow. Therefore, alternative MRI sequences for optimized detection of the target-associated liposomes are currently explored.

References ***[1]*** Cheng C. et al. *Circulation*; 113(23):2744-2753, 2006 ***[2]*** Nahrendorf M. et al. *Circulation*; 114:1504-1511, 2006 ***[3]*** Mulder W.J.M. et al. *Bioconjugate Chem*; 15(4):799-806, 2004.