MRI investigation of an iron contrast agent in a mouse model of myocardial ischaemia reperfusion

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Introduction Adverse remodeling after myocardial infarction (MI) is the most frequent cause of heart failure and may involve an inflammatory component [1]. To better understand post-MI remodelling, we investigated the role of the inflammatory response by *in vivo* cardiovascular MR imaging (CMRI) and supplementary histological studies. Macrophages were chosen as the imaging target because of their key roles in the inflammatory process that follows a myocardial insult and cardiac remodeling [2]. In this study, *in vivo* MRI protocols have been utilized to investigate the mechanisms and biological relevance of very small iron oxide particle (VSOP) uptake in a mouse model of MI induced by ischaemia reperfusion.

Methods 15µl of VSOP (250 µmol/kg, 15mg iron/kg) was injected intravenously. In the *post MI protocol*, VSOP was injected at different time points: a) 30 mice were injected 24 hours after MI and the MRI scan was performed 2 hours and 48 hours after injection of VSOP. After the imaging session, 10 mice were sacrificed. The remaining 20 mice were re-imaged 8 days after MI; b) 10 mice were injected 28 days post MI and MRI was performed 48 hours after VSOP injection. CMRI was performed on a 7T horizontal MR scanner (Varian Inc., Palo Alto, CA). Cine-FLASH was used to acquire temporally resolved dynamic T1 weighted images to achieve functional and volumetric parameters [3]. T2* images at end-diastole were used to identify VSOP enhanced areas. A T2* weighted imaging parameters included TR = 1 heartbeat; TE = 1, 2, 3, 5 ms; matrix = 128x128; FOV = 25x25mm; 1mm slice thickness; 5 slices; 3 averages and acquisition time = 10 minutes. T2* maps were created from MRI data. Complementary immunohistochemistry, Evan's blue staining, electron microscopy (EM) and macrophage counting were also performed to corroborate the MRI findings.

Results Figure 1 illustrates apex to base myocardial short axis images for an animal at 2 hours, 48 hours and 8 days post injection. Figure 2A reports T2* values and their percentage increments for the 2 hours, 48 hours and 8 days time points. The same approach is reported in Fig2B for VSOP areas. Areas of negative contrast decreased from early to late post MI time points. Figure 3C correlates the VSOP area extension with the T2* values calculated with a T2* map analysis. Significant correlation was observed at 2 hours (R^2=0.53) and 48 hours (R^2=0.503) after injection. Histology showed colocalisation of macrophages and VSOP particles, using MAC3 and Perls' Blue staining respectively, exclusively in the endocardial part of the left ventricle. Electron microscopy showed VSOP internalization (Figure 3). Figure 4B shows uptake of VSOP into two different clusters within a macrophage. A detailed image (Figure 4C) also shows single iron particles, of a size 7x7nm. The same behaviour was seen within cardiac myocytes where a VSOP cluster appears in the vicinity of a mitochondrion (Figure 4D). 3 days post MI, the number of white blood cells in the outer border of the infarct was larger (25±11%) than that seen at the inner border. A similar behaviour was seen at 8 days after MI where an increase in macrophages (18±9%) was seen between the inner and outer borders of the infarcted area. The total number of macrophages was reduced by 41±11% (43.2% in the inner border, 39.4% in the outer border) between 3 and 8 days. Evan's Blue showed higher permeability in the endocardium and in the pericardium of the myocardium in the infarcted areas when compared to healthy mice. No VSOP uptake was seen in MRI images and related histology in the 30 days experiment.

Discussion and Conclusion The work presented herein successfully reports *in vivo* detection of iron particles in a mouse model of MI by MRI. Iron particles were taken up by inflammatory macrophages in situ and a negative contrast was produced due to susceptibility effects. The inflammation site was therefore identified and studied over a time span of 4 weeks. Only macrophages close to the endocardium were identified to take up VSOP. The reason for such behaviour is probably due to two factors: endothelial permeability and perfusion. In conclusion, MRI provides information about the location of the iron particles, the extent of the latter and the T2* values related to VSOP areas. Such information determines the extent of the inflammation and the severity of the MI insult.

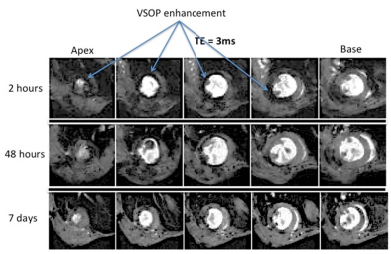

Figure 1. Apex to base MR images of mouse after reperfused MI. The VSOP area, identified as negative contrast in the left ventricle endocardium, diminishes from 2 hours to 48 hours to 8 days post injection.

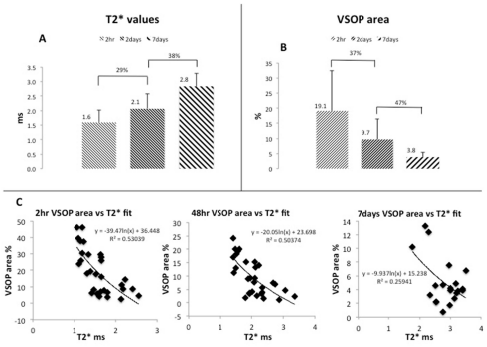

Figure 3. Diagrams and logarithmic correlation of T2* values and VSOP areas A) T2* values for the three studied time points. An increase in T2* is observed from early to later points. B) VSOP area for the three studied time points. A decrease in VSOP areas is observed from early to later points. C) VSOP areas versus T2* value fit for the three studied time points. Only the 8 days post MI data do not correlate significantly. 2 hours and 48 hours post injection data present a logarithmic correlation.

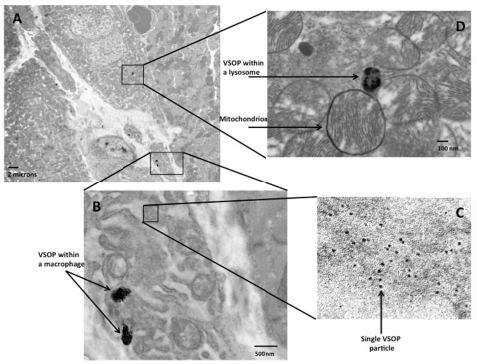

Figure 4. Electron Microscopy. A) 2 µm resolution view of a section of the infarcted myocardium. Two iron sites were identified. B) 100nm resolution view of VSOP within a lysosome. Mitochondrion is also identified. C) 500nm resolution view of VSOP within a microphage. Two VSOP clusters were created, probably due to lysosomes. D) Single VSOP particles were also found in the macrophage.

References

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