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Imaging seizure-induced inflammation using an antibody targeted iron oxide contrast agent

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SUMMARY

Early inflammation following status epilepticus has been implicated in the development of epilepsy and the evolution of brain injury, yet its precise role remains unclear. The development of non-invasive imaging markers of inflammation would enable researchers to test this hypothesis *in vivo* and study its temporal progression in relation to epileptogenic insults. In this study we have investigated the potential of a targeted magnetic resonance imaging contrast agent – vascular cell adhesion molecule 1 antibody labelled iron oxide – to image the inflammatory process following status epilepticus in the rat lithium–pilocarpine model. Intravascular administration of the targeted contrast agent was performed at approximately 1 day following status epilepticus. The control group received diazepam prior to pilocarpine to prevent status epilepticus. Magnetic resonance imaging of rats was performed before and after contrast administration. Comparison with quantitative T_2 measurements was also performed. At the end of the study, brains were removed for *ex vivo* magnetic resonance imaging and histology. Marked focal hypointensities caused by contrast agent binding were observed on *in vivo* magnetic resonance images in the post status epilepticus group. In particular these occurred in the periventricular organs, the hippocampus and the cerebral cortex. Relatively little contrast agent binding was observed in the control group. T_2 relaxation times were not significantly increased for the hippocampus or the cerebral cortex in post status epilepticus animals. These results demonstrate the feasibility of *in vivo* imaging of seizure-induced inflammation in an animal model of epilepsy. The antibody targeted MRI contrast agent identified regions of acute inflammation following status epilepticus and may provide an early marker of brain injury. This technique could be used to determine the role of inflammation in models of epileptogenesis and to study the potential for anti-inflammatory therapeutic interventions.

Introduction

There is mounting evidence to suggest that inflammation may be involved in seizure generation (ictogenesis) and the development of some partial epilepsies (epileptogenesis) (Ravizza et al., 2011). The mechanisms through which this occurs are becoming clearer and this knowledge is likely to lead to novel therapeutic approaches. Ictogenesis appears to be exacerbated by inflammatory cytokines, in particular interleukin-1 beta (IL-1β) (Dube et al., 2005; Rodgers et al., 2009) and tumour necrosis factor-alpha (TNF-α) (Riazi et al., 2008) which increase cellular hyperexcitability, possibly via enhanced NMDA-dependent Ca^{2+} influx into neurons (Viviani et al., 2003). Alongside this, it has been shown that blood brain barrier (BBB) disruption can induce epileptiform activity (Marchi et al., 2007a), the mechanism of which is thought to occur via astrocytic uptake of serum albumin resulting in impaired buffering of extracellular potassium (Ivens et al., 2007). Given this evidence, it has been hypothesised that inflammation could provide a crucial mechanism for the development of some partial epilepsies (Vezzani et al., 2011; Yang et al., 2010).

Evidence that inflammation contributes to epileptogenesis was demonstrated by blocking leukocyte adhesion using alpha-4 specific monoclonal antibodies following status epilepticus (SE). This reduced the frequency and severity of seizures in the chronic epilepsy period of the pilocarpine model (Fabene et al., 2008). Furthermore,

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administering the cyclooxygenase-2 (COX-2) inhibitor celecoxib following SE reduces the frequency of spontaneous recurrent seizures (SRS) in the pilocarpine model (Jung et al., 2006). Other work has shown that interactions between the innate and adaptive immune system may contribute to both seizure suppression and neuroprotective effects (Zattoni et al., 2011). The COX-2 inhibitor parecoxib did not alter the frequency or duration of SRS when administered following pilocarpine induced SE (Polascheck et al., 2010) and COX-2 inhibitors have been ineffective at preventing the development of epilepsy in electrically induced status epilepticus (Holtman et al., 2009). Taken together, this highlights the need to monitor the inflammatory process *in vivo* in order to provide greater understanding of the role of inflammation in epileptogenesis, as this could lead to the development of anti-inflammatory therapies as an anti-epileptogenic strategy (Kleen and Holmes, 2010).

Non-invasive imaging of inflammation would enable tracking of the temporal progression of inflammation following epileptogenic insults. Regional cerebral inflammation has previously been imaged *in vivo* using a novel intra-vascular magnetic resonance imaging (MRI) contrast agent, vascular cell adhesion molecule 1 (VCAM-1) conjugated to micron-sized iron oxide particles (McAteer et al., 2007), which is ideally suited as an imaging agent due to the low background expression of VCAM-1 in normal tissue and a large payload of iron; Akhtar et al., 2010; Hoyte et al., 2010). It is known that VCAM-1 is expressed on the walls of blood vessels and that it mediates the rolling and extravasation of leukocytes across the endothelium (Elices et al., 1990). As leukocyte adhesion is strongly correlated to BBB permeability (Stokes and Granger, 2000), we hypothesise that VCAM-1 expression occurs prior to vasogenic oedema and the associated MRI T_2 relaxation time changes (Choy et al., 2010a; Fabene et al., 2003). Imaging VCAM-1 expression could therefore provide an early and more sensitive marker of inflammation or subtle alterations in blood brain barrier permeability that cannot be detected clinically using gadolinium based contrast agents or T_2 weighted MRI (Hoyte et al., 2010). In this study we have imaged seizure induced endothelial activation using micron-sized particles of iron oxide conjugated to VCAM-1 antibodies (VCAM-MPIO) soon after termination of lithium–pilocarpine induced status epilepticus in rats.

Materials and methods

The contrast agent (VCAM-MPIO) was synthesised as described in the literature (McAteer et al., 2007). Briefly, (12.5 mg) 1 μm diameter tosylactivated Dynabeads (26% iron content, Invitrogen) were conjugated to (500 μg) monoclonal antibodies specific to rat VCAM-1 (eBioscience, San Diego, CA). IgG-MPIO was synthesised using the same method, except an isotype matched control antibody (Southern Biotech, Birmingham, AL) was used in place of the VCAM-1 antibody. The VCAM-MPIO contrast agent was then used in two different experiments.

In vitro experiment

The first experiment was carried out in cell culture to establish whether the contrast agent was bound *in-vitro* in the immortalised rat brain endothelial cell line (GPNT) in the presence of an inflammatory insult. The immortalised rat brain endothelial cell line (GPNT) was generously provided by Prof. John Greenwood, Institute of Ophthalmology, University College London (UCL). GPNT cells were cultured until confluent in 35 mm diameter well dishes. Cells were treated with 10 ng/ml human recombinant TNF-α (Invitrogen, UK) (n = 3) or saline (n = 3) in controls and incubated at 37 °C for 20 h. VCAM-MPIO was added at a concentration of 0.06 mg/ml media and cells were incubated for 2 h at 37 °C then washed several times with PBS (pH 7.4). Quantitation of contrast agent binding was performed on grayscale images by thresholding at an arbitrary value. Data is displayed as mean ± SD.

In vivo experiment

All animal procedures were carried out in accordance with the UK Animals (Scientific Procedures) 1986 Act and institutional ethics regulations. Adult male Sprague–Dawley rats weighing 180–230 g (n = 12) were obtained from the breeding colony of the University College London (UCL) animal facility. All rats were housed in a controlled temperature and humidity environment with a 12 h light/dark cycle with food and water provided *ad libitum*. Animals were separated into three groups:, lithium–pilocarpine control group which did not undergo status epilepticus given VCAM-MPIO contrast ($Control_{VCAM}$) (n = 4), lithium–pilocarpine induced status epilepticus with IgG-MPIO isotype matched contrast agent to control for non-specific binding and leakage through the blood brain barrier (SE_{IgG}) (n = 3) and lithium–pilocarpine induced status epilepticus with VCAM-MPIO contrast (SE_{VCAM}) (n = 5). It has been previously shown that lithium and pilocarpine may directly cause peripheral inflammation (Marchi et al., 2007b), and could conceivably also directly lead to central nervous system (CNS) inflammation. To control for this effect, the control group received diazepam injections prior to the injection of lithium and pilocarpine (Fig. 1). Injection of diazepam prior to the injection of pilocarpine has shown to completely prevent the development of SE (Turski et al., 1983). In the SE_{VCAM} group, animals were treated with lithium chloride (3 mEq/kg (per kilogramme of body weight), i.p., Sigma-Aldrich, Dorset, UK) 2 h prior to methylscopolamine (5 mg/kg, i.p, Sigma-Aldrich) injection. Methylscopolamine was administered to reduce the peripheral effects of pilocarpine. This was followed 15–20 min later by administration of pilocarpine hydrochloride (30 mg/kg, i.p., Sigma-Aldrich) in order to induce status epilepticus. Animals were behaviourally assessed on using the Racine scale (Racine, 1972). The onset of status epilepticus was defined as stage 3 on the Racine scale. Diazepam (10 mg/kg, i.p.) was administered 90 min after the onset of SE to terminate the seizure. Further injections of diazepam were administered as required. The $Control_{VCAM}$ group received a lithium chloride injection (3 mEq/kg, i.p., Sigma-Aldrich, Dorset, UK), a subsequent injection of diazepam (30 min prior to pilocarpine injection) to prevent SE onset (Turski et al., 1983), followed by methylscopolamine (20 min prior to pilocarpine injection) and pilocarpine (30 mg/kg).

In vivo MRI

MRI was performed using a 9.4 Tesla DirectDrive VNMRS horizontal bore system with a shielded gradient system (Agilent technologies, Palo Alto, CA) and a 4-channel rat head phased-array coil (Rapid Biomedical GmbH, Würzburg, Germany). MRI was performed before and after injection of the contrast agent. Animals were anesthetised with 4% isoflurane and maintained at 1.5% isoflurane in pure oxygen (1 L/min) throughout the imaging protocol. A physiological monitoring system (SA Instruments, Stony Brook, NY) was used to monitor respiration rate and rectal temperature. Temperature was maintained at 37 ± 0.5 °C using an air warming system. VCAM-MPIO (4 mg of iron/kg) was injected via the right external jugular vein 21.9 ± 0.2 h post-SE onset or 22.1 ± 0.2 h (mean ± SEM) post-pilocarpine administration. Animals were imaged 1 h post-injection to allow time for MPIO clearance. The imaging parameters were as follows: three-dimensional (3D) spoiled gradient-echo (SPGR), TR = 100 ms, TE = 6.5 ms, FA = 30°, matrix = 192 × 192 × 128, FOV = 22 × 22 × 20 mm, acquisition time = 40 min. The images were zero filled to 256 × 256 × 256, giving a final voxel size of 86 × 86 × 78 μm^3. 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 × 128, FOV = 25 × 25 mm slice thickness = 1 mm, acquisition time = 4 min.