Cellular MRI of monocyte infiltration: In vitro vs in vivo labeling

R. D. Oude Engberink[1], E. L. Blezer[1], E. I. Hoff[2], S. van der Pol[3], and H. E. de Vries[3]

[1]Image Sciences Institute, University Medical Center Utrecht, Utrecht, Netherlands, [2]Department of Neurology, Maastricht, Netherlands, [3]Molecular Cell Biology and Immunology, VU medical center, Amsterdam, Netherlands

Introduction

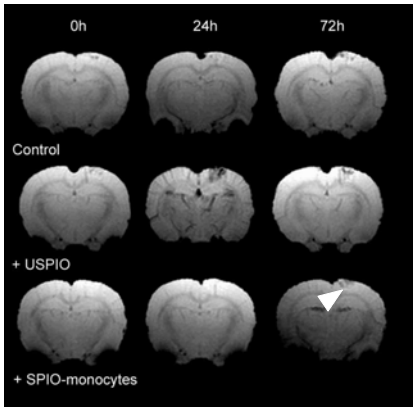
Infiltration of monocytes into the brain after ischemic damage plays an important role in ongoing inflammation and in the extent of tissue destruction [1]. The development of superparamagnetic particles of iron oxide (SPIO) has made MRI a valuable tool for in vivo cell tracking. Cellular MRI will provide a better insight into the inflammatory process longitudinally and may contribute to the development of anti-inflammatory therapeutics. In recent animal studies macrophage activity in cerebral ischemia was monitored after intravenous administration of ultra small SPIO (USPIO) [2-3]. It is believed that USPIO label monocytes in circulation and that hypointense spots on MR images represent actual cell infiltrates. However, it remains unclear what mechanism in vivo is responsible for USPIO entrance into the brain parenchyma. In addition to the infiltration of peripheral labeled monocytes, non-specific labeling and diffusion of extracellular USPIO over the blood brain barrier (BBB) may misleadingly contribute to MR enhancement. We hypothesized that transplantation of in vitro labeled monocytes may be a better tool. The purpose of this study was to compare MR enhancement in a focal brain lesion following transfusion of labeled monocytes to a single injection of USPIO. To further elucidate the mechanism of USPIO entrance into the brain, MRI was performed directly after USPIO injection up to 6h.

Material and Methods

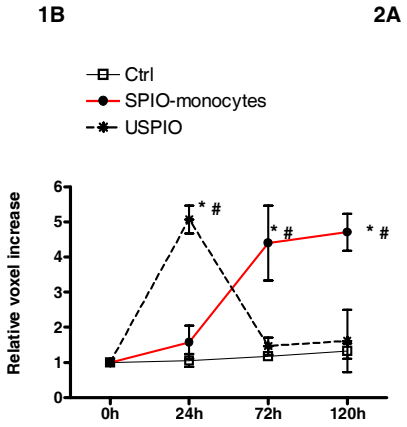
Focal ischemic damage was induced in 14 rats (male, 250g, Lewis Hannover) by photothrombosis of cortical microvessels using erythrosin B (20 mg/kg, 2.5min laser illumination) [4]. On day 5 post photothrombosis, rats were injected with USPIOs (n=3, Sinerem, Guerbet France, 17 mg Fe/kg, iv) or in vitro labeled monocytes (n=4, 5x 10^6 cells, iv). In vitro labeling of freshly isolated monocytes was done using SPIO (Feridex, Guerbet France, 4 mg Fe/ml, 1.5h). Control animals (n=4) were vehicle treated. Imaging (4.7T, Varian, Palo Alto, USA, FOV=3.2x3.2cm, matrix= 128x128, 21x 1mm) was performed before, 24h, 72h and 120h later. To assess acute USPIO dynamics, rats (n=3) received USPIO in the scanner and MRI was performed in a repeat loop of 12 cycles up to 6h. T_2-relaxation time images were calculated from a multi echo multi slice sequence (TR=3.2s, TE=17.5ms + 9*17.5ms, NEX=4). T_2*W images were acquired using a gradient echo sequence (TR=2.5s, TE=12.5, NEX=2). Hypointensities in brain lesions were analyzed by a voxel threshold based method on T_2*W (TE=12.5ms) and quantitative T_2 images. Data were evaluated by two-way ANOVA, followed by a multiple comparison procedure (SNK-Method). $P<0.05$ was considered statistically significant.

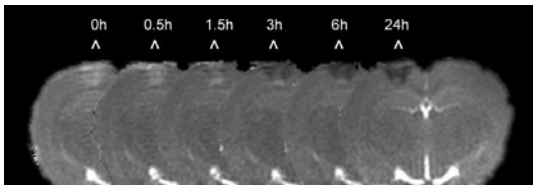
Results

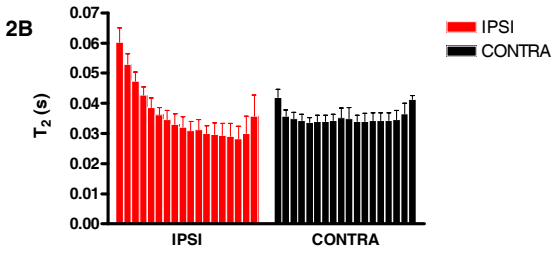
Cortical lesions are in the upper right hemisphere and T_2*W images show in the USPIO injected animals (Fig. 1A, middle row) hypointensities that covered the total lesion area at 24h and decreased at 72h. After transfusion of SPIO-monocytes (Fig. 1A; arrowhead, bottom row) a hypointense area was present only after 72h. Quantification of hypointense voxels in the lesion (Fig. 1B) showed a significant difference comparing transfusion of labeled monocytes to USPIO injection and to images before transfusion (0h). T_2 images in fig. 2A show an acute USPIO experiment where the hyperintense lesion readily turned hypointense after USPIO administration. Comparing lesion T_2 with an identical region of interest (ROI) at the contra-lateral side (Fig. 2B), revealed a large T_2 decrease in the lesion which persisted at 24h. In contrast, the control ROI showed a minor decrease in T_2 which restored after 24h.



1A







Discussion

This study demonstrates that monocyte infiltration into a brain area with ischemic damage can be visualized by MRI using in vitro labeled monocytes. The comparison with conventional USPIO injection revealed a different spatial and temporal pattern in signal loss which may point to separate events. We further showed that USPIO are present in the lesion in an early stage after injection. Most likely, USPIO are present extracellularly and may be taken up by phagocytic cells in a later stage. In conclusion, transfusion of labeled monocytes might be a more accurate tool to perform cellular MRI and study cell migration processes in vivo.

References

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