First Results of an ex-vivo Experiment on Human Plaques Using a Contrast Agent Targeting Activated Platelets

D. Paul[1], C. von zur Mühlen[2], J. Möller[2], T. Spehl[3], C. Bode[2], K. Peter[4], and D. von Elverfeldt[1]

[1]Dept. of Diagnostic Radiology, Medical Physics, University Hospital Freiburg, Freiburg, BW, Germany, [2]Department of Cardiology and Angiology, University Hospital Freiburg, Freiburg, BW, Germany, [3]Department for Neurology and Neurophysiology, University Hospital Freiburg, Freiburg, BW, Germany, [4]3 Department of Atherothrombosis and Vascular Biology, Baker Heart Research Institute, Melbourne, Victoria, Australia

Introduction

Targeted MRI contrast agents are a rapidly evolving technique. Specific imaging of activated platelets, such as found within a thrombus on the surface of ruptured atherosclerotic plaques, represents a promising tool for clinical diagnosis of acute coronary syndromes. Here, we are reporting on the application of a molecular MRI contrast agent consisting of microparticles of iron oxide (MPIO) and single-chain antibodies targeting ligand-induced binding sites (LIBS) on activated glycoprotein IIb/IIIa-receptors (1,2), and its use to selectively detect activated platelets on the surface of human carotid artery thrombendarterectomy (TEA) specimens ex vivo. The aim of this work was to evaluate 1.) the functionality of the specific contrast agent in human plaque and 2.) the concept of an ex-vivo experimental MRI setup.

Methods

For the contrast agent construction, LIBS single-chain antibodies were conjugated to 1 µm sized MPIOs, resulting in the LIBS-MPIO contrast agent. Endovascular specimens of human carotid arteries were removed by TEA during vascular surgery. The plaque was stored in a warm NaCl-bath (37° C) immediately after extraction and transported to the on-site MRI system (within 5 min walking distance). High resolution MRI was performed on a 9.4T system (Bruker BioSpin 94/20, Bruker, Ettlingen, Germany) including T2- and T2*-weighted image protocols:

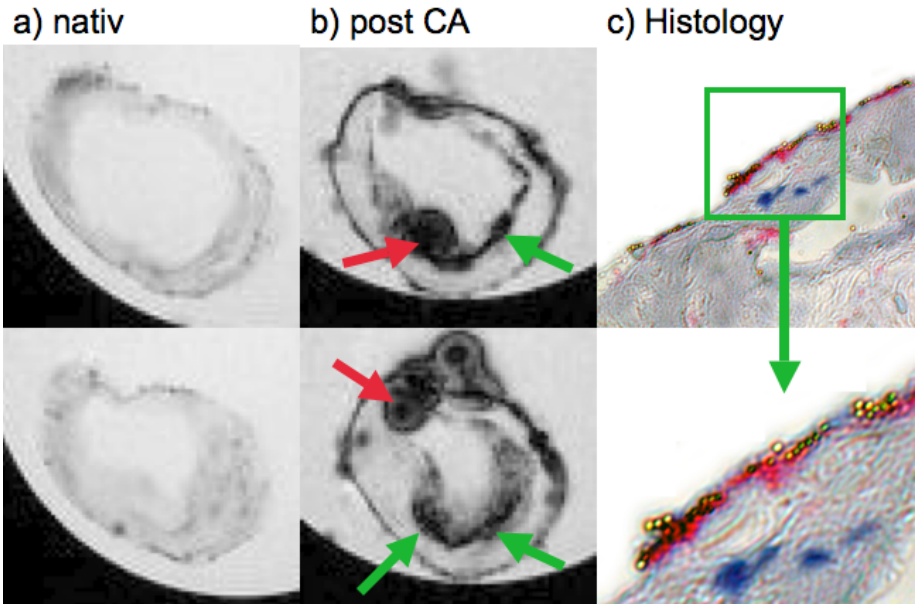
Geometry: 256x256matrix, 20 slices; Resolution: 117µm x 117µm x 1mm.
T2-w.: TE=60ms, TR=5000ms, 4av, TF=8; TA=8min;
T2*-w.: TE=7ms, TR=1000ms, a=45°; TA=4min;
Data acquisition was performed prior to (native) and several times after CA incubation:

1. native scan: T2-w and T2*-w imaging
2. contrast agent incubation: 15 min, swaying
3. washing I: 3 times for 5 min in clean PBS solution
4. post contrast agent scan I: T2-w and T2*-w imaging
5. washing II: 3 times for 5 min in clean PBS solution
6. post contrast agent scan II: T2-w and T2*-w imaging

As reference, plaque tissue was examined by histology for anatomic information, and by immunohistochemistry to detect platelets using antibodies against GPIIb/IIIa (anti-CD41 antibody).

Results & Discussion

Figure 1 shows exemplary results from T2* MR imaging and histology. In areas inside plaque lumen, a clear decrease in signal intensity can be observed after LIBS-MPIO incubation (compare Fig. 1a and 1b, green arrows). LIBS-MPIO binding in these areas was confirmed by histology (Fig. 1c), showing the yellow/blue appearance of MPIOs at areas of platelets deposition (stained red by immunohistochemistry).



Problems arose from air bubbles leading to artifacts (Fig. 1b, red arrows). In addition, image analysis was hindered by different positioning of the plaque due to washing procedures between imaging sessions and a missing plaque fixation.

In conclusion, these pilot data show the high potential of this ex-vivo MRI using human plaques. It also demonstrates that LIBS-MPIO binds to human plaques, proven by histology, and results in a clearly identifiable signal void in MRI.

Further studies involving larger numbers of TEA specimens will have to evaluate the correlation of the quantity of platelet aggregation with the initial clinical symptoms of the patient, as well as the prognostic value of activated platelet aggregation on the plaque surface.

Literatur

(1) Schwarz et al. Circ Res. 2006 Jul 7;99(1):25-33.
(2) Schwarz et al. FASEB J. 2004 Nov;18(14):1704-6.