Detection of intimal thickening by contrast-enhanced MRI using paramagnetic liposomes

W. J. Mulder[1], G. J. Strijkers[1], J. W. Habets[1], G. A. Koning[2,3], E. Lutgens[4], K. Douma[5], M. A. van Zandvoort[5], D. W. Slaaf[1,5], K. Nicolay[1]

[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands, [2]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Delft University of Technology, Delft, Netherlands, [3]Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands, [4]Department of Pathology, Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, Netherlands, [5]Department of Biophysics, Cardiovascular Research Institute Maastricht, University of Maastricht, Maastricht, Netherlands

Introduction

Magnetic resonance imaging (MRI) is starting to become a key imaging modality in the detection of atherosclerosis and might become the standard imaging modality to evaluate and quantitate (the progression of) atherosclerosis[1]. Several studies have shown that conventional high resolution MRI is capable of detecting atherosclerotic plaques, both in human atherosclerosis and animal models of atherosclerosis[2], but is not capable to visualize atherosclerotic lesions. Previously, contrast enhanced MRI with Gadofluorine has been used for improved plaque detection[3].

In this study we demonstrate a method to use contrast enhanced MRI for the detection of a lesion in a model of atherosclerosis (ApoE-/- mice) following injection of fluorescently labeled, paramagnetic liposomes.

Materials and Methods

Paramagnetic liposomes containing a Gd-DTPA based lipid and rhodamine-PE were prepared by lipid film hydration as described previously[4].

As a model for an early atherosclerotic lesion ApoE-/- mice were used, in which accelerated atherosclerosis was induced by placing a stiff polyethylene cuff around the right carotid artery proximal to the bifurcation. Two mice were assessed with histology using hematoxylin-eosin staining to evaluate the model. 9-12 Days after placement of the cuff the mice were scanned on a 6.3 T MR scanner. A careful planning procedure was used to avoid partial volume effects and to enable good comparison between the different time points. The procedure started with acquiring low resolution scout images to determine a plane parallel to the carotid arteries. Next, high resolution MR images perpendicular to this plane were acquired. In all experiments the FOV was 3x3 cm^2, matrix size was 256x196, number of signal averages was 10, and the slice thickness was 0.5 mm. After zero-filling to 512^2 the in plane resolution was 59 µm. 2 Mice were evaluated with conventional MRI; T1-weighted images (TR 800 ms, TE 12 ms), T2-weighted (TR 2500 ms, TE 30 ms) and PD-weighted images (TR 2500 ms, TE 12 ms) of the neck region were acquired. For contrast enhanced MRI a T1-weighted (TR 800 ms, TE 12 ms), black blood, spin echo sequence was used. The neck area of the mice was imaged pre and 15 minutes, 45 minutes and 24 hours post contrast agent injection. 6 Mice were injected with fluorescently labeled, paramagnetic liposomes and 3 mice were injected with Gd-DTPA. After MRI, the mice were sacrificed and the carotid arteries were surgically isolated. Two-photon laser scanning microscopy of intact vessels may be used to localize the liposomal contrast agent at submicron resolution. MRI signal intensities were analyzed by placing a ring shaped ROI around the border of the arterial lumen, using Mathematica.

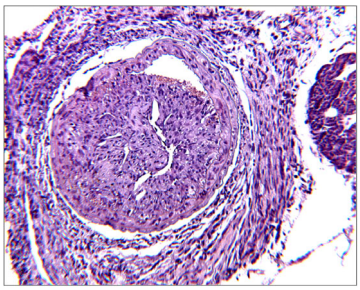

Figure 1: Histological transverse cross-section of right carotid artery proximal to the cuff.

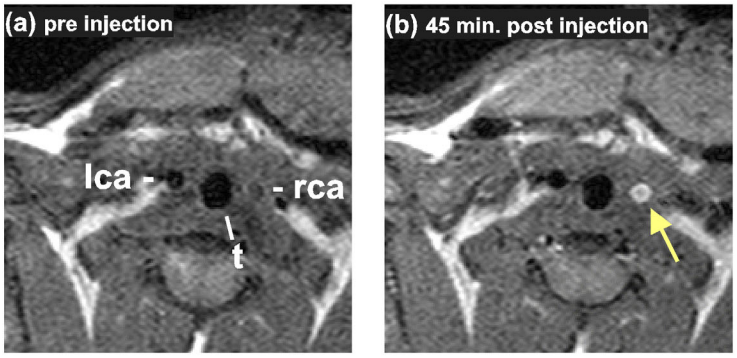

Figure 2: Transversal MR images through neck area, as measured (a) pre and (b) 45 min. post injection with Gd-liposomes. (lca=left carotid artery, rca=right carotid artery, t= trachea).

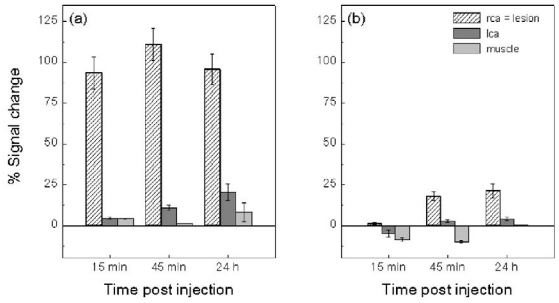

Figure 3: Change in signal intensities (%) of right carotid artery (rca) wall, left carotid artery wall (lca) and muscle ROI, 15 min., 45 min., and 24 hours after injection with (a) Gd-liposomes and (b) Gd-DTPA.

Results and Discussion

The model for cuff-induced lesion formation in the mouse carotid artery of was evaluated by histopathology (figure 1). 9-12 Days after placement of the cuff intimal thickening was observed proximal to the cuff and an increase of smooth muscle cells and foam cell deposition was seen. In figure 2a a transversal slice through the neck area is depicted. The right carotid artery has a narrowed lumen compared to the left carotid artery as a result of the cuff placement. Upon injection of liposomes the lesion site becomes clearly visible as a bright ring (figure 2b, arrow). Quantitative analysis (n=6) revealed a dramatic signal enhancement in the lesion area (figure 3a) within 15 minutes after contrast agent injection. This signal enhancement was still preserved 24 hours after contrast agent injection. On the contrary, upon injection of Gd-DTPA only minor contrast difference between the healthy carotid artery and the cuffed carotid artery was observed at all time points.

Conclusions

We demonstrated the detection of intimal thickening by contrast enhanced MRI upon injection of paramagnetic liposomes. Since liposomes are used as a drug carrier vehicle this approach can also be used to deliver anti-inflammatory drugs to atherosclerotic lesions. Furthermore, these liposomes can be functionalized by conjugating a targeting ligand[4] for MRI detection of molecular targets expressed at these lesion sites.

References

1. Yuan and Kerwin, J Magn Reson Imaging. 2004; 19.
2. Choudhury et al., Arterioscler Thromb Vasc Biol. 2002; 22.
3. Barkhausen et al., Circulation. 2003 ; 5.
4. Mulder et al., Bioconjug Chem. 2004 ; 15.