Quantitative DWI Analysis of Neuroprotection Against Soman-Induced Neuropathology

Yusuf A Bhagat[1], Andre Obenaus[1], Murray G Hamilton[2], Edward J Kendall[1]
[1]Department of Medical Imaging, College of Medicine, University of Saskatchewan, Saskatoon, Canada; [2]Medical Countermeasures Section, Defence Research Establishment Suffield, Medicine Hat, AB Canada;

Introduction

The organophosphate compound soman (pinacolylmethylphosphonofluoridate) is an irreversible inhibitor of acetylcholinesterase (AChE) [1]. Exposure to soman increases extracellular levels of acetylcholine (ACh) and glutamate. The occurrence of cell death in this model is attributed to glutamate-mediated toxicity [1]. Symptoms of soman intoxication include seizures that may progress to status epilepticus (SE) contributing to profound neuropathology in the limbic structures [1]. We have recently examined these neuro-degenerative sequelae using diffusion-weighted (DW) and T2-weighted imaging (T2WI) with excellent temporal and spatial resolution [2]. However we have not determined if pharmacological intervention can be quantitatively assessed using similar techniques. Current treatment of soman-induced SE includes injection of atropine sulphate, a muscarinic antagonist in combination with diazepam [3], or in experimental settings, phenobarbital [4]. Recently, others [5] have shown that Gacyclidine (GK-11), a non-competitive antagonist of NMDA receptors prevents soman-mediated injury challenge by reducing levels of extracellular glutamate. Furthermore GK-11 is superior to diazepam because its window of effectiveness extends beyond the 10-minute after seizure limit of diazepam. Here we report our findings using DWI and T2WI to compare the protection provided by these 3 drugs.

Methods

Drug Administration: Male Sprague Dawley rats (n=20, 200-250 grams) were pretreated with atropine sulphate (17 mg/kg, i.p.) and bis-pyridinium oxime, HI-6 (125 mg/kg, i.p.) 30 minutes apart. A single dose of soman (180-200 µg/kg, s.c.) was administered 1 minute following the HI-6 treatment. Diazepam (0.2 mg/kg, i.m., n=5) was administered 1 minute after the soman injection.; phenobarbital (120 mg/kg, i.v., n=5) or GK-11 (0.5 mg/kg, i.v., n=5) was administered 3 hours post soman. Soman (n=5) was the control treatment group. Animals were imaged at 3, 12 and 24 hours and 7 days post treatment on an SMIS 3.0T, 50 cm (130.6 MHz) scanner. Imaging details have been reported [2]. ADC maps were computed using b = 1000 s/mm^2. Image/Data Analysis: Image analysis was conducted as previously described [2]. Regions of interest (ROI) selected were: hippocampus, amygdala, thalamus, piriform cortex (PC) and retrosplenial cortex (RC). No significant ($p<0.05$) differences were found between hemispheres. All data was normalized to pre-treatment values in all groups according to *Observed value/Pre-treatment value x 100* (Observed value = mean ADC or T2 at any time point).

Results

Soman- The hippocampus exhibited a significant (*, $p<0.05$) decline in mean ADC from normal values at 3 hours post soman exposure (Fig.1). Twelve hours later there was still a decrease in mean ADC that was significant (75.8 ± 1.3; $p<0.05$). At 24 hours, mean ADC returned to control values but by 7 days post soman treatment, the values again declined ($p<0.05$) (33% below normal) (Fig. 1). A highly significant (**, $p<0.01$) decline in mean T2 relaxation was observed 12 hours after soman treatment (35% below normal) (Fig. 2). T2 values returned to control levels by 7 days post treatment. **GK-11**- At 7 days post GK-11 therapy there was a highly significant ($p<0.01$) decrease in mean ADC in the PC (17% below normal) and the amygdala (25% below normal) (data not shown). Mean T2 relaxation values in the PC significantly ($p<0.01$) increased at 7 days post GK-11 treatment (25% above control levels). A significant ($p<0.05$) increase (17%) in mean T2 was also observed in the amygdala at the same time point (data not shown). No significant changes in ADC or T2 were observed for the hippocampus (Figs. 1 and 2), RC and thalamus. **Diazepam**- At 7 days mean ADC and T2 declined significantly ($p<0.05$) within the hippocampus (18% below normal) (Figs. 1 and 2). **Phenobarbital**- At 24 hours there was a highly significant ($p<0.01$) increase (29%) in mean T2 in the hippocampus. These values declined at 7 days, but continued to remain significantly ($p<0.05$) above normal (19%) (Fig. 2). No significant ADC changes were observed (Fig. 1).

Discussion

Early ADC changes after soman treatment within the hippocampus correlate with reports of neuronal necrosis; the decline in ADC could be a result of cellular swelling [6]. Secondary changes correspond with reported neuronal necrosis [1]. GK-11 treatment prevented the ADC changes within the hippocampus but not within the PC and the amygdala. We speculate that these tissues experience a non-specific delayed neuronal response to the toxin. The ADC values after soman intoxication are consistent with those of previous imaging studies in kainic acid and pilocarpine induced SE [7,8]. The data indicates that GK-11, diazepam and phenobarbital provide significant neuroprotection against soman. GK-11 appears most effective when evaluated using ADC and T2 changes as the metric.

Acknowledgements- Supported by Holliston and Associates Ltd., MRV Systems Inc. of Saskatoon and the Defence Research Establishment Suffield, AB, Canada.

Fig. 1- ADC Changes within the Hippocampus

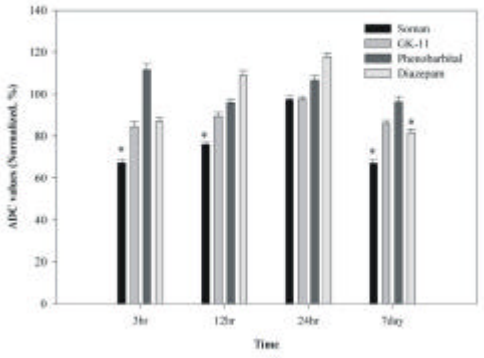
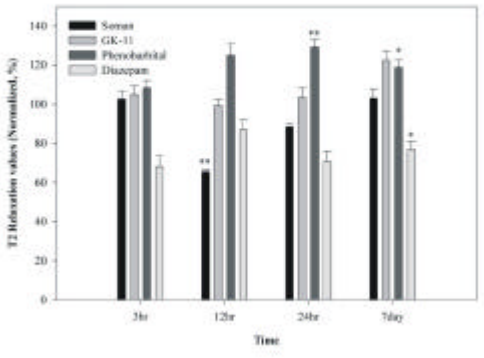


Fig.2- T2 Relaxation Changes within the Hippocampus



References

1- Lemercier et al., Acta Neuropathol. (Berl), 61: 123-129 (1983), 2- Bhagat, Y.A. et al., ISMRM Proc. 2000, 2: 1276, 3- Shih, T-M, Brain Res Bull., 26: 565-573 (1991), 4- Clement J.G., Biochem. Pharmacol., 32(8): 1411-1415 (1983), 5- Lallement, G. et al., Neurotoxicology, 18(3): 851-856 (1997), 6- Zimmer, L.A. et al., J. Comp. Neurol., 378: 482-492 (1997), 7- Nakasu, Y. et al., Am. J. Neuroradiol., 16: 1185-1192 (1995), 8- Wall, C. et al., Am. J. Neuroradiol., 21(10) (2000)