Neuroprotection From Soman-induced Seizures In The Rodent: Evaluation With Diffusion- And $T_2$-weighted Magnetic Resonance Imaging

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Abstract

Exposure to the organophosphate nerve agent soman produces seizures that in turn lead to neuropathology. This study describes the temporal and spatial evolution of brain pathology following soman-induced convulsions and the attenuation of these alterations after neuroprotective intervention with magnetic resonance imaging (MRI). Neuroimaging 12 h after soman exposure, the hippocampus and thalamus exhibited significant decreases (23%) in apparent diffusion coefficients (ADC). These acute effects were resolved by 7 days. In addition, $T_2$ measurements declined significantly at 12 h (37%) returning to near normal values by 24 h. Histopathological analyses confirmed moderate cell loss within the hippocampus and piriform cortex. Together these findings suggest that initial cell death was resolved through regional cellular remodeling. Pharmacological countermeasures were administered in the form of diazepam, a benzodiazepine anticonvulsant, or gacyclidine (GK-11), an anti-glutamatergic compound. Diazepam therapy applied immediately after soman exposure prevented acute ADC changes. However the presence of edema, using $T_2$ measurements, was detected at 3 h within the retrosplenial, amygdala and piriform cortices and at 12 h in the thalamus (34% below normal). GK-11 therapy appeared to prevent most of these changes. However at 7 days after soman, a decrease (17%) in ADC was observed in the piriform cortex. Pathology was confined to the piriform cortex suggesting that this region is more difficult to protect. This is the first report that provides temporal and spatial resolution using MRI with histological correlation of pharmacological interventions against soman-mediated seizure-induced neuropathology.

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INTRODUCTION

Soman (pinacolyl methylphosphonofluoridate) is an irreversible inhibitor of acetylcholinesterase (AChE) and a chemical warfare agent (Shih and McDonough, 1997; Shih and McDonough, 1997). Soman readily

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crosses the blood–brain barrier where its acts to increase extracellular levels of acetylcholine (ACh) and glutamate. This in turn leads, within 12–24 h, to glutamate-mediated neurotoxicity (Shih and McDonough, 1997).

Soman intoxication is characterized by a progression of hypersecretions, respiratory distress and cardiovascular dysfunction to muscular convulsions and paralysis that often result in death. These signs are attributed to hyperactivity of the cholinergic system actuated by increased levels of acetylcholine at central and peripheral neuronal synapses (Lemercier et al., 1983; Shih and McDonough, 1997). The seizures and convulsions progress to status epilepticus (SE) contributing to profound neuropathology in the limbic structures of the hippocampus, amygdala and piriform cortex (Lemercier et al., 1983). We have recently examined these neurodegenerative sequelae using diffusion-weighted and T2-weighted (T2W) magnetic resonance imaging (MRI) with excellent temporal and spatial resolution (Bhagat et al., 2001a,b). However, we have not determined if pharmacological intervention can be quantitatively assessed using these same techniques.

Current opinion to counter soman intoxication favors drugs possessing anti-muscarinic and anti-N-methyl-D-aspartate (NMDA) properties (Lallement et al., 1998). McDonough and Shih (1993) suggested that pre-treatment with a tertiary anticholinergic such as atropine could block the recurrent seizure activity and concomitant brain damage in the event of soman intoxication. However, seizures recruit other neurotransmitter systems that may be refractory to anticholinergic control. So far at least three groups of compounds involving three neurotransmitter systems, tertiary anticholinergics, γ-aminobutyric acid (GABA) agonists and NMDA antagonists have been shown to effectively moderate the development of soman-induced seizures and associated brain damage (McDonough and Shih, 1993; Shih et al., 2003).

Diazepam, a GABAergic agonist, is widely used as an anticonvulsant (Hobbs et al., 1995). It functions by increasing the amount of chloride current generated by GABA\(_A\) receptor activation, thereby potentiating the effects of GABA throughout the nervous system (Hobbs et al., 1995). Previous therapy using diazepam reported that soman-induced seizures were terminated rapidly in most of the animals with few after-effects (McDonough and Shih, 1993).

GK-11 is a non-competitive NMDA antagonist that blocks cell activation induced by excitatory amino acids such as glutamate (Hirbec et al., 2001). Lallement et al., 1997 observed no evidence of soman-induced neuronal necrosis in a primate study following GK-11 therapy regardless of the dose administered. Therapy was effective when GK-11 was administered within 45 min.

T2- and diffusion-weighted imaging (DWI) techniques have been used to study the kainic acid, pilocarpine and bicuculline models of limbic seizures in animals (Zhong et al., 1993; Wall et al., 2000). DWI encodes the rate of Brownian motion of water molecules; maps representing the apparent diffusion coefficient (ADC) of the water in each pixel can be computed from these images. Positive intensity changes in DW images have been associated with acute ischemia (Nakasu et al., 1995; Wang et al., 1996). In contrast, similar changes in T2W images are considered to reflect downstream edema events (Righini et al., 1994). Based on this background and previous work from our laboratory (Bhagat et al., 2001; Wall et al., 2000) we hypothesized that DWI and T2WI can quantitatively distinguish attenuation of seizure-induced neuropathology following drug therapy.

**METHODS**

**Animals**

Adult male Sprague–Dawley rats (200–250 g, Charles River Laboratories, QC, Canada) were housed under standard laboratory conditions in plastic cages. They were separated into four groups (\(n = 5\)): saline control, soman, diazepam and GK-11. The experimental protocol was in accordance with the guidelines of the Animal Resources Center at the University of Saskatchewan.

**Soman Intoxication Protocols and Controls**

Soman intoxication was performed as previously described (Bhagat et al., 2001). Soman and HI-6 were provided by the Defence Research Establishment Suffield (DRES; Ralston, AB, Canada). Soman was diluted in ice-cold saline to a concentration of 200 \(\mu\)g/mL aliquoted to 400 \(\mu\)L per vial and stored at \(-80^\circ\)C (Shih et al., 1999).

The animals in seizure groups were pretreated with atropine sulfate (J.T. Baker Chemical; Phillipsburg, NJ, USA) (17 mg/kg; i.p.) and 30 min later with the bis-pyridinium oxime (HI-6, 125 mg/kg; i.p.) to reduce mortality from the direct effects of soman. Soman was injected 1 min after the oxime in a concentration of
180–200 μg/kg (1.8–2.0 LD<sub>50</sub>), s.c. This dose was sufficient to induce robust seizures as reported previously (Bhagat et al., 2001).

Control animals were treated with a single injection of saline (1 mL/kg; i.p). The animals were observed continuously after soman treatment and scored for motor manifestations of seizure activity for 45 min and thereafter every 15 min until the first imaging time point (Table 1). Rats exhibiting signs of repeated facial clonus, tonic clonic seizures (TCS), body tremors, body jerks, head tremors and Straub tail were prepared for imaging at 3-h post treatment. Animals scoring ≥4 were included.

Therapy Administration Post Soman Intoxication

Therapeutic drugs were prepared at the same time as soman and its treatment adjuncts. Diazepam (Hoffman La Roche; Vadreuil, QC, Canada) (0.2 mg/kg; i.m.) was dissolved in dimethyl sulphoxide (BDH Inc.; Toronto, ON, Canada) and administered 1 min after soman treatment. GK-11 (Beaufor Ipsen, Dreux, France) (1 mg/kg; i.v.) was prepared in saline (0.9%) and delivered as a slow intravenous injection through a tail vein canula, 3-h post soman-induced convulsions.

Imaging Protocols

Imaging was performed at 3, 12, 24 and 168 h (7 days) post treatment on a SMIS 3.0T, 500 mm horizontal-bore scanner operating at 130.6 MHz for proton imaging. Rats were placed in a plastic stereotactic head holder fitted into a 50 mm diameter quadrature saddle coil (Morris Instruments Inc.; Gloucester, ON, Canada) under a light level of anesthesia (3.5% isoflurane (Abbott) in 95% oxygen/5% air). Acquisition parameters were as previously reported (Bhagat et al., 2001).

Diffusion and T<sub>2</sub> Map Generation

Apparent diffusion coefficient maps are two-dimensional gray scale representations of computed ADC values. ADC were computed from the equation,

$$\text{ADC}(x_n,y_n) = \ln \left( \frac{S(x_0,y_0)}{S(x_n,y_n)} \right)$$

where $S(x_0,y_0)$ is the pixel intensity from a $b=0$ image and $S(x_n,y_n)$ the pixel intensity from a $b=1000$ s/mm<sup>2</sup> image.

$T_2$ maps were obtained from the six echo T<sub>2</sub>W images in which relaxation constants were calculated for each pixel using non-linear least squares curve fitting to the data from the equation,

$$M_{x,y} = M_0 e^{-\frac{TE}{T_2}}$$

where $M_0$ is the initial magnetization before decay, TE is the echo time and $T_2$ is the spin–spin relaxation time.

Region of Interest Analysis (ROI)

Quantitative analysis was performed on the hippocampus and the piriform cortex as previously described (Bhagat et al., 2001). In addition, the thalamus, amygdala and retrosplenial cortex were similarly analyzed. Raw data were normalized to pre-treatment values in both treatment groups. This permitted the correction of non-specific fluctuations in pre-treatment values between all bilateral ROIs. Computed mean values were normalized according to (observed value/pre-treatment value) × 100, where the observed value is the mean ADC or $T_2$ relaxation value at any given time point.

Statistics

A comparison of the left and right bilateral regions was performed for each animal using a one way analysis of variance (ANOVA) with repeated measures. No significant differences ($p < 0.05$) were found allowing us to combine both sides for subsequent analysis.

For the soman exposure and control groups, a two-way ANOVA with repeated measures was conducted on normalized ADC and $T_2$ values to detect significance differences between the treatment and the pre-scan values at each observation time.

Tissue Fixation and Histological Protocols

After the 7-day imaging protocol, all animals were anesthetized with ketamine hydrochloride (Ayerst

### Table 1

Convulsions scale for animals

<table>
<thead>
<tr>
<th>Scale number</th>
<th>Key features</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No activity, quiet periods (QPs)</td>
</tr>
<tr>
<td>2</td>
<td>QPs with chewing and facial clonus</td>
</tr>
<tr>
<td>3</td>
<td>Head tremors and body jerks</td>
</tr>
<tr>
<td>4</td>
<td>Tonic clonic seizures (TCS) = 1 or 2 and paw waving</td>
</tr>
<tr>
<td>5</td>
<td>TCS (3–4) and body jerks</td>
</tr>
<tr>
<td>6</td>
<td>TCS (&gt;5), body jerks and body tremors</td>
</tr>
<tr>
<td>7</td>
<td>TCS (&gt;5), P4s and P5s</td>
</tr>
<tr>
<td>8</td>
<td>Severe TCS often leading to death</td>
</tr>
</tbody>
</table>

P4: phase 4, Racine scale: rearing with no loss of balance; P5: phase 5, Racine scale: rearing with loss of balance.
Veterinary Laboratories; Guelph, ON, Canada) (125 mg/kg, i.p.) and xylazine hydrochloride (Bayer Inc.; Toronto, ON, Canada) (10 mg/kg, i.p.) for transcardial perfusion (1 mL/g body weight) by 4% paraformaldehyde (PFA) (BDH, Inc.) dissolved in 0.12 M Millonig’s phosphate buffer solution (pH 7.3) (Obeanaus et al., 1993; Wall et al., 2000). The fixed brains were removed and placed in 4% PFA for 1 h, after which they were rinsed 3 times for 30 min in 0.12 M Millonig’s buffer solution. They were then placed in 20% sucrose in 0.12 M Millonig’s buffer and stored at 4°C. Brain sections, 30 μm thick were obtained from all 4 treatment groups at the level of the hippocampus and piriform cortex. Standard histological stains to visualize neuronal cells (cresyl violet) and neuronal degeneration (silver impregnation) were obtained.

Every tenth section was stained with cresyl violet to determine the general histologic features of the tissue. Sections at similar levels were then processed to identify neuronal degeneration by using silver impregnation methods. Neuropathology was evaluated in all five ROIs within the images. These observations compared well with published studies (Wall et al., 2000; McDonough et al., 1995).

RESULTS

Physiological Effects

Atropine pretreatment, used to protect the peripheral nervous system, induced short-term hyperactivity in the animals. Soman injection elicited a rapid cascade with animals scratching and exhibiting signs of facial clonus followed by generalized tonic-clonic seizures (TCS). This behavior was interspersed with fasciculation of the back and flank musculature, body jerks, body tremors and Straub tail. After the first hour of TCS activity the animals adopted a prone posture and exhibited continuous body jerks or body tremors.

Diazepam (0.2 mg/kg; i.m.) administered 1-min post soman treatment was at least partially antidotal. This treatment prevented severe convulsions but not the hyperactivity, head nodding, facial clonus, and occasional body tremors. This was followed by quiet periods during which the animals were silent and did not undergo any convulsive activity. G inhibitory effects when administered at 3 h after the soman intoxication were masked by the ongoing seizures.

Magnetic Resonance Imaging

Control scans were collected 1–5 days prior to soman exposure as a basis for comparison (Fig. 1, 0 h). Three hours after the onset of soman-mediated seizures, a general increase in diffusion-weighted (DW) signal intensity was observed throughout the brain (Fig. 1; 3 h). At 12-h post treatment, the DW signal intensity increased in the amygdala and piriform cortex but decreased in the hippocampus (Fig. 1). By 24-h post soman treatment, DW images featured near normal intensities in the piriform cortex and the hippocampus. However, at 7 days after soman-induced seizures, signal intensity increased again within the piriform cortex and adjacent amygdala but varied in the hippocampus (Fig. 1).

ADC and $T_2$ Relaxation Results

ADC values are proportional to molecular mobility; mobility is reduced in viscous or tortuous environments. $T_2$ values decline with increasing solute concentration.

Hippocampus

The hippocampus exhibited a significant decline ($p < 0.05$) in mean ADC (33%) from normal values at 3 h after soman exposure (Fig. 2) that persisted for at least twelve hours (75.8 ± 1.3; $p < 0.05$). At 24 h, mean ADC returned to control values, but by 7 days, the values again declined (33% below normal; $p < 0.05$) (Fig. 2). The mean $T_2$ relaxation values declined ($p < 0.01$) at 12 h after soman treatment (35% below normal) (Fig. 2) but returned to control values by 7 days.

No significant changes in mean ADC values were observed in the hippocampus (Fig. 2), after GK-11 treatment. Mean $T_2$ values did not change significantly in any region observed throughout the time course following GK-11 therapy (Fig. 2).

Following diazepam therapy, ADC values in the hippocampus did not vary significantly from normal (100 ± 2.1) throughout the time course (Fig. 2). Mean $T_2$ relaxation values did not change significantly until 7 days when a highly significant ($p < 0.01$) decrease (23% below normal) was observed (Fig. 2).

Piriform Cortex and Amygdala

ADC values did not change significantly until 7 days post soman when a highly significant ($p < 0.01$) decrease (39%) was observed (Fig. 3). Mean $T_2$ relaxation values decreased at 12-h post treatment (37%)
returning to control levels by 7 days (Fig. 3). The amygdala paralleled the piriform cortex with a significant \((p < 0.05)\) decline (36\%) at 7 days post treatment. Mean \(T_2\) relaxation values in the amygdala declined significantly \((p < 0.05)\) at 12 h after soman exposure (31\%); but were otherwise unchanged.

No significant changes in mean ADC values after GK-11 treatment were observed in the amygdala, but at 7 days post therapy, a significant \((p < 0.05)\) decrease in ADC values was observed in the piriform cortex (17\% below normal; 82.6 ± 1.6) (Fig. 3). Mean \(T_2\) values did not change significantly in this region following GK-11 therapy (Figs. 2 and 3).

No significant ADC changes were observed after diazepam in either the piriform cortex (Fig. 3) or the amygdala regions. Mean \(T_2\) values declined significantly \((p < 0.05)\) at 3-h post therapy in the amygdala (13\% below normal) and returned to normal by 7 days. The piriform cortex featured a significant \((p < 0.05)\) decrease in mean \(T_2\) at 3-h post therapy (20\% below normal; 79.7 ± 1.9) and returned to control values at 12 h. \(T_2\) values in this region again declined (14\%) significantly \((p < 0.05)\) at 7 days post therapy (Fig. 3).

**Thalamus**

Three hours to 12 h after the onset of soman-induced seizures a decrease in mean ADC (31\%) was observed. However, at 24-h ADC values were similar to those observed in untreated animals. But at 7 days the mean ADC (44\% of control) once again declined \((p < 0.01)\). A transient decline (28\%; \(p < 0.05\)) in \(T_2\) values was observed at 12-h post soman treatment with other time points exhibiting values near normal.

No significant changes in mean ADC values were observed in the thalamus, after GK-11 treatment. Mean \(T_2\) values did not change significantly in any region observed throughout the time course following GK-11 therapy.
After diazepam treatment ADC values did not differ significantly from normal in thalamus. At 12-h post diazepam, there was a significant ($p < 0.05$) decrease in mean $T_2$ values, continuing to 24 h (36% below normal; 64.1 ± 2.0). Mean $T_2$ increased to 22% of normal by 7 days post therapy.

**Retrosplenial Cortex**

Mean ADC in the retrosplenial cortex declined significantly at 3 h (20%; $p < 0.05$) from control and remained there to the 12-h time point. The ADC values returned to near normal at 24 h and as noted in other ROIs decreased once again (40% of normal; $p < 0.01$) at 7 days post treatment. $T_2$ values decreased significantly ($p < 0.01$) to 28% of normal at 12 h after soman-intoxication and remained below control levels for 7 days after soman treatment.

No significant changes in ADC values were observed in the retrosplenial cortex (Fig. 2), after GK-11 treatment. Likewise, $T_2$ values did not change significantly in any region observed throughout the time course following GK-11 therapy.

No significant ADC changes after diazepam treatment were detected in the retrosplenial cortex. There was however, a significant ($p < 0.05$) decrease in mean $T_2$ at 3 h following therapy. This decrease continued at 12 and 24 h, and remained significantly ($p < 0.05$) below normal at 7 days post diazepam (21% below normal; 79.3 ± 1.0).

**Histological Findings**

Brain tissue stained by cresyl violet in saline control animals featured recognizable neuronal morphology in
all regions of the brain, including the hippocampus (Fig. 4A). In all animals examined at 7 days after soman treatment, cell density decreased within the hilar regions of the dentate gyrus and in the CA3 area of the hippocampus (Fig. 4B). Higher magnification revealed shrunken and condensed nuclei lacking a nucleolus in hippocampal neurons along with swollen neurons. This finding was consistent in all soman-treated animals.

Hippocampal cell density was apparently normal in all regions after diazepam therapy (Fig. 4C). However in some cases, neuronal swelling was observed within the CA1 and CA3 areas. After GK-11 treatment hippocampal sections featured moderate thinning in the CA3 region (Fig. 4D). Decreased neuronal density and swelling among neurons in the CA3 areas and the granule cell layer of the dentate gyrus was also observed.

Soman mediated neuronal changes within the thalamus appeared as regions of darkly stained nuclei, suggestive of cell death. Moderate cell loss was also evident in the retrosplenial cortex where dense staining revealed pyknotic nuclei.

Robust cellular density was observed in the piriform cortex of saline treated control animals (Fig. 5A). In contrast, after soman-induced seizures, the piriform cortex exhibited decreased neuronal density along the length of cortical layers II and III (Fig. 5B). Similar to the hippocampus, higher magnification revealed decreased neuronal density and swollen neurons within the piriform cortex, extending into the amygdala. The amygdaloid nuclei followed a similar pattern of neuronal loss.

Treatment with diazepam, slightly moderated decreases in neuronal density within piriform cortex sub-regions of layers II and III (Fig. 5C). Higher magnification still showed the presence of shrunken and condensed nuclei without a nucleolus and swollen neurons. This finding was consistent in all diazepam treated animals.

Similarly, diazepam therapy moderated cell loss in the retrosplenial cortex where swollen neurons within layers III and IV at 7 days were also evident. Normal neuronal density was observed in the thalamus.

Moderate losses in cell density were also visualized in cortical layers II and III of the piriform cortex and some regions of the amygdala after soman and GK-11 treatment (Fig. 5D). Higher magnification confirmed this decrease in cell density and revealed diffuse clumps of distended neurons.
GK-11 treatment showed moderate cell damage in the thalamus in the form of distended neurons. However, this feature did not correlate with any changes in cell density. The retrosplenial cortex did not show any abnormal changes in neuronal density or morphology in the GK-11 treatment group.

Silver impregnation methods reveal dying neurons as black or brown stained neurons. Control (saline-treated) tissue contained virtually no darkly stained neurons after silver impregnation. Tissue from soman intoxicated animals at 7 days revealed a moderate number of darkly stained cells within the hippocampus, and a thinning of cells within the CA3 layer and in the dentate gyrus. The piriform cortex contained a moderate number of dying neurons in layer II, with the presence of dark brown cell clusters in layer III. Amygdaloid nuclei also exhibited dark brown to black cell clusters in a similar pattern to that observed in the piriform cortex. Marked increases in the density of dark brown cells were also observed in the thalamus. The retrosplenial cortex also had a moderate number of darkly stained neurons in layers II and III.

Diazepam treatment still had darkly stained cells within layers II and III of the piriform cortex. In contrast, the amygdala displayed very few dark brown nuclei. The retrosplenial cortex showed in layer II a few dark brown nuclei were present at the border between layers I and II.

After soman and GK-11 treatment, silver impregnation revealed moderate numbers of dark brown nuclei were also present in layers II and III of the piriform cortex and dark brown cell clusters extending to the amygdala. Higher magnification confirmed the presence of darkly stained nuclei in layer III of the piriform cortex. No overt changes were seen in the thalamus or retrosplenial cortex.

**DISCUSSION**

This report details the temporal and spatial development of neuropathology arising from soman-mediated seizures using diffusion-weighted imaging as a sensitive non-invasive imaging modality. Five novel findings are reported. (1) The evolution of injury within the hippocampus and thalamus was rapid (<3 h) after soman intoxication, (2) the initial soman response was transient, (3) initial injury presaged secondary effects, (4) diazepam was effective as an anticonvulsant and prevented most of the neuropathology observed in the soman group, and (5) GK-11 was partially effective in ameliorating the neuropathology. It is important to
note that while diazepam was administered at the same time as soman, GK-11 was administered 3 h after the initial seizure onset.

In neuroimaging, DWI has emerged as the method of choice for early detection of short-term changes that arise from brain edema and attendant pathology. In DW images intensity increases arise from restricted water mobility. Likely causes of restriction are the presence of reflective boundaries, increased matrix viscosity and disruption of cytoplasmic streaming (Nicholson and Sykova, 1998; Thomas et al., 2000). \( T_2 \)-weighted imaging provides an additional measure of tissue alterations, and is suggestive of edematous changes.

**Neuroimaging of Soman: Comparison to Seizure Models**

Previous MRI studies have shown marked alterations in \( T_2 \)-WI and ADC values in limbic structures after kainic acid and pilocarpine treatment. Our imaging results demonstrate that ADC values in the hippocampus and the piriform cortex decline during the first day following intoxication. \( T_2 \) values in intoxicated animals were only significantly decreased at 12 h in the hippocampus and piriform cortex; this is consistent with transient edema formation.

Similar observations were reported after kainic acid (Righini et al., 1994; Wang et al., 1996) and pilocarpine (Wall et al., 2000) induced status epilepticus. In many of these reports, the ADC changes preceded measurable \( T_2 \) effects. In addition, an association with decreased ADC and subsequent increased \( T_2 \) values was not reported.

Ebisu et al. (1996) reported that \( T_2 \) signal intensity increased at 26 h in the piriform cortex, and ADC decreased at both 12 and 26-h time points post seizure. They noted that the early decrease in ADC did not correlate temporally with any early changes in \( T_2 \) signal intensities (Ebisu et al., 1996). The findings reported here support those observations. \( T_2 \) relaxation values did not correlate with ADC changes observed at 3- and 12-h post soman exposure (Fig. 5).

Finally, the consensus is that in the kainic acid model of status epilepticus, most regions return to normal ADC and \( T_2 \) values by 7 days after seizures (Wang et al., 1996; Nakasu et al., 1995). This contrasts with the model reported here. Our observations indicate that the soman intoxication model results in decreased ADC values in virtually all regions at 7 days. However, soman \( T_2 \) results are similar to those reported with a return to control values at 7 days (Wang et al., 1996; Nakasu et al., 1995).

Pilocarpine and soman mediated seizures are similarly modulated by agonist activity within the cholinergic system. Our own recent studies generally agree in substance with the results described here (Wall et al., 2000). In that model an increase in hippocampal ADC was observed 24 h after seizure onset. This coincided with decreased neuronal density and degenerating hilar neurons. In the pilocarpine model, the retrosplenial and piriform cortices exhibited significant ADC declines at 12 h. Values subsequently returned to near normal within the retrosplenial cortex, but remained below normal within the piriform cortex (Wall et al., 2000). Again, histopathologic observations revealed marked neuronal losses within layers II and III of the piriform cortex, and neuronal swelling in retrosplenial layers III–VI at 12 h. By 24 h, cells in the retrosplenial cortex increased in density and appeared normal in terms of morphology. These findings mirror the present study of soman-induced seizures. The highly significant decrease in mean ADC (Fig. 2) at 7 days correlates with evidence of neuronal loss and swelling within the hippocampus and piriform cortex (Figs. 4 and 5).

**GK-11 Therapy**

Recall that GK-11 therapy 3-h post soman-induced convulsions prevented significant ADC changes within the retrosplenial cortex, hippocampus, thalamus and amygdala (Fig. 2) but not the piriform cortex at any time points. Mean \( T_2 \) values did not significantly vary from normal following GK-11 treatment (Figs. 2 and 3). However, histological analysis revealed moderate pathology within the hippocampus (Fig. 4).

The decrease in ADC within the piriform cortex at 7 days post therapy temporally correlates with neuronal swelling and degeneration (Figs. 3 and 5). These changes may be due to neuropil swelling and/or decreased intracellular water mobility. Both effects will decrease the observed diffusion coefficient of water (Nakasu et al., 1995; Righini et al., 1994). Although, we observed moderate levels of pathology within areas of the hippocampus, no significant diffusive or nuclear relaxation changes were noted. Thus, the GK-11 therapy apparently prevented at least in part those changes in ADC and \( T_2 \) relaxation associated with soman mediated seizures (Figs. 2 and 3). Histopathology within the piriform and hippocampal regions was moderate compared to that after soman exposure (Figs. 4 and 5). These observations, when combined with the MR results, suggest that GK-11 provides some neuroprotection to several brain regions.
These studies have not been optimized for therapeutic intervention. For example, earlier reports determined that GK-11 should be administered between 10 and 45 min after soman intoxication to achieve a positive response (Lallement et al., 1998, 1999). Our protocol required that GK-11 be administered 3-h post soman exposure. Our results extend the reported window of efficacy. However, they underline that protection is not uniform. The piriform cortex experienced significant ADC changes at 7 days post GK-11 therapy. Nevertheless, our results indicate that GK-11 prevents severe neuronal changes in some brain areas even when administered 3 h after seizure onset.

**Diazepam Therapy**

Diazepam administration 1 min after soman treatment prevented significant ADC alterations in all ROIs examined (Figs. 2 and 3). However, the declining $T_2$ values obtained suggest water influx from the interstitial to the intracellular space (Thomas et al., 2000).

Pathological changes in this group occurred within the piriform cortex and retrosplenial cortex at 7 days after treatment (Fig. 5). Although the ADC results from this group suggest that diazepam therapy prevented soman-induced-seizure changes, the $T_2$ values suggest otherwise. The pathological changes in this group combined with the $T_2$ relaxation results suggests that extracellular water migrates to the intracellular matrix.

At present, there are no MR studies on the effects of diazepam or related benzodiazepines against cholinergic agonists in animals. However, previous histological studies with diazepam therapy have showed that diazepam can prevent soman-induced neuropathology when administered in sufficiently high doses at early time points (<40 min) post exposure (Shih et al., 2003, 1999).

**Piriform Cortex Sensitivity to Seizures**

Our data show that the piriform cortex exhibits ADC and $T_2$ relaxation changes following soman induced seizure activity that correlates with the observed histopathology in this group. Together our data reveal that of all the limbic structures analyzed, the piriform cortex has the highest sensitivity to soman poisoning and that injury could not be prevented, but only attenuated with therapeutic interventions.

Zimmer et al. (1997) investigated the occurrence of astrocytosis within limbic structures of the rat brain from 1- to 24-h post soman-induced seizures. They found that the piriform cortex showed the earliest changes in the form of intense glial fibrillary acidic protein (GFAP) staining in layers II and III, and by 24 h, intense cellular pathology was evident within the rostral-caudal extent of layer II. This suggests intense sustained neural activity within this region following convulsions (Zimmer et al., 1997, 1998).

In the pilocarpine model of seizures, we have also noted marked sensitivity of the piriform cortex to seizures (Wall et al., 2000). Acute (<24 h) ADC changes and pathology within this region following pilocarpine administration were observed. The rationale for enhanced sensitivity was based largely on the reports of others showing that neurons of this region have electrophysiological properties that allow for rapid depolarization and cell firing. The studies here confirm that the piriform cortex is the limbic structure most sensitive to soman intoxication. Our current therapeutic protocols were ineffective in preventing all of the neuropathologic sequelae in this region. Future experiments should investigate earlier time points for GK-11 intervention to evaluate the viability of neurons in the piriform cortex in comparison to other limbic structures. In addition, therapy with multiple compounds may also be effective.

**Correlations Between Pathophysiology and Imaging**

Multiple factors may contribute to ADC changes arising from epileptic-induced lesions. Unlike ischemia, seizure-induced lesions do not possess characteristics such as: (1) cessation of perfusion, (2) energy depletion and (3) a decrease in tissue temperature resulting in ADC decreases (Wall et al., 2000; Thomas et al., 2000). Both ischemia and seizure-mediated lesions regionally reduce water mobility. Soman-induced neuronal cell death begins as early as 3 h after the onset of status epilepticus. The ensuing neurodegeneration leads to macrophage invasion and astrocytic proliferation. Neuronal swelling may be caused by cytotoxic edema due to repetitive excitation of postsynaptic membranes after cholinergic overstimulation (Solberg and Belkin, 1997).

Neuronal cell death may also lead to proliferation and hypertrophy of macrophages and astrocytes (Wall et al., 2000). Neuronal membranes damaged after seizures may result in the release of amino acids and ions and result in long-term glial and astrocytic swelling. The end result is a decrease in extracellular volume and an increase in tortuosity. Increases in tortuosity contribute to the rise of intracellular barriers and connectivity of the extracellular space resulting in...
decreased ADC (Nicholson and Sykova, 1998). Other factors such as temperature (in ischemic lesions), transmembrane molecular exchange, macroscopic bulk motion, viscosity, restrictive diffusion, net movement of compartmental water and cytoplasmic motion may also affect ADC (Le Bihan, 1991).

In our study, ADC values increased to near normal by 24 h and then again declined at 7 days post soman intoxication. The underlying reasons for this mechanism require further investigation. The ADC increase at 12 h may result from increased cerebral blood flow (CBF) to regions undergoing severe hypoxic insults. As a corollary, a decline in CBF at 7 days post seizures may account for decreased ADC from a mismatch between CBF and metabolic rates in the hyper-metabolic regions (Thomas et al., 2000). Beaulieu and colleagues (Beaulieu et al., 1999) point out that if perfusion is restored after ischemic infarctions, cytotoxic edema can resolve through restoration of ATP and Na+/K+ pumps normalizing leading to normalized ADC.

Increased $T_2$ values, the MR transverse relaxation time of water, seen on $T_2$W images and $T_2$ relaxation maps, suggest vasogenic edema and infarcted areas (Loubinoux et al., 1997). $T_2$ relaxation maps detect neuronal insults, but only at later time points. Quantitative $T_2$ relaxation is a useful marker of vasogenic edema development (Loubinoux et al., 1997). Here, the interpretation is difficult. $T_2$ values declined after soman exposure in all regions arguing against an increase in total water at the 7-day observation point.

**Anticonvulsants in Soman Poisoning**

GK-11 prevents the deleterious effects of excessive glutamate activation by blocking ion channels permeable to Ca$^{2+}$ (Hirbec et al., 2001). GK-11 therapy did not significantly protect the piriform cortex against soman-induced pathology as indicated by ADC changes and histopathologic alterations (Figs. 3 and 5). Other pathological changes were observed within the hippocampus in terms of decreased neuronal density and increased neuronal swelling. The most significant observation in our study was that GK-11 is an efficacious treatment against soman exposure, even when administered 3 h after the onset of convulsions when compared to untreated soman controls. However, our data revealed moderate pathology within two structures that can be attributed to the delayed intervention with this drug. A possible means to counter the deleterious changes within those regions, would be to intervene at earlier time points (<30 min post soman) as shown previously (Lallement et al., 1997, 1999).

Another option may be to combine GK-11 therapy with that of diazepam. In our hands, diazepam and GK-11 therapy prevented some of the ADC alterations that were observed following soman treatment alone. Furthermore, pathology within limbic structures observed after both treatments was relatively moderate compared to that of the soman group. This implies that both drugs are somewhat efficacious, but combined therapy, barring any adverse effects, may be more beneficial to counter soman effects. In a recent review of additional medical therapy to conventional treatments against soman intoxication, Lallement et al. (1999) argued for adding GK-11 to conventional treatments. GK-11 administered early after soman poisoning showed rapid control of seizures, total normalization of EEG, recovery at 3 days and no neuropathological damage at 5 weeks post soman exposure (Lallement et al., 1999). Future work incorporating such an experimental protocol may yield better MRI and histopathologic results after soman exposure in rats.

**CONCLUSION**

Soman-induced status epilepticus was accompanied by immediate MRI visible changes within limbic structures. Non-invasive imaging, in particular DWI, permitted multiple measures of the pathology resulting in improved sampling and increased sensitivity in the detection of the pathology. The data revealed significant ADC changes by 12-h post treatment, persisting for at least 24 h and in some regions for 7 days. This suggests that meaningful pharmacological intervention would have to occur before 12 h, and not after 24 h, in order to mitigate the observed effects.

Diazepam and GK-11 when given after soman treatment, prevented most of the changes in mean ADC and $T_2$ relaxation values, which were observed after soman-induced seizures. Histological analyses also revealed that all these therapeutic measures offered significant neuroprotection to regions that were affected by soman exposure. $T_2$ relaxation decreased following diazepam therapy in all regions, but again only moderate neuronal changes were observed within the piriform cortex. GK-11 therapy still had decreased ADC values and neuronal changes within the piriform cortex as visualized from histological sections. Thus, the piriform cortex appears to be the most difficult region to rescue following soman intoxication. Our
data and prior studies (Lallement et al., 1997, 1998, 1999), suggest that diazepam and GK-11 combined therapies may offer more protection against soman-mediated seizures.

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