Ex vivo and *in vivo* MR imaging of ischemia reperfusion injury in mouse hearts using microparticles of iron oxide targeting VCAM-1

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Introduction: Ischemia-reperfusion (IR) injury is a major cause of tissue damage in vascular syndromes of the heart. Myocardial ischemia in the myocardium leads to oedema, inflammation and eventually to cell-death. T_2-weighted magnetic resonance imaging (T2w-MRI) has been shown to visualize and to quantify edema in the acutely infarcted myocardium, while Gadolinium (Gd)-based delayed enhancement methods identify the area of necrosis/scar. Sensitive markers of early inflammation in reversible myocardial injury are lacking. The aim of our study is to investigate whether antibody-conjugated microparticles of iron oxide (MPIO) targeting VCAM-1 would enable molecular MR imaging of endothelial activation in murine IR hearts.

Materials & Methods: The left coronary artery was occluded in C57BL/6 mice for 30 minutes. Following a 22-24 hour reperfusion period, the mice were subjected to MRI on a 9.4T VNMRS DirectDrive MR system (Varian Inc. USA). *Ex vivo protocol*: 16µl antibody-conjugated MPIO's in 84 µl saline were injected via the jugular vein into anesthetized control and IR mice (n=3 each). Following a 30min circulation period, the hearts were excised, perfused with heparinised saline, perfusion fixed and stored in PFA. After embedding in 1% agarose (spiked with 2mM Gd), high-resolution MRI was performed using a 28 mm quadrature driven birdcage coil and a spoiled gradient-echo sequence (TE/TR=6.6/30ms, FOV=26×26×40mm, matrix size=512×512×1024, 4 averages). The left-ventricular myocardium was segmented in 16 equidistantly spaced slices throughout the entire heart. The number of low-intensity pixels (LIP) in septum and free wall, normalized to the total number of pixels for each compartment (threshold: $SI_{Control} - 2\times SD_{Control}$), was calculated on a slice-by-slice basis. *In vivo protocol*: a double-gated, flow-compensated, segmented 3D GE-sequence (TE/TR=2.5/4.1ms, 8 k-space lines per cardiac cycle, FOV=25.6x25.6x12mm, 8mm short-axis slice, flip angle 10°, matrix size 256x256x64, 2 averages) was applied pre- and post tail or jugular vein injection of 16µl antibody-conjugated MPIO's in 84 µl PBS, flushed with 100 µl PBS in 2 IR mice. In addition, T2w-imaging was performed (multi-slice 2D spin echo, TE = 20 ms, TR = 1× respiratory cycle).

Results: Figure 1 shows a mid-ventricular slice through a control (left) and an IR heart (right). Part of the free wall exhibits signal drop-out, as indicated by the arrows. Figure 2 illustrates the mean LIPs for the free wall of the IR hearts (open bars), which are consistently higher than the mean LIP for the control hearts (filled bars). The error bars depict the standard deviation. The mean LIPs for the septal compartment were similar for both groups and comparable to the free wall data for the controls (data not shown). Figure 3 shows *in vivo* image of an IR heart (a) pre and (b) post application of MPIO, resulting in defined signal voids within the myocardium (arrows in 3b) and within the area of edema as illustrated by the T2w-image in Figure 3c.

Figure 1: Ex vivo MRI of a control and an IR heart.

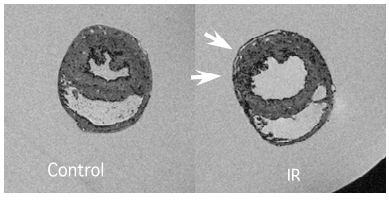


Figure 2:LIP quantification in control (filled bars) and IR (open bars) hearts ex vivo (mean ± SD).

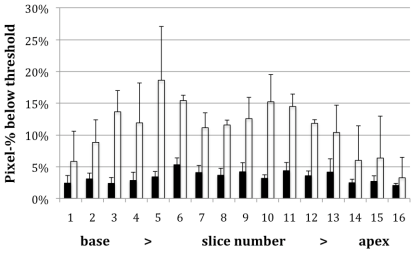
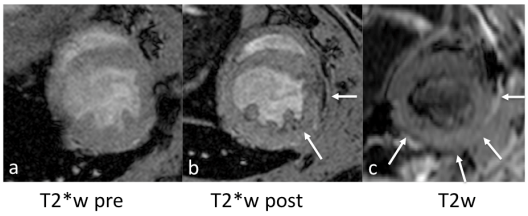


Figure 3: In vivo MRI on an IR heart pre (left) and post (middle) MPIO application. The right panel shows a T2w image.



Discussion & Conclusion: *Ex vivo* MRI on IR hearts subjected to injection of antibody-conjugated microparticles of iron oxide (MPIO) targeting VCAM-1, showed a consistent decrease in signal in the free wall compared to controls. Signal drop-outs were also found in the pilot experiments *in vivo* (n=2). This is a first indication of the feasibility of molecular imaging of IR injury in the mouse heart by targeting VCAM-1. Future experiments will use an irrelevant antibody such as IgG to verify the specificity of these initial findings, and will relate signal drop-out to histological assessment of IR injury.

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