USPIO-enhanced MR imaging of macrophage recruitment into the rat brain following MCP-1 microinjection

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Introduction: Recruited hematogenous macrophages and microglia resident in CNS tissue play a key role in the pathophysiology of a number of neuroinflammatory diseases such as neuropathic pain, stroke and multiple sclerosis. Under activated conditions, microglia and macrophages will phagocytose invading cells and CNS debris. It has been shown that ultrasmall superparamagnetic particles of iron oxide (USPIO), such as Sinerem, injected systemically, are engulfed by macrophages, which in turn migrate to sites of tissue injury. USPIOs can be visualised as a distinct reduction in signal intensity on T2* weighted MR images. This technique therefore provides a method of tracking macrophage recruitment to sites of neuroinflammation. However, there are still some issues regarding the distinction between iron-laden recruited macrophages and the entry of free iron across a permeable blood brain barrier (BBB) in disease cases. In addition, a systematic study of macrophage numbers compared to contrast changes has not been reported. Therefore, the aim of the current study was to induce macrophage recruitment to the brain through microinjection of monocyte chemoattractant protein (MCP-1) in a quantifiable manner across an intact BBB and to detect the relationship between the number of macrophages and USPIO-enhanced MRI signal change.

Materials and Methods: Previous experiments using GdDTPA-enhanced MRI (n=6) have shown that BBB breakdown occurs at 3-5 hours following MCP-1 microinjection. Male Wistar rats (n=2) were injected with 0.2ml of Sinerem (Guerbet, France, 2.6mg/Fe/kg) into the tail vein. With a fine glass pipette, 1µl MCP-1 (AbD Serotec, Oxford, 100ng/ml) was injected (over 10 minutes) stereotactically into the left striatum at coordinates of 1 mm anterior and 3 mm lateral to the bregma, at a depth of 4 mm, 24 hours following Sinerem infusion. Imaging was then performed at 1h, 2h, 3h and 4h post MCP-1 injection in a horizontal bore 7T scanner (Varian, Palo Alto, USA). Single slice T2 weighted images (TR=3s, TE=16ms) were acquired to localise the injection site in transverse and coronal planes. T2* maps were created from a gradient echo sequence with 6 different echo times (TR=0.1s, TE=0.008s, 0.016s, 0.024s, 0.032s, 0.040s, 0.048s). Following each T2* acquisition, permeability of the BBB was determined by pre- and post-contrast (GdDTPA, 0.1ml, i.v.) T1 weighted images (TR=0.5s, TE=0.02s).

Results: Figure 1, (a) T2-weighted image shows the presence of a hyperintense region in the left striatum 1 hour following injection of MCP-1. (b) T2* map 1 hour post-injection showing no signal changes in the left striatum. (c) T2* map 2 hour post-injection showing a focal area of signal hypointensity in the left striatum close to the injection site. The reduction in signal intensity indicates recruitment of iron laden macrophages to the injection site. The region of hypointensity remained at the same site and same intensity at 3 hours (d) and 4 hours post-injection (e). Comparison of pre- and post-GdDTPA T1w images revealed that BBB breakdown was not a feature at any time point as shown at 4 hours post injection (f). This finding indicates that the Sinerem enhancement in the injection site seen on (c), (d) and (e) is not due to leakage of the agent through a compromised BBB and further confirms that the signal change is due to the recruitment of USPIO laden macrophages. Figure 2, shows an activated microglial cell in the left striatum at 2 hours post MCP-1 microinjection.

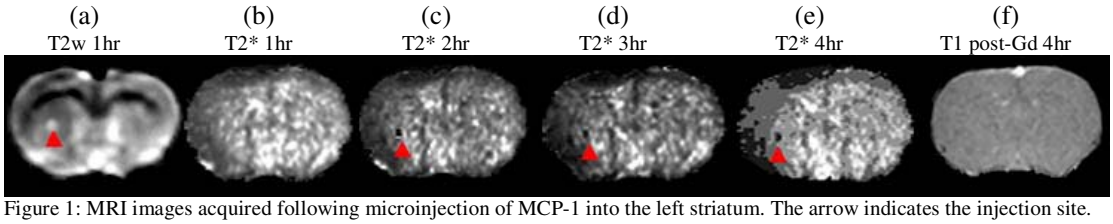

Figure 1: MRI images acquired following microinjection of MCP-1 into the left striatum. The arrow indicates the injection site.

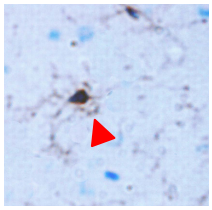

Figure 2: OX42 immunohistochemical stain following MCP-1 microinjection into the left striatum. The arrow indicates an activated microglial cell.

Conclusion: This approach can be used to determine the relationship between the number of recruited macrophages to area of tissue damage and signal change by USPIO-enhanced MRI. Further work will look to quantify the number of macrophages and microglia present and to compare with the degree of signal hypointensity at MCP-1 injection sites.