Imaging of inflammation using VSOP at multiple time points in a mouse model of myocardial infarction

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Introduction Myocardial infarction (MI) represents an acute injury of the myocardium and triggers the recruitment of monocytes and subsequent accumulation of macrophages at the site of injury. Despite intensive research in this field it is still poorly understood how many and which type of monocytes are involved in the migration into the infarct zone. The use of non-invasive imaging methods is therefore of great interest for the in-vivo investigation of the inflammatory response post MI as demonstrated recently [1]. MRI of post MI inflammation was performed with a dextran coated iron oxide nanoparticle, which have been shown to be phagocytosed by macrophages and to generate dark spots due to their T2* enhancing properties [1]. In this work, we sought to investigate the merits of a very small superparamagnetic iron oxide particle (VSOP) [2] for direct imaging of inflammation in a mouse model of MI. We investigated several time points after MI and with different injection protocols in order to understand the mechanism underlying iron-oxide particle uptake in this setting.

Method Two experiments, related to three time points, were performed in C57Bl6 female mice. Five mice per group were subjected to occlusion reperfusion (30 min) injury of the myocardium to generate an inflammatory response in the myocardium. 18µl of VSOP (250 umol/kg, 15mg iron/kg) were injected intravenously. The two protocols corresponded to: injection of VSOP 7 days pre MI and injection of VSOP 24h after MI. 1) In the pre MI protocol, VSOP was injected 7 days before surgery and MRI scan was performed 2 days after LAD ligation. 2) In the post MI protocol, VSOP was injected on day 1 post MI and MRI scan was performed 3 and 10 days post MI. The half-life time of VSOP in blood was estimated to be of few hours, thus allowing complete clearance of VSOP in any of the experiments. Histology was performed in all animals. After sedation of the animals with an isoflurane-oxygen mixture, images were acquired on a 7T horizontal-bore MR scanner (Varian, USA), with a gradient strength of 1000mT/m and a send/receive 39mm RF coil (Rapid, Germany). A T2* weighted gradient echo (FLASH) sequence was used to detect VSOP uptake in the infarct area. Imaging parameters included TR = 1 heartbeat; TE = 3 ms; matrix = 128x128; FOV = 25x25mm; 1mm slice thickness; 7 slices; 3 averages and acquisition time = 5 minutes. ECG triggering and respiration gating were used for the T2* weighted acquisition.

Results No VSOP uptake was observed in the infarct zone following the pre MI injection protocol in any of the mice but reduced functional/volumetric values were observed indicating myocardial damage.

In the post MI injection protocol, VSOP uptake was observed at 3 and 10 days post MI as a negative contrast due to susceptibility effects. No correlation was found between functional/volumetric values and the estimated size of the inflamed area by T2* weighted MRI. The signal void total area was greatest at day 3 by MRI and a 60±5% decrease was observed at day 10 (Figure 1). Figure 2 shows a Prussian Blue and macrophage (MAC3) stain of the infarct area at day 10 demonstrating colocalisation of VSOP and macrophages.

Discussion & Conclusion In this work we investigated the mechanism of VSOP uptake in a model of occlusion reperfusion injury of the myocardium. The findings from the pre injection experiment suggest that either VSOP is not taken up by blood born monocytes as reported for larger iron-oxide particles using a similar injection protocol [3], or the delay between injection and imaging was too long. Furthermore, monocyte recruitment may differ between MI occlusion reperfusion and occlusion only models. If VSOP was injected 1 day after coronary ligation, iron-oxide particles were found in the infarct area suggesting leakage of VSOP through injured endothelium at the infarct site and subsequent phagocytosis by macrophages. At day 10, the area of negative contrast decreased in size suggesting lower VSOP concentrations in the infarct zone. This finding suggests that VSOP laden macrophages either underwent apoptosis or were diminished due to migration. In conclusion, this work provides first insights into the uptake mechanism of very small iron particles by monocytes and macrophages in the infarct zone using different injection protocols and for different imaging time points post MI. VSOP uptake in the occlusion reperfusion MI model seems to behave differently from a more severe model of coronary occlusion.

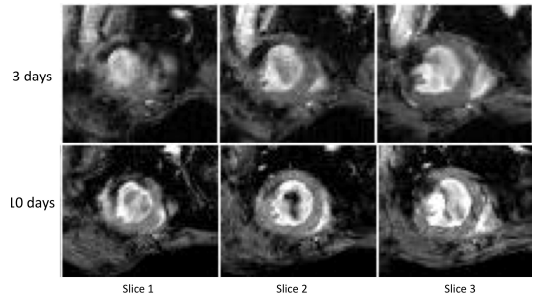

Fig1. T2* weighted images at day 3 and 10 after occlusion reperfusion MI and following VSOP injection at day 1. VSOP uptake can be observed in the infarct zone with a decrease in VSOP area at day 10.

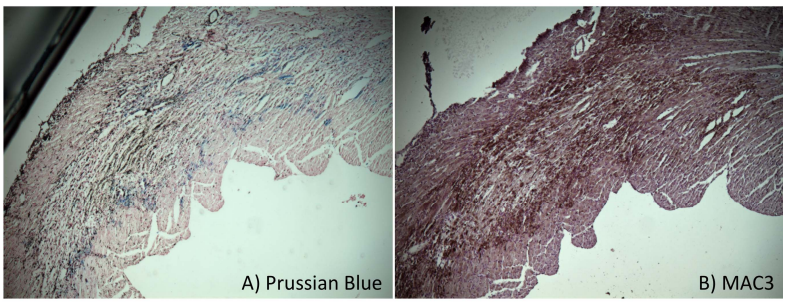

Fig2. A) Prussian Blue and B) MAC3 stain. VSOP positive areas (blue staining) colocalize with areas of increased macrophage density (brown staining) and are primarily seen in the periphery of the infarct area. Neither VSOP uptake nor macrophages are seen in the necrotic core.

References

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