MR measurements of diffusion, perfusion and T2 with proteomic analysis in the hippocampus following status epilepticus in the rat lithium-pilocapine model

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Introduction

Status epilepticus (SE) in humans may be associated with hippocampal injury, epileptogenesis and development of temporal lobe epilepsy. However, the precise relationships between these events remain unclear. Currently, there remains no effective treatment for the prevention of TLE and therefore identifying the processes that occur following SE will be important for developing such a therapy. Diffusion (ADC), perfusion (CBF) and T_2 changes have been previously reported in both clinical and experimental settings following SE[1,2], but the temporal progression of these changes have yet to be defined. In this study, we investigated diffusion, perfusion and T_2 from day 0 to day 21 after SE in the lithium-pilocarpine model of SE in rat using MRI. MR changes peaked 2 days following SE and proteomic analysis was used to investigate the underlying status of the tissue at this time.

Methods

Twelve adult Sprague-Dawley rats were administered lithium chloride (3mEq/kg) intraperitoneally (i.p.) 18 to 20h prior to either pilocarpine (30mg/kg) (n=6) or saline (n=6). Methylscopolamine i.p. (1mg/kg) was given to reduce mortality. Diazepam (10mg/kg) was administered i.p. 90 min after the onset of SE. Imaging was performed before injections and on days 0, 1, 2, 3, 7, 14, 21 after SE. Animals were anaesthetised with 3% isoflurane and maintained on 1.5% isoflurane with 60/40% N_2O/O_2. Coronal images were obtained approximately 3.3mm from bregma on a 2.35T horizontal bore SMIS system. For the T_2 maps, a MASAGE-IEPI[3] sequence was used with FOV 40 x 40mm, 128 x 64 pixels, 2mm slice thickness and 16 averages. For CBF maps, continuous arterial spin labelling sequence was used with 88 averages and T_1 fits using 8 different TI times with 22 averages, the same FOV, pixels and slice thickness as for the T_2 maps. For diffusion maps, trace-weighted single shot spin-echo EPI was used with TR=1500ms, TE=56ms; b=38 and 872s/mm^2. 2-way repeated-measures ANOVA was used for statistical analysis. Main effects were treatment (saline, pilocarpine) and time (pre, 0, 1, 2, 3, 7, 14, 21). For the proteomics study six animals were imaged prior to SE and on days 0, 1 and 2 at which point the animals were sacrificed for proteome analysis using 2D gels and mass spectrometry.

Results and discussion

There were significant interactions in both CBF (F=8.529 p=0.0001) and T_2 (F=7.561 p=0.001). Figure 1 shows that hippocampal CBF and T_2 increase following SE and that these changes peak around day 2. There were no significant differences in whole hippocampal ADC (F=1.33 p=0.284). However, visual inspection indicated that there was a region with a decreased ADC which corresponded to the CA1 subfield of the hippocampus in the lithium-pilocarpine animals (see fig. 2); this was most evident on days 2 and 3. By day 7 all parameters appeared to return to pre-SE levels and remained so until day 21. Using proteomic analysis, we identified two proteins in the hippocampus that were most abundantly up-regulated, when compared to controls, on day 2: heat shock protein 27 (HSP-27) and dihydropyrimidinase related protein-2 (DRP-2).

We have demonstrated that time-dependent changes in ADC, CBF and T_2 changes occur following SE in the lithium-pilocarpine model. These changes occur within the first week and subsequently return to pre-SE levels. Traditionally it has been viewed that a decrease in ADC and a subsequent increase in T_2 occurs during or after an ischaemic event[4]. The delayed regional ADC change may therefore reflect cell swelling leading to cell damage in CA1[5]. However it is also possible that the ADC decrease is not due to cytotoxic oedema but instead is caused by hypercellularity due to an inflammatory process[6]. This inflammatory response may also be driving the concomitant increase in CBF. The observed T_2 increases are likely to be due to transient vasogenic odema[1,2].

The MR changes peaked 2 days after SE and we have identified a concomitant increase in DRP-2 and HSP-27 in the hippocampus during this period. HSP-27 is up-regulated following physiological stress and has been shown to have neuroprotective properties following SE[7]. DRP-2 has been identified as a marker of newborn neurons[8] and thus indicates an increased level of neurogenesis following SE, which has been previously described[9].

Conclusion

In conclusion, we have observed time-dependent changes in ADC, CBF and T_2 in the hippocampus after SE that peaked on day 2. Our preliminary proteomics analysis suggests that a number of processes occur during this period including cell stress and neurogenesis. The time-dependence of these changes may indicate an opportunity for early intervention. Further studies are necessary to elucidate the mechanisms that underlie these changes and the role that they may play in epileptogenesis.

References

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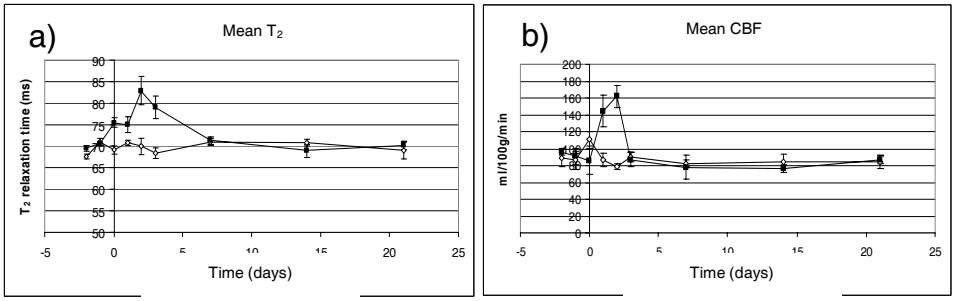

Figure 1: Mean hippocampal time-courses from pre- to day 21 post-injection a) CBF; b) T_2. ■ = pilocarpine-injected, ◊ = saline-injected animals

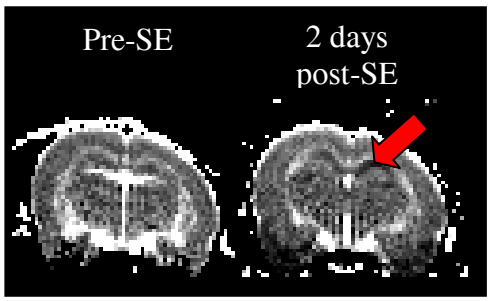

Figure 2: ADC maps from a single pilocarpine-injected animal imaged pre-injection and 2 days after SE