Imaging Seizure-Induced Inflammation using an Antibody Targeted Iron Oxide Contrast Agent

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Introduction

Acute inflammation following status epilepticus has been implicated in the development of epilepsy and subsequent evolution of brain injury, yet its precise role remains unclear. New therapies to directly reduce inflammation following seizures are being developed and there is an urgent need for readily available, non-invasive methods of detecting and quantifying inflammation in vivo. Furthermore, there is a need for longitudinal imaging studies to help elucidate the role of inflammation in brain injury following seizures. Quantitative T_2 measurements are currently used as a marker of blood brain barrier impairment following status epilepticus. However, this is a relatively insensitive and nonspecific measure of neuroinflammation. In this study we demonstrate that imaging VCAM-1 expression using a targeted contrast agent (VCAM-MPIO) [1] may provide an earlier, more sensitive and more specific measure of seizure-induced neuroinflammation.

Materials and Methods

Contrast Agent Synthesis: The contrast agent (VCAM-MPIO) was synthesised as described in the literature [1]. Briefly, 1 µm diameter tosylactivated Dynabeads (26% iron content, Invitrogen) were conjugated to monoclonal antibodies specific to rat VCAM-1. Animal Model: Adult male Sprague-Dawley rats (n = 9) were separated into three groups: lithium-pilocarpine induced status epilepticus with VCAM-MPIO contrast (SE_{VCAM}) (n = 3), lithium-pilocarpine control group in which status epilepticus was prevented using diazepam with VCAM-MPIO contrast ($Control_{VCAM}$) (n = 3) and lithium-pilocarpine induced status epilepticus with IgG-MPIO isotype matched contrast agent to control for non-specific binding and blood brain barrier leakage (SE_{IgG}) (n = 3). ***In vivo* MRI**: MRI was performed using a 9.4 Tesla VNMRS horizontal bore system with a shielded gradient system (Agilent technologies, Palo Alto, CA) and a 4-channel rat head phased-array coil. MRI was performed before and after injection of the contrast agent. Animals were imaged 1 h post-injection to allow time for MPIO clearance. The Imaging parameters were as follows: 3D spoiled gradient-echo (SPGR), TR = 100 ms, TE = 6.5 ms, FA = 30˚, matrix = 192 x 192 x 128, FOV = 22 x 22 x 20 mm. T_2 measurements were performed before injection of the contrast agent with a multislice multi-echo spin-echo sequence across 13 contiguous slices: TR = 2 s TE = 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, 104, 112, 120 ms, matrix = 128 x 128, FOV = 25 x 25 mm slice thickness = 1 mm. **Segmentation of Contrast Volume:** Brain regions were manually segmented on 3D SPGR images on 70 contiguous slices. Regions were classified as hypointense using an automated segmentation program based on adaptive thresholding.

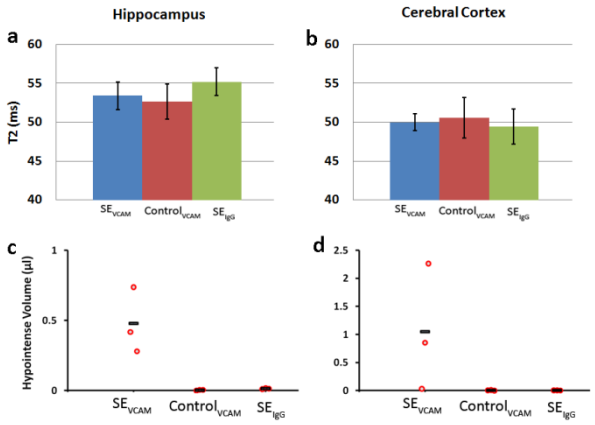

Figure 1: Quantitative T_2 measurements prior to contrast administration (a,b) and contrast agent binding (c,d).

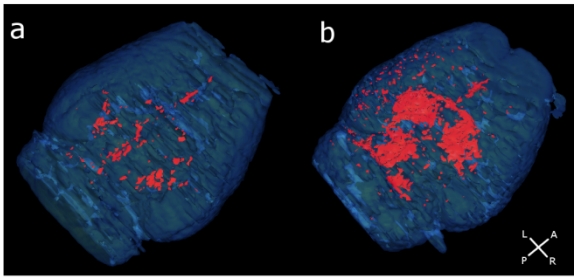

Figure 2: 3D reconstruction of segmented hypointense regions (shown in red). (a) $Control_{VCAM}$ (b)SE_{VCAM}. L-left, R-right, A-anterior, P-posterior.

Results

All animals in the SE_{VCAM} group and the SE_{IgG} group progressed to status epilepticus. All animals displayed akinesia and facial automatisms which progressed to tonic-clonic seizures and status epilepticus within 60 min of pilocarpine administration. None of the animals in the control group displayed any signs of behavioural seizures. Contrast agent binding (volume of hypointensity) was significantly higher in the hippocampus of the SE_{VCAM} group compared to control groups (Figure 1c), whereas T_2 measurements were not significantly increased in either SE group (Figure 1a). VCAM-MPIO was visible on *in vivo* MRI images in the SE_{VCAM} group most notably in the choroid plexus and the periventricular organs. Additionally, the contrast agent was predominantly in the hippocampus and the cerebral cortex on *in vivo* images (Figure 2b). Hypointensities were not identified in the pre-injection images. A similar distribution was observed on high resolution *ex vivo* 3D SPGR images, corroborating that these hypointensities are caused by VCAM contrast agent in similar brain regions. The $Control_{VCAM}$ and SE_{IgG} groups showed very little contrast agent binding on MR images on both *in vivo* and *ex vivo* images, demonstrating that VCAM-1 expression was seizure induced and also that there is little nonspecific binding or leakage across an impaired BBB (Figure 1c,1d).

Discussion

In this study we have used antibody targeted superparamagnetic iron oxide particles to track the distribution of VCAM-1 expression *in vivo.* This is the first study to identify regions of inflammation following seizures using *in vivo* MRI detection of VCAM-1 expression. We have also demonstrated that binding of the contrast agent occurred prior to the more commonly used MRI biomarkers (T_2 measurements) of seizure induced brain injury [2]. Hypointensities caused by contrast agent binding were clearly present in the regions which are widely associated with neuronal damage following seizures. In particular, the hippocampus and the cerebral cortex appeared to be the most significantly affected regions. VCAM-MPIO is a sensitive marker of acute neuroinflammation and molecular imaging of inflammatory processes could provide an early biomarker for epileptogenesis in clinic.

Conclusions

Imaging the activated vascular endothelium using a VCAM-1 targeted contrast agent provides an early and sensitive marker of acute neuroinflammation.

References:

[1] M. A. McAteer *et al.*, Nature Medicine **13** (2007). [2] M. Choy *et al.*, Epilepsy Research **88** (2010).