MR imaging of rat monocytes homing towards an area of cerebral infarction

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

The development of superparamagnetic particles of iron oxide (SPIO) has made MRI a valuable tool for in vivo cell tracking. It opens up the possibility to monitor the migration of inflammatory cells towards a site of tissue injury in a non invasive manner. The infiltration of monocytes in the brain after ischemic stroke plays a dominant role in the inflammation process and to the extent of tissue destruction [1]. Labelling monocytes with SPIOs and subsequent MR tracking in vivo 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 SPIOs (USPIO) [2,3]. Possible disadvantages of this in vivo labelling method include aspecific labelling and leakage of USPIOs which misleadingly contribute to the MR signal. Therefore isolating monocytes, label them in vitro with SPIOs and re-inject them into circulation may be a better tool. The purpose of this study was to monitor the homing of labelled rat monocytes to areas of cerebral infarction over time and compare the in vivo labelling with the in vitro labelling method.

Material and Methods

Cerebral infarctions were induced in 11 rats (male, Lewis Hannover) by photothrombosis of cortical microvessels using erythrosin B (20 mg/kg, 2.5m laser illumination) [4]. On day 5 post photothrombosis, rats were administered USPIOs (n=3, Sinerem, Guerbet France, 17 mg Fe/kg, iv) or in vitro labelled monocytes (n=4, 5x 10^6 cells, iv). Control animals (n=4) were vehicle treated. From a different set of rats, monocytes were freshly isolated by perfusion and labelled in vitro with SPIOs (Endorem, Guerbet France, 4 mg Fe/ml, 1.5h). Imaging was performed before, 24h and 72h after iv injection in an animal scanner (4.7T, Varian, Palo Alto, USA). T_2-maps, sensitive for lesion size, were recorded with a multi echo multi slice sequence (FOV=3.2x3.2 cm, matrix= 128x128, 21x 1mm, TR=3.2 s, echo-spacing=17.5 ms, echo train length=10, NEX=4). T2*W images, sensitive for iron, were acquired using a gradient echo sequence (TR=2.5s, TE=12.5 and 30 ms, NEX=2). Areas of signal loss were analyzed by a voxel threshold based method on T2*W (TE=12.5 ms) images. Data were evaluated by two-way ANOVA, followed by a multiple comparison procedure (SNK-Method). $P<0.05$ was considered statistically significant.

Results

Lesions are in the upper right hemisphere and they appear as hyperintense areas in the T2-maps (Fig.1). T2*W images show in the USPIO treated rats (middle row) hypointensities that cover the total lesion area at 24h and decrease at 72h. In addition at 24h, bloodvessels appear hypointense indicating the presence of USPIOs still in circulation. SPIO-monocytes transplanted rats (bottom row) show a more delineated area of signal loss, only after 72h (Fig 1).The relative increase in the area of hypointensity over time shows a significant increase after 24h for USPIO treated rats, which returns to baseline after 72h (Fig. 2). The area with hypointensities at 72h in rats transplanted with SPIO-labelled monocytes is significantly different from control animals as well as baseline values (0h).

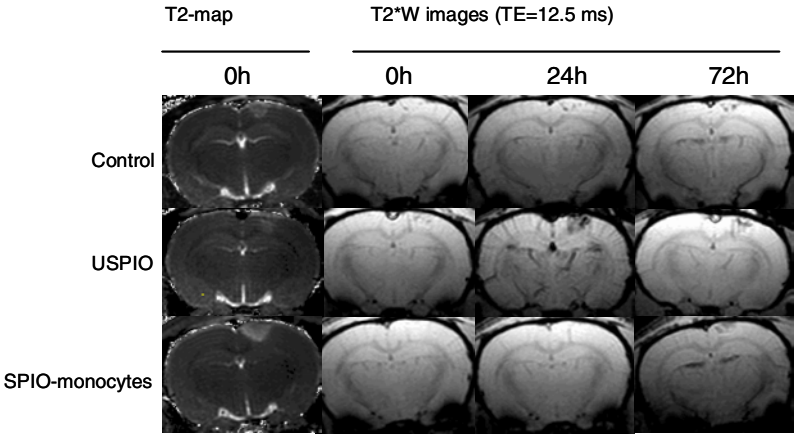

Figure 1: T2-maps and T2*W images of brain slices through the middle of the lesion.

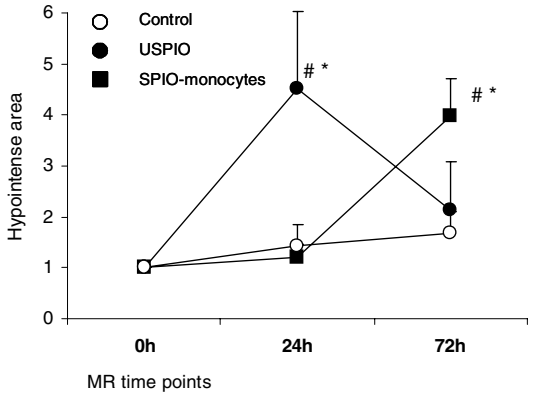

Figure 2: The relative area of hypointense pixels before (0h) and after (24h and 72h) transplantation. # $P<0.05$ versus control animals, * $P<0.05$ versus baseline (0h).

Discussion

The results of this experiment suggest that in vitro labelled monocytes migrate towards an area of neuro-inflammation and can be visualized by MR imaging. The difference in timing and shape in the areas of signal loss between the two labeling methods may point to separate events. We think that USPIOs enter the lesion without any cellular interaction by passive leakage (24h) and are washed out after 72h. The results after administration of pre-labeled monocytes indicate that we are more specifically looking at cellular migration processes.

References

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