MRI measurements after status epilepticus can be used as an early biomarker for the hippocampal injury in the rat lithium-pilocarpine model

M. Choy[1], R. C. Scott[1], D. L. Thomas[2], K. K. Cheung[2], D. G. Gadian[1], and M. F. Lythgoe[1,3]

[1]Radiology and Physics, UCL-Institute of Child Health, London, United Kingdom, [2]Department of Medical Physics and Bioengineering, UCL, London, United Kingdom, [3]UCL Centre for Advanced Biomedical Imaging, UCL, London, United Kingdom

Introduction

There is a relationship in humans between convulsive status epilepticus (SE), hippocampal injury and subsequent temporal lobe epilepsy. There is currently no effective means of identifying individuals at risk of adverse outcomes, which potentially could be minimised with early intervention. It is therefore important to develop biomarkers or predictors of later injury, and in this context animal studies could prove invaluable. Diffusion (ADC), perfusion (CBF), T_2, and hippocampal volume changes have previously been reported in both clinical and experimental settings following SE[1,2], but the temporal progression of these changes has yet to be fully defined. In this study, diffusion, perfusion and T_2 from day 0 to day 21 after SE was characterised in the lithium-pilocarpine model of SE in rat using MRI, and the relationship between early hippocampal injury and later outcome was investigated.

Methods

Sixteen adult Sprague-Dawley rats were administered lithium chloride (3mEq/kg) intraperitoneally (i.p.) 18 to 20h prior to either pilocarpine (30mg/kg) (n=9) or saline (n=7). Methylscopolamine i.p. (1mg/kg) was given to reduce mortality. To terminate the SE that was induced by the pilocarpine, 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, a 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). Principal component analysis was carried out on the peak multiparametric MRI data on day 2, after which a regression analysis was performed to investigate the relationship between early hippocampal injury and hippocampal volumes on day 21.

Results

Significant time-dependent pilocarpine effects were found for 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 both increase following SE, with peak changes 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; this was most evident on days 2 and 3. By day 7, T_2, CBF and ADC appeared to return to pre-SE levels, and they remained at these levels until day 21. Principal component analysis (PCA) of the peak changes on day 2 extracted two components: component 1 was weighted towards T_2 and CBF, whereas component 2 was weighted towards ADC. Regression analysis on these components with hippocampal volumes on day 21 revealed a strong relationship between component 1 ($R^2=0.899$, $p=0.001$) (fig.2) but not for component 2 ($R^2=0.007$, $p=0.854$).

Discussion

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, with a subsequent return to pre-SE levels. Our previous work has demonstrated that various physiological processes are modulated in the hippocampus on day 2 post-SE; including neurogenesis, cell stress, and inflammation[4]. The delayed regional ADC change may reflect cell swelling leading to cell damage in CA1[5]. The observed T_2 increases are likely to reflect the formation and resolution of oedema[1,2]. The temporal nature of both T_2 and CBF changes parallel the transient expression of inflammatory cytokines following SE, which can cause vasodilatation[5]. Post-SE hippocampal volume reductions following SE have been found in experimental models and in humans, and have been reported to continue for up to 2 months after SE[1,2,7]. These data indicate that MRI measurements on day 2 may be an early biomarker for subsequent injury.

Conclusion

In conclusion, we have used MRI to characterise early SE-induced hippocampal injury in the lithium-pilocarpine rat model and we have identified an early biomarker of subsequent injury.

References

(1) Scott RC *et al.* (2002) *Brain* **125**:1951-1959; (2) Roch *et al.* (2002) *Epilepsia* **43** (4):325-35; (3) Thomas DL *et al* (2002) *Neuroimage* **15** (4):992-1002; (4) Greene *et al.* (2007) *Proteomics* **7**(8):1336-44; (5) Lythgoe MF *et al.* (2003) *Br Med Bull* **65**:235-57; (6) Vousinos-Porche *et al.*(2004) *Neurobiol Dis.* **17**(3):385-402; (7) Pitkanen *et al.* (2002) *Prog Brain Res.* 135:67-83.

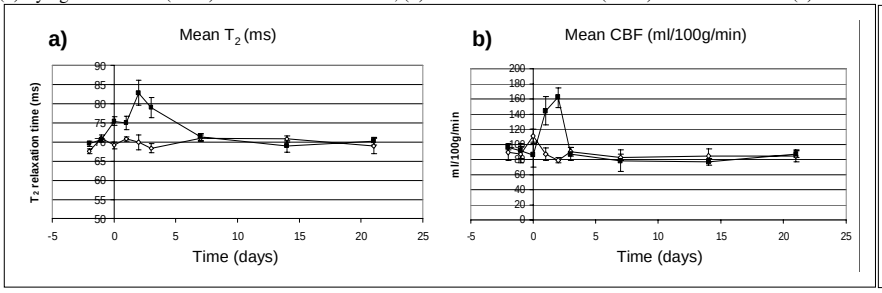

Figure 1: Mean (sem) hippocampal time-courses from pre- to day 21 post-injection a) CBF; b) T_2. ■ = pilocarpine-injected, ◊ = saline-injected animals

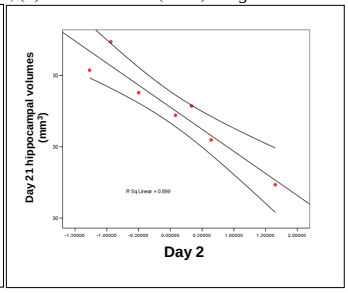

Figure 2: Relationship between component 1 extracted from MRI measurements on day 2 after PCA and hippocampal volumes on day 21