A Magnetic Resonance Imaging Contrast Agent Targeted Towards Activated Platelets Allows Detection Of Platelets On Symptomatic Human Carotid Plaques

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1. Introduction: Platelets play an important role in the rupture of atherosclerotic plaques. Especially in late stages of atherosclerosis platelets can attach to the endothelial surface and lead to secondary damage such as stroke or heart attack. So far with imaging techniques like MRI/CT and coronary angiography, only the degree of atherosclerotic narrowing, but not the stage, and thus the potential risk of arterial wall changes can be observed. It would be an important advance in the diagnosis, to identify late stages of atherosclerosis to initiate early treatment measures. In this study, the imaging of activated platelets in symptomatic human atherosclerotic carotid plaques with a 9.4 Tesla MRI is under investigation.

2. Methods: Contrast agent: The contrast agent is a single-chain antibody (LIBS) to the activated form of GPIIb/IIIa receptor which is coupled to a micro-particle of iron oxide (MPIO). The contrast agent (LIBS-MPIO) binds to activated platelets attached to the endothelium of the carotid interna plaques and results in signal loss on MRI. As a control group, MPIOs were coupled to a non-functioning single chain antibody (Control-MPIO). Samples: The human carotid plaques (n= 16; 8 LIBS-MPIO, 8 Control-MPIO) obtained by carotid endarterectomy were incubated under dynamic conditions with the contrast agent. For the dynamic conditions the plaque was connected to a roller pump and incubated under flow conditions (100 ml/min) with the contrast agent (Fig.1). MR-methodology: A 9.4 Tesla small-animal MRI system (Bruker) is used for the measurement (TR: 500 ms, TE: 7 ms, Flip-angle 45°, No of averages: 4, Total activation time: 4min16 sec, resolution: 105 \(\mu\)m). A measurement before and after contrast agent administration was processed. Evaluation: The difference (pre- vs. post contrast agent) of the SNR of the average voxel intensity in the MR data was measured in MIPAV by using regions of interest (Fig.2). After the measurement the plaques were histologically processed and treated with an immunohistochemical platelet staining. This is followed by microscopically quantification of the platelet areas and the bound MPIOs.

3. Results: A significant signal loss was observed when incubated with LIBS-MPIO compared to control (Fig.3). The Difference of SNR in average voxel intensity was 4.635 for LIBS-MPIO compared to 0.7535 for Control-MPIO (p<0.05). The specific binding of LIBS-MPIO to activated platelets was confirmed by the immunohistochemical staining (Fig.4). The specific bound MPIOs for LIBS-MPIO was 97.66/mm² platelet area compared to 39.49/mm² platelet area for Control-MPIO (p<0.05). The correlation of MRI data with histology shows a coefficient of determination \(r^2\) of 0.68.

4. Discussion and outlook: Based on the results so far we showed that the used contrast agent enables the display of activated platelets on symptomatic human carotid plaques under dynamic conditions. A correlation of the MR-data with the histology-data was confirmed. In the course we will increase the number of plaque. Furthermore the comparison of the MR- and histological-data with the clinical symptoms of the patients will be in the focus of our work.