VCAM-1-targeted contrast agent delineates acutely activated cerebral vasculature in neuropathology

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Introduction:

Currently used magnetic resonance imaging (MRI) techniques in neurological disease are often limited in two important respects: (1) they reflect downstream injury, owing to relatively advanced pathology, and (2) while providing an indication of *severity*, they can not assess disease *activity*. Urgently needed are methods that accelerate diagnosis, quantify disease activity and guide specific therapy. Molecular imaging has the potential to realize this goal[1]. Inflammation is the body's reaction to infection or injury, and is often associated with the acute stages of neurological disease. One of the earliest events in an inflammatory response is the localized expression of specific adhesion molecules on endothelial cells which enables the migration of leukocytes across the blood-brain barrier. Endothelial vascular cell adhesion molecule-1 (VCAM-1) and its ligand, $\alpha_4\beta_1$ integrin, are key mediators of leukocyte recruitment. Moreover, new selective inhibitors that bind to the α_4 subunit of $\alpha_4\beta_1$ substantially reduce clinical relapse in multiple sclerosis (MS) the archetypal inflammatory disease of the brain. Consequently, in the current study we have determined the utility of a microparticle of iron oxide (MPIO) targeted to vascular cell adhesion molecule 1 (VCAM-1) in detecting early endothelial activation.

Methods:

Targeted MPIO Synthesis: 1µm diameter MPIO (26% Fe) were covalently conjugated to purified monoclonal rat anti-mouse antibodies, against VCAM-1, IgG-1 or a 50:50 combination of VCAM-1 and P-selectin, at 37°C for 20h with constant rotation. Antibody conjugated MPIO were resuspended in PBS buffer containing 0.5% BSA and 0.05% tween 20 (pH 7.4) and incubated at 37°C overnight with constant rotation, to block any remaining active sites on the MPIO surface.

Animal Preparation: Adult male NMRI mice were anesthetized with 2.5% isoflurane in 70% N_2O: 30% O_2. Animals were stereotaxically injected in the left striatum (co-ordinates from Bregma: anterior 0.5mm, lateral 2mm, depth 2.5mm) with 1ng of recombinant mouse IL-1β in 1µl saline, to induce endothelial activation. After 3h, anaesthetized mice were injected via a tail vein with either (a) anti-VCAM-1-MPIO, (b) anti-VCAM-1/anti-P-selectin-MPIO, or (c) isotype anti-IgG negative control MPIO ($4x10^8$; approx. 4.5mg Fe/kg) (n=3 per group). Two groups of control mice (i) naïve and (ii) injected intracerebrally with 1µl saline (n=3 per group) were also injected intravenously with anti-VCAM-1-MPIO.

MRI: MRI was performed in a 7T horizontal-bore magnet with a Varian Inova spectrometer. A T_2*-weighted 3D gradient-echo dataset was acquired; flip angle 35º, TR=50ms, TE=5ms, field of view 22.5x22.5x31.6mm, matrix size 192×192×360, 2 averages, total acquisition time ~1h. The mid-point of the acquisition was 4.7±0.3h after IL-1β injection and 1.5±0.3h after MPIO injection. Data were zero-filled to 256x256x360 and reconstructed off-line, with a final isotropic resolution of $88\mu m^3$. The brains were masked and low signal areas segmented and quantified.

Immunocytochemistry: VCAM positive vessels were identified using an anti-mouse VCAM-1 monoclonal antibody. Cresyl violet-stained sections were also examined for the presence of MPIO by light microscopy (LM).

Results:

Following injection of anti-VCAM-1-MPIO, areas of intensely low signal were observed on T_2*-weighted images that appeared to delineate blood vessels on the IL-1ß injected side of the brain (Fig 1a). There was almost complete absence of non-specific retention in the non-injected hemisphere (Fig 1a). To mimic leukocyte binding more closely, dual antibody-conjugated MPIO were constructed targeting both VCAM-1 and P-selectin. These dual-conjugated MPIO also bound specifically, but did not appear to further enhance contrast effects (Fig 1b). IL-1β-injected mice that were subsequently injected with the irrelevant isotype antibody(anti-IgG)-conjugated MPIO showed no contrast effect (Fig 1c). Similarly, control animals showed no retention of the anti-VCAM-1-MPIO contrast agent (Fig. 1d). Segmented areas of low signal intensity were volume rendered to create a 3D map of agent binding, which clearly demonstrates the architecture of the activated vessels in the IL-1β-injected hemisphere (Fig.2). Compared to control animals, specific contrast (mean ± SD x $10^{-6}\mu m^3$) was significantly increased (P=0.02) following administration of VCAM-1-MPIO. (Fig 3).

Fig. 1

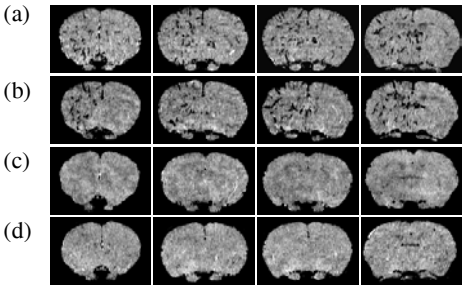


Fig. 2

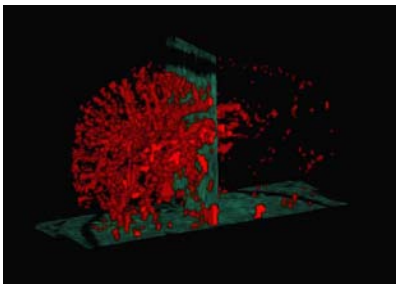
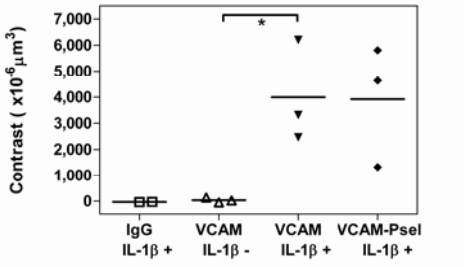


Fig. 3



Discussion:

In this study we demonstrate *in vivo* MRI detection of VCAM-1 expression during acute brain inflammation at a time when pathology is otherwise undetectable. The use of microparticles carrying a high payload of iron oxide (as opposed to the ultrasmall iron oxide particles used previously) provide potent and quantifiable contrast effects, which clearly delineate the architecture of activated cerebral blood vessels. Rapid clearance from blood results in minimal background contrast. This technology is adaptable to monitor expression of endovascular molecules *in vivo* in a range of pathologies. Such an approach could potentially provide more precise and earlier diagnosis, together with much-needed insights into disease activity, prognosis and response to specific therapy.

References: [1] Sibson *et al* (2004) *Magn Res Med* 51:248-252 *these authors have contributed equally to this work.