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# Dexamethasone exacerbates cerebral edema and brain injury following lithium-pilocarpine induced status epilepticus ☆

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ABSTRACT

Anti-inflammatory therapies are the current most plausible drug candidates for anti-epileptogenesis and neuroprotection following prolonged seizures. Given that vasogenic edema is widely considered to be detrimental for outcome following status epilepticus, the anti-inflammatory agent dexamethasone is sometimes used in clinic for alleviating cerebral edema. In this study we perform longitudinal magnetic resonance imaging in order to assess the contribution of dexamethasone on cerebral edema and subsequent neuroprotection following status epilepticus. Lithium-pilocarpine was used to induce status epilepticus in rats. Following status epilepticus, rats were either post-treated with saline or with dexamethasone sodium phosphate (10 mg/kg or 2 mg/kg). Brain edema was assessed by means of magnetic resonance imaging ($T_2$ relaxometry) and hippocampal volumetry was used as a marker of neuronal injury. $T_2$ relaxometry was performed prior to, 48 h and 96 h following status epilepticus. Volume measurements were performed between 18 and 21 days after status epilepticus. Unexpectedly, cerebral edema was worse in rats that were treated with dexamethasone compared to controls. Furthermore, dexamethasone treated rats had lower hippocampal volumes compared to controls 3 weeks after the initial insult. The $T_2$ measurements at 2 days and 4 days in the hippocampus correlated with hippocampal volumes at 3 weeks. Finally, the mortality rate in the first week following status epilepticus increased from 14% in untreated rats to 33% and 46% in rats treated with 2 mg/kg and 10 mg/kg dexamethasone respectively. These findings suggest that dexamethasone can exacerbate the acute cerebral edema and brain injury associated with status epilepticus.



## Introduction

Inflammation has been suggested to play a major role in epileptogenesis (Ravizza et al., 2011; Vezzani et al., 2013). Mechanisms for this are thought to occur via leakage of blood serum components into the parenchyma across an impaired blood–brain barrier (BBB) leading to impaired astrocyte function and altered potassium homeostasis (Cacheaux et al., 2009; David et al., 2009; Friedman et al., 2009; Ivens et al., 2007; Seiffert et al., 2004; van Vliet et al., 2007). This leads to the hypothesis that anti-inflammatory therapies which help to alleviate vasogenic edema are likely to be anti-epileptogenic or neuroprotective following convulsive status epilepticus (CSE).

A variety of anti-inflammatory drugs have been shown to be neuroprotective or anti-epileptogenic following status epilepticus. For example, blockade of leukocyte–endothelial interactions following status epilepticus (SE) via administration of α4 integrin specific antibodies reduces the occurrence of spontaneous seizures in the chronic epileptic phase (Fabene et al., 2008). Non-steroidal anti-inflammatory drugs (NSAIDs) given after SE have wide-ranging effects depending on the animal model used and schedule of administration. Parecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor administered for 18 days following lithium-pilocarpine induced SE is neuroprotective (but not anti-epileptogenic) (Polascheck et al., 2010), and Celecoxib reduces neuronal injury and microglia activation when administered

*Abbreviations:* BBB, blood–brain barrier; CSE, convulsive status epilepticus; SE, status epilepticus; NSAIDs, non-steroidal anti-inflammatory drugs; COX-2, cyclooxygenase-2; MRI, magnetic resonance imaging; DEX, dexamethasone; IκB, inhibitor of kappa-B; $T_2$, transverse magnetisation relaxation time constant; rHCV, relative hippocampal volume; fse, fast spin-echo; TR, repetition time; FOV, field of view; TE, echo time; TEeff, effective echo time; etl, echo-train length; ROIs, regions of interest.



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one day after lithium-pilocarpine induced status epilepticus (Jung et al., 2006). Conditional ablation of COX-2 in forebrain neurons leads to reduced hippocampal injury at 4 days post pilocarpine induced status epilepticus. However there is also some evidence to suggest that COX-2 is neuroprotective at 24 h following the insult (Serrano et al., 2011). SC58236, another selective COX-2 inhibitor, has no effect on cell death or microglia activation in the hippocampus when administered following electrically induced SE (Holtman et al., 2009). Therefore, there is conflicting evidence on whether modulation of inflammatory cascades can influence brain injury following status epilepticus in rats. In order for these findings to be translated into a clinical setting, there needs to be a biomarker for therapy monitoring. $T_2$-weighted magnetic resonance imaging (MRI) can be used as a biomarker of vasogenic edema (Batchelor et al., 2007) and has been observed to be elevated within 2 days of childhood status epilepticus (Scott et al., 2002). In this study we investigate whether using a broad-spectrum anti-inflammatory agent (dexamethasone) can reduce vasogenic edema, assessed by quantitative transverse magnetization relaxation time constant ($T_2$) measurements following pilocarpine induced SE in rats, and whether these changes predict final hippocampal volumes.

Corticosteroids such as dexamethasone (DEX) act on the glucocorticoid receptor and are highly effective in reducing BBB permeability. DEX does not readily cross the BBB (Meijer et al., 1998) and the mechanisms by which DEX is BBB protective are still not well understood and are likely to be numerous. However one mechanism by which this effect may occur could be the inhibition of NF-kappa-B activity via induction of inhibitor of kappa-B (IκB) proteins (Auphan et al., 1995). DEX appears to reduce infarct volume when administered following cerebral ischemia in rats (Bertorelli et al., 1998), supporting the view that DEX may also alleviate injury following status epilepticus. Two recent studies have found that dexamethasone is neuroprotective when administered prior to pilocarpine or lithium-pilocarpine induced status epilepticus (Al-Shorbagy et al., 2012; Marchi et al., 2011), the mechanisms of which are thought to occur via the alleviation of vasogenic edema and subsequently less severe status epilepticus. However, the clinical relevance of these studies is debatable as administration of dexamethasone preceded the insult. In the current study we investigate whether DEX is protective when administered following SE. It was found that dexamethasone led to increased $T_2$ at 2 days and 4 days following SE compared to controls and to a subsequent worsening of brain injury.

## Materials and methods

### *Animal model*

All animal procedures were carried out in accordance with the UK Animals (Scientific Procedures) 1986 Act and institutional ethics regulations.

### *Experiment 1*

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich, UK. Male Sprague–Dawley rats (170–210 g) were obtained from Charles River Laboratories (Margate, UK) (n = 42) and were kept under controlled environmental conditions including a 12 h light–dark cycle and the provision of with food and water ad libitum. Lithium chloride (3 meq/kg, intraperitoneal (i.p.)) was administered approximately 3 h prior to pilocarpine. Rats were then pre-treated with methyl scopolamine nitrate (5 mg/kg, i.p.) in order to reduce the peripheral effects of pilocarpine. 30 min later, pilocarpine hydrochloride (30 mg/kg, i.p.) was given in order to induce status epilepticus. Saline was administered in control animals in place of pilocarpine (n = 4). Seizure severity was assessed every 10 min using the Racine scale (Racine, 1972). Status epilepticus was defined as stage 3 on the Racine scale. Diazepam (10 mg/kg, i.p., Hameln Pharmaceuticals, Gloucester) was administered 90 min following SE onset in order to terminate the seizure. Additional diazepam (10 mg/kg) was administered 30–40 min later. Following status epilepticus, rats were randomly assigned to one of two groups: SE-DEX10 rats (n = 13) received dexamethasone sodium phosphate (10 mg/kg, equivalent to 7.6 mg/kg dexamethasone) immediately following status epilepticus and at 24 h (10 mg/kg) following SE. This dose is comparable to doses that have shown or attempted to demonstrate efficacy in other rat models of brain edema (Altman et al., 1984; Bertorelli et al., 1998; Mima and Shigeno, 2000; Shapira et al., 1988; Tran et al., 2010). SE rats (n = 15) received saline injections in place of dexamethasone. Dexamethasone is known to have a diuretic effect (Liu et al., 2010), therefore SE-DEX10 and SE animals received subcutaneous saline and saline/glucose solution for the first few days following SE.

### *Experiment 2*

As a high mortality rate was observed in the SE-DEX10 group, the experiment was repeated using the same protocol except a single dose of dexamethasone sodium phosphate (2 mg/kg) was administered i.p. at 1 h following SE (n = 16). A single dose was used in Experiment 2 in order to minimise exposure to possible side effects or the stress caused by such side effects. Following SE rats were randomly assigned to one of two groups: SE (n = 6) and SE-DEX2 (n = 6).

### *Magnetic resonance imaging*

MRI relaxometry was performed at 48 h and 96 h following SE. A subset of animals in Experiment 1 were imaged prior to SE induction (n = 17) and all of these rats were used in the data analysis. High resolution structural imaging was conducted between 18 and 20 days following SE but was not performed at the earlier time points in order to keep the imaging protocol and exposure to isoflurane short. The extremely short (5 min) protocol enabled rats to be imaged as close as possible to the 48 h and 96 h time points. All imaging was achieved using a 9.4 Tesla DirectDrive VNMRS horizontal bore scanner with shielded gradient system (Agilent Technologies, Palo Alto, CA) and a 4-channel rat head phased-array coil (Rapid Biomedical GmbH, Würzburg, Germany). Animals were anesthetised with 4% isoflurane and maintained at 1.5–2% 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 and water tubing warming system. $T_2$ measurements were performed across 15 contiguous slices using a multi-slice multi-echo spin-echo sequence using the following parameters: repetition time (TR) = 2.5 s, field of view (FOV) = 25 × 25 mm, slice thickness = 1 mm, matrix = 128 × 128 and echo time (TE) = 8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88, 96, 104, 112, 120 ms. $T_2$-weighted high resolution structural imaging was performed using a 3-dimensional fast spin-echo (fse) sequence with 150 μm isotropic resolution (TR = 1.8 s, FOV = 24 × 24 × 24 mm, matrix = 160 × 160 × 160, effective echo time (TEeff) = 41.8 ms, echo-train length (etl) = 16, acquisition time = 48 min).

### Quantitative $T_2$

Regions of interest (ROIs) were identified by coregistration of the multi-echo images to a rat brain MRI template (Schwarz et al., 2006). This was achieved in SPM 8 (UCL Wellcome Trust Centre for Neuroimaging, www.fil.ion.ac.uk) using a 12 parameter affine registration with normalized mutual information as the cost function. Following coregistration, the transformation matrix was used to transform the regions of interest to the image space. ROIs included right and left somatosensory cortices, anterior dorsal hippocampus, caudate putamen, cingulate cortices, piriform cortices and the thalamus. Quantitative $T_2$ measurements were performed by calculating power images (Miller and Joseph, 1993). For each region, the mean value from each echo