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Full Length Article

Time dependent dual effect of anti-inflammatory treatments on sarin-induced brain inflammation: Suggested role of prostaglandins

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ABSTRACT

A common consequence of exposure to organophosphate nerve agents is the centrally mediated seizure activity that appears even after conventional treatment with atropine and oximes. We have previously demonstrated a major inflammatory response with subsequent brain damage which was correlated with the duration of the sarin-induced seizures (Chapman et al., 2006). In the present work seizures were induced by the nerve agent sarin (1.2 LD50) insufficiently treated 1 min later by atropine and trimedoxime bromide (TA), with additional midazolam treatment either 5 or 30 min after continuous seizure activity. The efficacy of both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs), as well as other drugs that were reported as beneficial in neuroprotection, were evaluated for their contribution as adjunct treatment against sarin induced seizures and the ensuing inflammatory brain damage. Results show that both steroids and NSAIDs were harmful when administered during convulsions, and steroids were at best ineffective if administered at their termination. However, if administered at termination of convulsions, the NSAID ibuprofen, the selective COX 2 inhibitor nimesulide and the PLA2 inhibitor quinacrine were partially effective in reducing brain inflammatory markers. Administration of exogenous analogs of prostaglandins (PGE2) immediately following sarin-induced convulsions was found to have a beneficial effect in reducing brain inflammatory markers measured at 24 h and one week post sarin exposure. These findings support the hypothesis that elevated levels of PGE2 have a beneficial role immediately following sarin induced seizures, and that early inhibition of PGE2 production by both steroids and NSAID is contraindicative.

1. Introduction

The nerve agent sarin is a highly toxic, irreversible organophosphate (OP) cholinesterase (ChE) inhibitor used in the terror attacks in Japan (Okumura et al., 2003) and, more recently, in the civil war in Syria (Eisenkraft et al., 2014; Rosman et al., 2014). Sarin exposure results in a dose dependent hyper-secretion, fasciculation, tremor, convulsions, respiratory failure and death (Munro et al., 1994). In animals, OP intoxication was shown to induce a dose-dependent, wide spread and specific brain damage (Kadar et al., 1995; Lazar et al., 2016), the severity of which was found to correlate with the extent and duration of convulsions (McDonough and Shih, 1997; McDonough et al., 1999, 2000; Chapman et al., 2006; de Araujo Furtado et al., 2010). We have previously demonstrated a specific, time dependent inflammatory response following sarin-induced seizures (Chapman et al., 2006, 2015, Lazar et al., 2016). Exposure to the nerve agent soman also resulted in a microglia activation (Zimmer et al., 1997), induction of COX-2 (Angoa-Pérez et al., 2010) and an increase in the mRNA of the pro-inflammatory markers IL-1β, TNFα, and IL-6 within 6 h following exposure (Svensson et al., 2001; Williams et al., 2003). However, the effects of anti-inflammatory treatments following nerve agents have not been previously reported. In various models of seizures and brain injury there are conflicting reports as to the efficacy of different anti-inflammatory treatments. While some report ameliorating effects of steroids in reducing lipid peroxidation and providing neuroprotection (Hall, 1993), others were skeptical of their beneficial effect (Gomes et al., 2005). Steroids were helpful in reducing vasogenic edema (Sztriha et al., 1986), and in inhibiting COX-2 and PGE2 production following seizures (Ciceri et al., 2002). In contrast, there are reports of aggravation of seizures following steroid treatment (Duffy et al., 2014; Supko and Johnston, 1994; Lee et al., 1989) which was also seen in cell cultures (Semba et al., 1996). The same conflicting effects have been reported for non-steroidal anti-inflammatory drugs (NASIDs). While neuroprotective effects of ibuprofen and indomethacin have been reported following cerebral ischemia (Iwata et al., 2010; Girgis et al., 2013; Lopes et al., 2016; Tutak et al., 2005), exacerbation

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of seizures following NSAID have also been documented (Régnier et al., 2010; Auriel et al., 2014). The documentation of the effect of the COX2 inhibitors nimesulide and rofecoxib on seizures have been predominantly positive. Both are reported as protective either by elevation of seizure threshold (Akula et al., 2008), or by neuroprotection (Wang et al., 2012). The present study evaluated the efficacy of anti-inflammatory treatments, as adjunct therapy to the standard treatment of OP poisoning, in ameliorating inflammatory markers and the ensuing brain injury that resulted from sarin-induced prolong convulsions. The role of prostaglandins was delineated as timing of COX inhibition was found to be crucial for the efficacy of treatments.

2. Method

2.1. Animals

All procedures involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 2011, and were approved by the IIBR animal care and use committee. Male albino Sprague-Dawley rats weighing 280–300 g were purchased from Charles River (England). Animals were housed on bedding in plastic cages, 3 per cage, in a controlled environment with a constant temperature of 21 ± 2 °C and 12 h light/dark cycle, lights on at 6 a.m.. Food pellets and water were available ad lib. Animals were allowed at least 5 days of acclimatization before onset of experiment.

2.2. Materials

2.2.1. Sarin

(isopropyl methylphosphono-fluoridate) was supplied by the Department of Organic Chemistry at the Israel Institute for Biological Research (IIBR). Sarin (at least 96% pure, based on 1H and 31 P NMR) was dissolved in propylene glycol and kept frozen. For intramuscularly (i.m.) injections, fresh dilute solutions in saline were prepared for each experiment.

2.2.2. Drugs

Atropine sulfate, trimedoxime bromide (TMB4), Nimesulide, Quinacrine Ibuprofen and Misoprostol (Sigma-Aldrich). Midazolam solution (Dormicum; Hoffman-LaRoche, 5 mg/ml), Solu-Medrol (Methylprednisolone, 125 mg/2 ml) Dinoprostone (Prostine E2, 10 mg/ml) and Iloprost (Ilomedine 0.1 mg/ml) purchased from a local pharmacy. Atropine and TMB4 were dissolved in saline and administered i.m. Nimesulide, ibuprofen, quinacrine and misoprostol were dissolved in DMSO and administered intraperitoneally (i.p.), Methylprednisolone, dinoprostone (Prostin) and iloprost (Ilomedin) were administered i.p. in their provided solutions. All the treatments were administered in neuroprotective doses determined based on published literature. Solutions of atropine (20 mg/ml) and TMB4 (30 mg/ml) were prepared in sterile cold saline.

2.3. Brain processing for markers

Brains were dissected out, the cerebellum was removed and discarded and the rest of the brain was weighed, flash frozen in liquid nitrogen, and stored at -80 ℃ until assayed. 100 mg tissue/ml, were homogenized for 30 s in ice-cold Tris − HCl (pH 7.4, 50 mM), buffer contained protease inhibitor cocktail (diluted 1:300, Sigma, P 8340), using Polytron (Ultra Turrax, 15 s, setting 5–6). Following homogenization sample were centrifuged for 15 min at 15,000 *g* and aliquots of the supernatant were frozen at − 80 ℃ until analyzed.

All ingredients for preparing Tris buffer were purchased from Sigma.

2.4. Inflammation markers

2.4.1. Prostaglandine (PGE2)

PGE2 was assayed according to SIGMA protocol for PGE2 antibodies (p 5164). Each sample tube contained 25–100 µl of brain homogenate and a reaction mixture containing 0.5 ml PGE2 antibodies (sigma) and 100 µl of [^{3}H]- PGE2 (Amersham, UK) was added.

Cytokines were assayed by DuoSet ELISA development systems for rats, TNFα (DY510), IL-1β (DY501) and IL-6 (DY506), R&D Systems, according to the protocols provided with the kits.

2.4.2. TSPO

The glial cell marker 18kDTranslocater protein (TSPO, formerly termed PBR) was assayed using labeled PK-11195 ([3 H]PK-11195, specific activity 83.5 Ci/mmol, purchased from Perkin Elmer, USA). Binding of [3 H]PK-11195 to rat brain membranes was performed with minor modifications as previously described (Benevides et al., 2001). Briefly, reaction mixture (in 12 × 75 test tubes), at a final volume of 1 ml, contained 300 µl Tris − HCl buffer (50 mM, pH 7.4), 100 µl [3 H] PK-11195 solution (1 nM), 100 µl PK-11195 solution (10 µM final concentration for nonspecific binding) or buffer and 500 µl membrane suspension (diluted 1:10 from the initial brain homogenate). Non-specific binding amounted to 5–15% of total ligand bound. Tubes were incubated for 1 h at 0–4 °C and the reaction terminated by rapid filtration over GF/B filters. Radioactivity was assessed using Packard liquid scintillation analyzer (1600 TR). (Chapman et al., 2015)

2.5. Histological staining and scoring

Brains were rapidly removed from the skull, immersed in 4% neutral buffered paraformaldehyde (pH = 7.0; Gadot, Israel) at 4 °C, and processed routinely for paraffin embedding. Serial 7µm-thick sections were cut in the coronal plane, mounted on positively charged plus slides and air dried overnight. Next, sections were deparaffinized [xylene(2 x 5 min), 100% ethanol (2 x 3 min), 95% ethanol (3 min), 80% ethanol (3 min) and distilled water for 2 min] and rehydrated through 100 and 95% alcohol to distilled water. Slides were stained with hematoxylin and eosin (H & E) for light microscopy examination.

The severity of brain damage was semi-quantitatively scored according to a modified histological scoring scale previously described (Bloch-Shilderman et al., 2005). The score is based on the typical neuropathology following OP poisoning (Kadar et al., 1995) and includes the hippocampus, piriform and thalamus. For each of these brain areas the score ranged from 0 (intact brain), 1- for minor changes, 2 – moderate changes, 3 –severe damage. Maximum score per brain was therefore-9

2.6. Experimental outline

2.6.1. Exposure and initial treatment

Rats were exposed to sarin by i.m. injection (1.2 LD50, 108 µg/kg) and 1 min later were treated with TA (TMB4, 7.5 mg/kg and atropine, 5 mg/kg, i.m.) to ensure survival, and with midazolam i.m. (1 mg/kg) at 5 or 30 min following onset of convulsions. The 30 min delayed administration of midazolam was previously shown to induce inflammatory response in the brain and consequent brain pathology (Chapman et al., 2006, 2015). All surviving rats exhibited toxicity signs (immobility, hyper-secretion, fasciculation, tremor, convulsions and respiratory distress).

2.6.2. Additional treatments

Since both steroids (e.g. methylprednisolone and dexamethasone) and NSAID (e.g. indomethacin, and ibuprofen) exacerbated the seizure activity and worsened the clinical severity score when administered immediately following onset of seizures (not shown), the administration of the anti-inflammatory drugs was delayed. Thus, the anti-

inflammatory drugs were administered i.p. at 4 h, concomitant with the attenuation of seizure activity, (if sacrificed 8 h post exposure) or repeatedly at 4 h and 20 h (when scarified at 24 h post sarin exposure) and addition treatments at 30 and 44 h (when scarified at 48 h post sarin exposure).

2.6.3. The study of PGE2 analogs on sarin induced convulsions

This study was designed to decipher the mechanism of early PGE2 involvement in sarin toxicity. Thus, neither TA nor midazolam were administered in order to exclude drug interactions, and the dose of sarin was reduced to ensure survival of half of the animals. Rats were exposed to 1 LD50 sarin by i.m. injection (1 LD50, 90 μg/kg) and 2 min later PGE2 analogs were administered i.p. and this was repeated 2 h later. The doses of all treatments were selected based on their effective protective doses in previous publications (Abakay et al., 2018, Wu et al., 2015). No further administrations were added since previous studies showed elevated PGE2 levels in rat brains starting at 5–6 h following sarin induced seizures (Chapman et al., 2006).

At the indicated time points (8 h and 24 h for NSAIDs, 24 h and 1 week after PGE2 analogs, and 24 and 48 h for methylprednisolone) following sarin exposure, surviving animals were sacrificed, their brains removed and divided: one hemisphere was frozen in liquid nitrogen and kept at −80 °C for biochemical analysis and the other hemisphere was immersed in 4% paraformaldehyde for histological screening.

2.6.4. Clinical severity score

The severity score ranged from 0 (no signs) to 20 (death). Each of six categories: hyper-secretion, fasciculation, tremor, convulsions, respiratory distress and posture were scored from 0 to 3 points according to the severity of the clinical symptoms and the sum of these scores was defined as the severity score for each animal at each measured time point.

2.7. Statistical analyses

Data are presented as mean ± SEM, and differences between groups was assessed by analysis of variance (ANOVA), followed by an appropriate ad hoc analyses (Bonferroni, Dunnett). A value of $p < .05$ was accepted as statistically significant.

3. Results

3.1. Toxicity

Toxicity signs (hyper-secretion, fasciculation, tremor, convulsions, or respiratory distress) following sarin exposure appeared within 1–5 min and peaked at 20–30 min post exposure (Fig. 1).

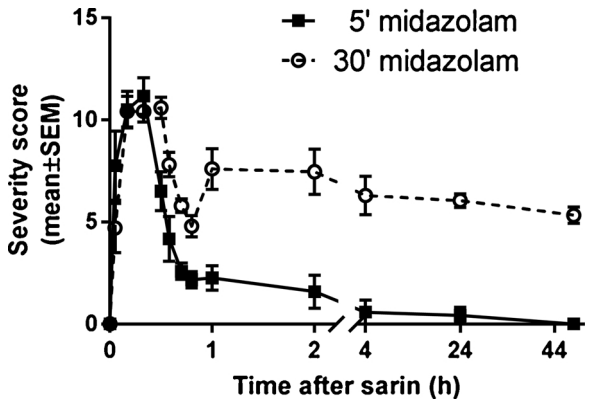

Fig. 1. Severity score following sarin exposed rats (1.2 LD50) treated immediately with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 5 or 30 min later with midazolam (1 mg/kg, i.m.). n = 6/group.

Administration of midazolam (1 mg/kg, i.m.) 5 min after seizure onset resulted in a decrease in the clinical severity score with no toxicity signs recorded after 4 h, and complete survival was noted. If midazolam administration was delayed by 30 min, some recovery and attenuation of seizures was seen immediately afterward and up to 4 h, but toxicity signs were still present 48 h post exposure. Sporadic deaths were recorded only before midazolam treatment (10–15%).

3.2. Histology

As can be seen in Fig. 2, brains of rats exposed to sarin and treated immediately with TA and 5 min later with midazolam, displayed no histological damage (A, C). In contrast, when the anticonvulsive treatment was delayed to 30 min from the onset of seizures, severe brain damage was seen. This included extensive loss of cells, laminar degeneration and vacuolization in the piriform cortex (PC), vacuolar necrosis in the dorsolateral thalamic nuclei, enlargement of ventricles and cell damage in the CA1 and CA3 layers of the hippocampus (B,D).

3.3. Effect of anti-inflammatory treatments

3.3.1. Immediate steroids and NSAIDs

In preliminary studies, rats exposed to sarin (1.2LD50, followed by suboptimal treatment of immediate TA and 1 mg/kg midazolam at 30 min) were administered the steroids methylprednisolone or dexamethasone (20 mg/kg) or the NSAIDs indomethacin or ibuprofen (10 mg/kg) at the onset of convulsions (1–2 minutes). These treatments seemed to exacerbate the seizure activity and increased the clinical severity score and the study was thus terminated (not shown).

3.3.2. Delayed methylprednisolone

The administration of the anti-inflammatory treatments was postponed to 4 h, following attenuation of convulsions. When methylprednisolone (20 mg/kg i.p.) was repeatedly administered at 4 h, 20 h, and when applicable additional treatments were administered at 30 and 44 h post sarin (1.2 LD50) exposure with suboptimal treatment, no clinical effect or biochemical improvement in inflammatory markers was measured. A representative graph of the effect of methylprednisolone on the inflammatory marker PGE2 is presented in Fig. 3. One way ANOVA supported the observation: PGE2 at 24 h $F(2,12) = 7.63$, $p < .01$. PGE2 at 48 h $F(2,13) = 4.33$, $p < .041$. *post hoc* Dunett test showed both groups differ from their respective controls $p < .007$-.048. The addition of methylprednisolone had no effect (*post hoc* Bonferroni analysis). Similar failures were recorded with dexamethasone (not shown).

3.3.3. Delayed NSAIDs

The non-selective COX inhibitor ibuprofen (10 mg/kg), the selective COX-2 inhibitor nimesulide (6 mg/kg), and the PLA2 inhibitor quinacrine (5 mg/kg), were administered 4 h and 20 h following suboptimal treatment of sarin exposure (1.2LD50, immediate TA and midazolam at 30 min). No change in clinical score was observed. The effects on the inflammatory markers were measured 8 and 24 h following sarin. Eight hours following sarin exposure, nimesulide resulted in a significant reduction of the inflammatory markers compared to the one seen after the suboptimal delayed midazolam treatment (Fig. 4). This observation was supported by one way ANOVA for each of the markers: TNFα $F(3,12) = 14.4$, $p < .001$; PGE2 $F(3,12) = 22.9$, $p < .0001$; Il1β $F(3,12) = 11.0$, $p < .002$; IL6 $F(3,12) = 8.5$, $p < .005$ followed by *post hoc* Bonferroni analysis that showed that the suboptimal midazolam treatment at 30 min, significantly increased all inflammatory markers compared to controls ($p < .001$–.004) and the beneficial effect of nimesulide was seen as a significant reduction in TNFα and PGE2 compared to the suboptimal delayed midazolam ($p < .01$-.0001). Partial reduction of PGE2 levels only were seen after ibuprofen and quinacrine treatment (not shown).

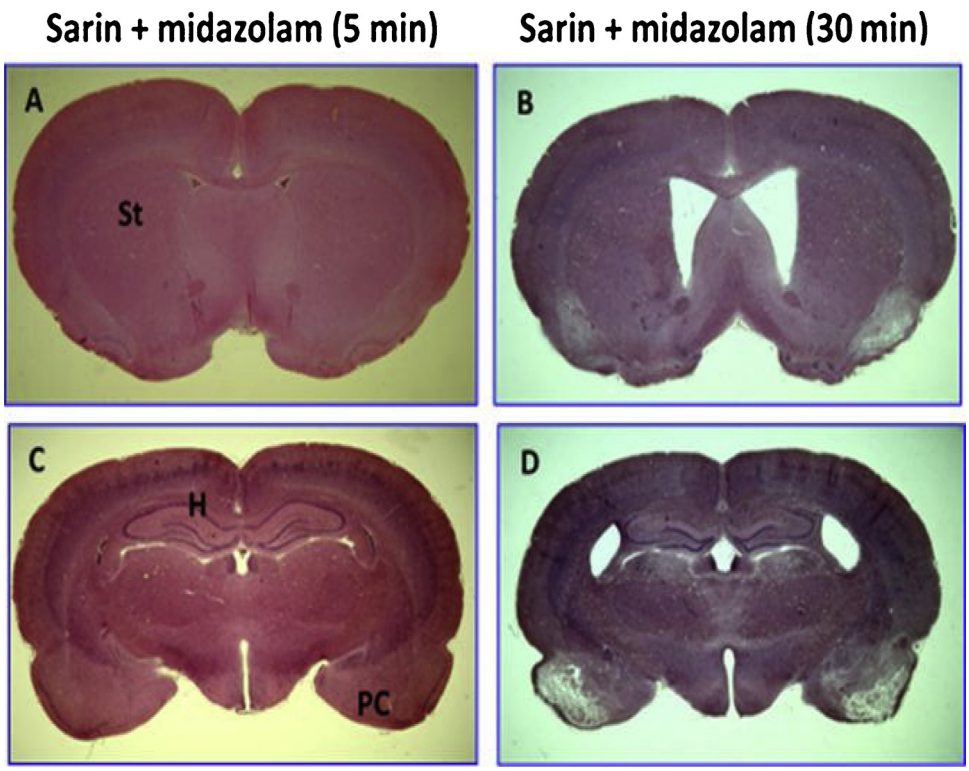

Fig. 2. Representative sections demonstrating histological damage 1 week following sarin exposure (1.2 LD50), treated 1 min later with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 5 (A,C) or 30 (B,D) min later with midazolam (1 mg/kg, i.m.) St-striatum, H-hippocampus and PC-piriform cortex.

24 h following sarin exposure, all three treatments were compared in their effects on inflammatory markers to that induced by the sub-optimal delayed midazolam treatment (Fig. 5). Of the three treatments, only nimesulide was effectively reducing all four inflammatory markers tested. Quinacrine reduced both IL-1β and IL-6 and ibuprofen reduced IL-6 only, compared to the levels seen in the sub-optimally treated delayed midazolam group.

This was supported by one way ANOVA for each of the markers: TNFα $F(4,29) = 8.35$, $p < .0001$; PGE2 $F(4,25) = 7.31$, $p < .001$; Il1β $F(4,29) = 7.58$, $p < .0001$; IL6 $F(4,29) = 11.97$, $p < .0001$ followed by post hoc Bonferroni analysis that showed the beneficial effect of nimesulide seen as a significant reduction in all markers compared to the suboptimal delayed midazolam ($p < .02$-$.0001$). The Bonferroni analysis also showed the significant reduction in both IL1β and IL6 by Quinacrine ($p < .009$, $.0001$) and reduction in IL-6 by ibuprofen ($p < .0001$) compared to the levels seen in the sub-optimally treated delayed midazolam group. In contrast, the histological severity score of the sarin-induced brain pathology (see Fig. 2B, D) obtained following delayed adjunct treatment with these anti-inflammatory drugs was unaffected by these treatments (Fig. 6).

3.4. Immediate PGE2 analogs

Since immediate treatment with COX inhibitors aggravated sarin-induced seizures, the role of PGE2 on the acute sarin toxicity was investigated. PGE2 analogs ilomedin (iloprost, 5 μg/kg), prostin (dinoprostone, 0.5 mg/kg) or misoprostol (1 mg/kg) were injected immediately (2 min) following sarin (1LD50 with no additional treatment), and again at 2 h after sarin, a reduction in inflammatory markers was seen 24 h later (Fig. 7). The analogs had no effect on the observed convulsions or on survival. In this experiment, the administered PGE2 analogs may interfere with the accurate measurement of brain PGE2 levels. Instead, we opted to measure TSPO, a marker of activated brain microglia previously shown to correlate with PGE2

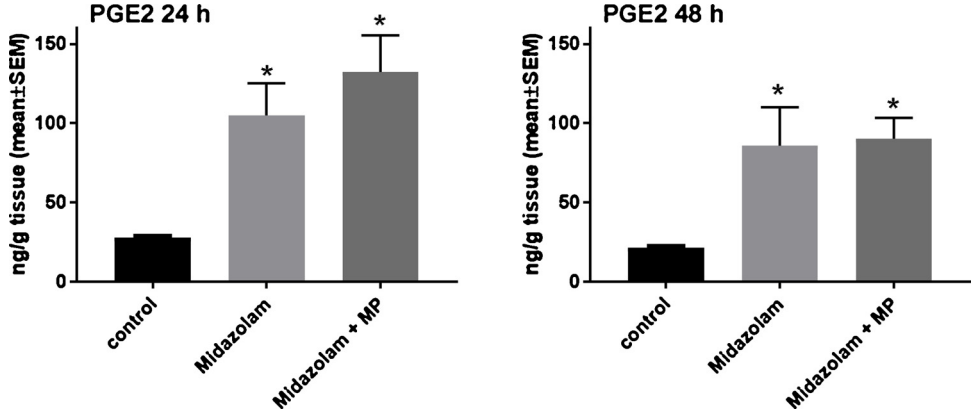

Fig. 3. PGE2 levels following delayed methylprednisolone (MP, 20 mg/kg) treatment of sarin exposure (1.2 LD50, treated immediately with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 30 min later with midazolam (1 mg/kg, i.m.)). n = 3–6/group. * vs. controls, $p < .007$-$.048$.

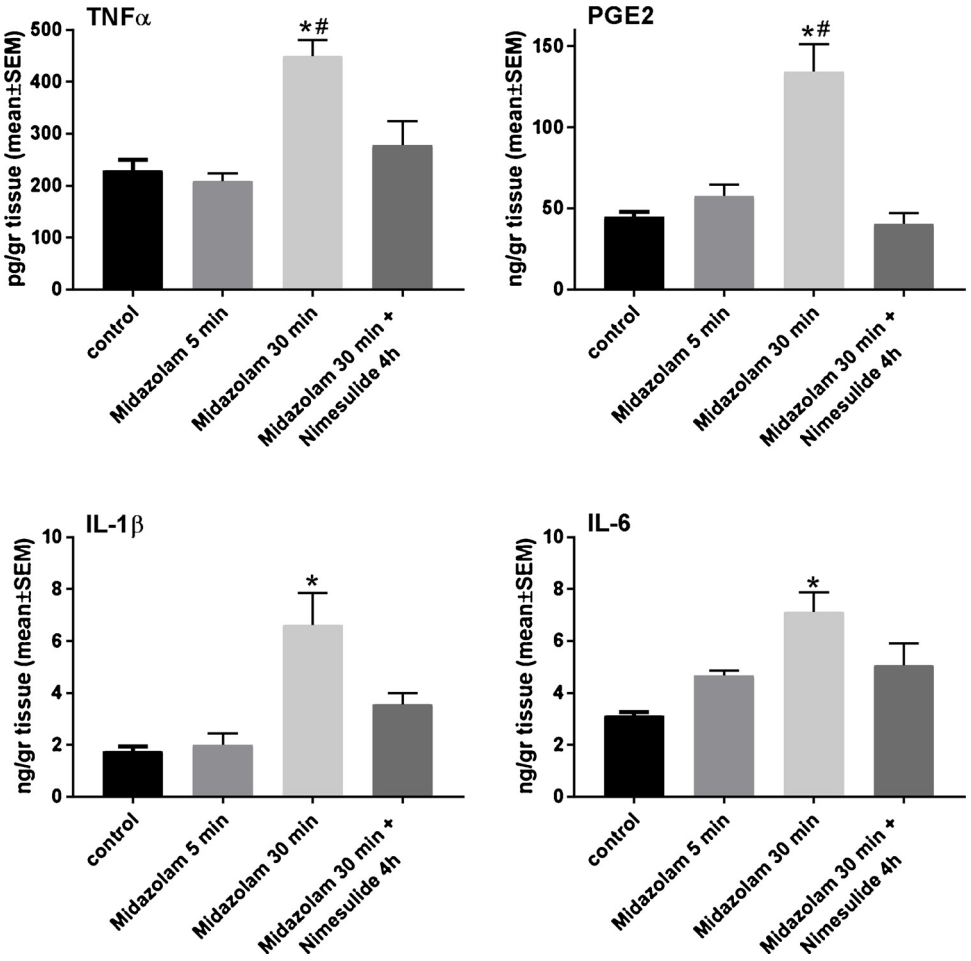

Fig. 4. TNFα, PGE22, IL-1β, and IL-6 in brain of rats 8 h after exposure to sarin, treated 1 min later with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 5 or 30 min later with midazolam (1 mg/kg, i.m.). 4 h later part of the group of rats from the delayed midazolam treatment was additionally treated with the selective COX-2 inhibitor nimesulide (6 mg/kg i.p.). n = 6/group, *vs. control $p < .001$-.004. #vs. nimesulide $p < .01$-.0001.

levels in the brain after sarin exposure (Grauer et al., 2008; Chapman et al., 2015). As can be seen, the PGE2 analogs significantly reduced the inflammatory markers and partially the marker for activated microglia (TSPO) at 24 h. These observations were supported by one way ANOVA for each of the tested markers: TNFα $F(4,24) = 8.75$, $p < .0001$; TSPO $F(4,23) = 10.41$, $p < .0001$; IL-1β $F(4,24) = 14.08$, $p < .0001$; IL-6 $F(4,25) = 13.68$, $p < .0001$, followed by *post hoc* Bonferroni analysis that showed significant difference between sarin exposed non treated group and all treated groups in TNFα, IL1β and IL6 ($p < .04$-.0001), and between sarin exposed non treated group and prostin or misoprostol treated groups in TSPO ($p < .04$-.0001).

Since the reduced level of TSPO, an indicator of microglial activation, suggested amelioration of some of the brain pathology following sarin, rats were further tested for TSPO 1 week after sarin exposure (the level of all other cytokines tested subside by 1 week, Chapman et al., 2006). Sarin exposed rats were treated with PGE2 analogs at 2 min and 2 h, with no additional treatment. Brain TSPO levels in sarin poisoned untreated rats were previously shown to increase over a period of 1–2 weeks post sarin exposure, and remain high for a prolonged period of time (months) (Grauer et al., 2008). This is also seen here in the increase in TSPO levels from 1 day (Fig. 7) to 1 week (Fig. 8). These high levels of TSPO are eliminated in some, but not all, of the sarin exposed animals treated immediately with PGE2 analogs (Fig. 7). This "all or none" effect was positively correlated with amelioration of brain pathology in the histological analysis of these same animals (not shown).

4. Discussion

We have repeatedly shown that brain pathology following sarin exposure is associated with an increase in inflammatory markers (Chapman et al., 2006, 2015, Lazar et al., 2016). In the present study the effects of anti-inflammatory treatments were evaluated following sarin induced convulsions and the consequent brain inflammation. Unexpectedly, the immediate treatment with either steroids or NSAIDs was deleterious and resulted in aggravation of the toxic signs severity score. Conflicting reports on the effects of anti-inflammatory drugs in the attenuation of seizures, suggested that their benefit depend on either or both the animal model of seizures used and the timing of treatment (Temp et al., 2017; Girgis et al., 2013; Holtman et al., 2009, 2010, 2014; Régnier et al., 2010; Salvadori et al., 2012). In the acute phase following sarin exposure, endogenous PGE2 may be beneficial in reducing the inflammatory reaction of the seizures and its ensuing neuropathology. Thus, we report here for the first time that sarin exposed rats treated immediately with PGE2 analogs showed decreased inflammatory markers. This is in accord with reports on neuroprotective effects of PGE2 analogs in other models of brain injury like ischemia and closed head injury (McCollough et al., 2004, Santos et al., 2017; Li et al., 2008; Tian et al., 2016). We further showed that the partial efficacy of immediate PGE2 analogs treatment in reducing sarin induced brain inflammation lasted for at least one week. This effect seem to be an "all or none" effect: some of the treated rats showed normal level of TSPO while others showed high TSPO levels similar to that of sarin poisoned-untreated rats. This result may suggest

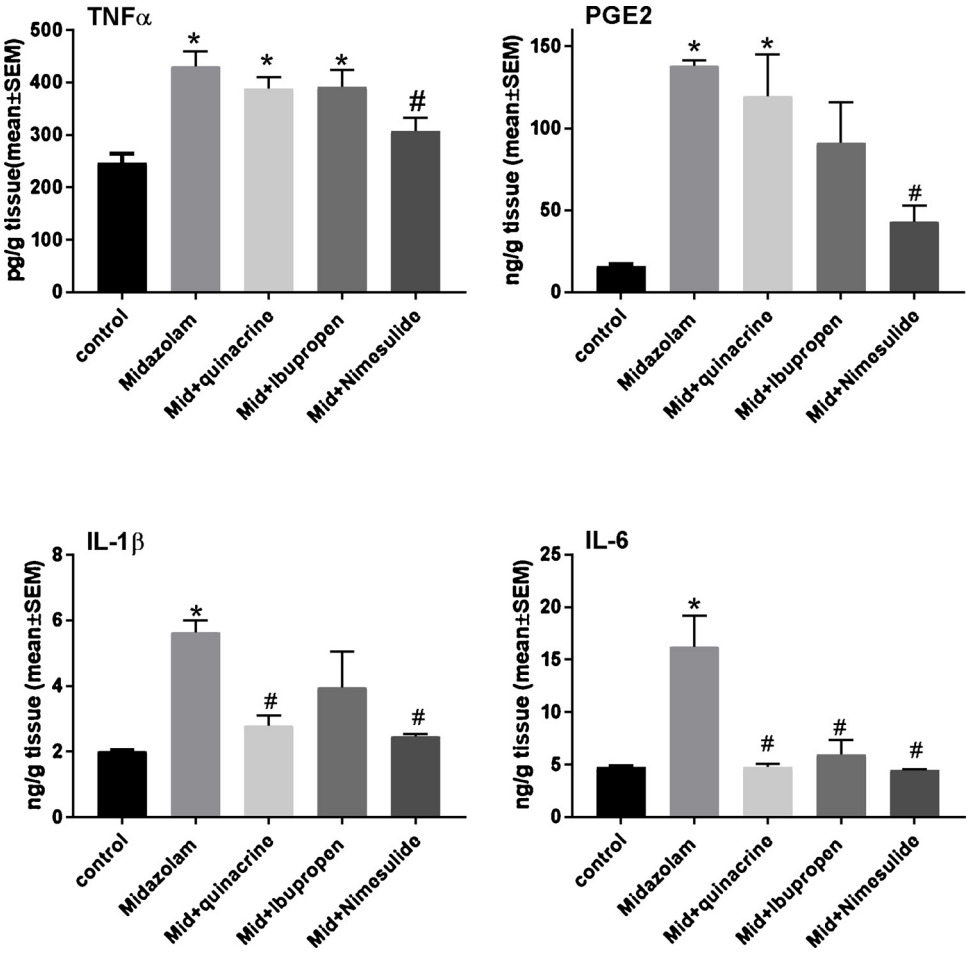

Fig. 5. TNFα, PGE22, IL-1β, and IL-6 in brain of rats 24 h after exposure to sarin, treated 1 min later with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 30 min later with midazolam (1 mg/kg, i.m.). 4 h and again 20 h later part of the group of rats from the delayed midazolam treatment was administered with the selective COX 2 inhibitor nimesulide (6 mg/kg i.p.), or the PLA2 inhibitor quinacrine (5 mg/kg i.p.), or with the NSAID ibuprofen (10 mg/kg i.p.). n = 6/group, *vs. control, # vs. midazolam, $p < .02$.

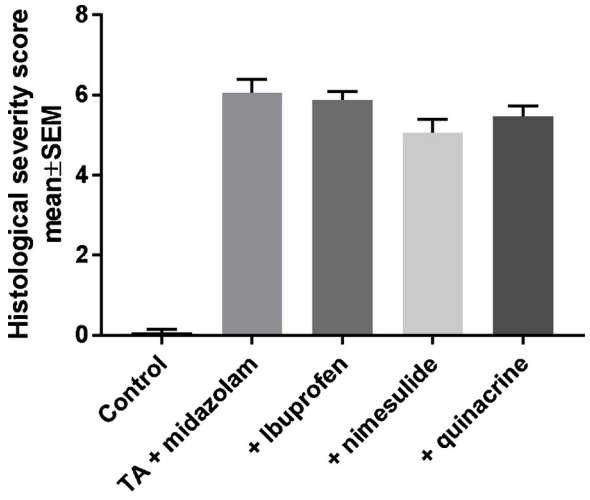

Fig. 6. Histological severity score of brains of rats exposed to sarin, treated 1 min later with TA (TMB4 7.5 mg/kg and atropine 5 mg/kg, i.m.) and 30 min later with midazolam (1 mg/kg, i.m.). 4 h and again 20 h later, groups of rats from the delayed midazolam treatment were administered with the selective COX 2 inhibitor nimesulide (6 mg/kg i.p.), or the PLA2 inhibitor quinacrine (5 mg/kg i.p.), or with the NSAID ibuprofen (10 mg/kg i.p.). n = 6/group.

suboptimal treatment either in dose or in schedule of treatment as these drugs are often continuously administered.

Since we hypothesize that PGE2 may be necessary for the immediate counteracting of seizure associated damage, while their persistent high levels may be detrimental, the efficacy of the delayed NSAIDs treatment (4 h post sarin exposure) was evaluated. Their partial beneficial effect may be symptomatic, reducing only the inflammatory markers with no significant amelioration of the underling brain pathology. One possible reason for the failure of delayed NSAIDs to improve brain pathology is that the delayed treatment allowed for prolonged convulsions previously shown to induce severe brain pathology (McDonough and Shih, 1997, 1998, Chapman et al., 2015) which the anti-inflammatory treatment was unable to repair.

There are conflicting reports that COX-2 inhibitors, which do not interfere with the constitutive COX1 PGE2 production, do ameliorate brain pathology following seizures (Tu and Bazan, 2003; Radu et al., 2017; Temp et al., 2017). In addition, there is a recent report of a beneficial effect of PGE2 receptor antagonist against DFP exposure in rats (Rojas et al., 2019). In our study the most effective anti-inflammatory treatment was the COX-2 inhibitor nimesulide which significantly reduced all of the inflammatory markers both at 8 h and at 24 h post sarin. This is in accord with reports of the beneficial effect of COX-2 inhibitors in other models of brain injury (Candelario-Jalil et al., 2002, 2003; Candelario-Jalil, 2008; Polascheck et al., 2010). Further evaluation of various doses, time and duration of administration following exposure may reveal its beneficial effects on histopathology

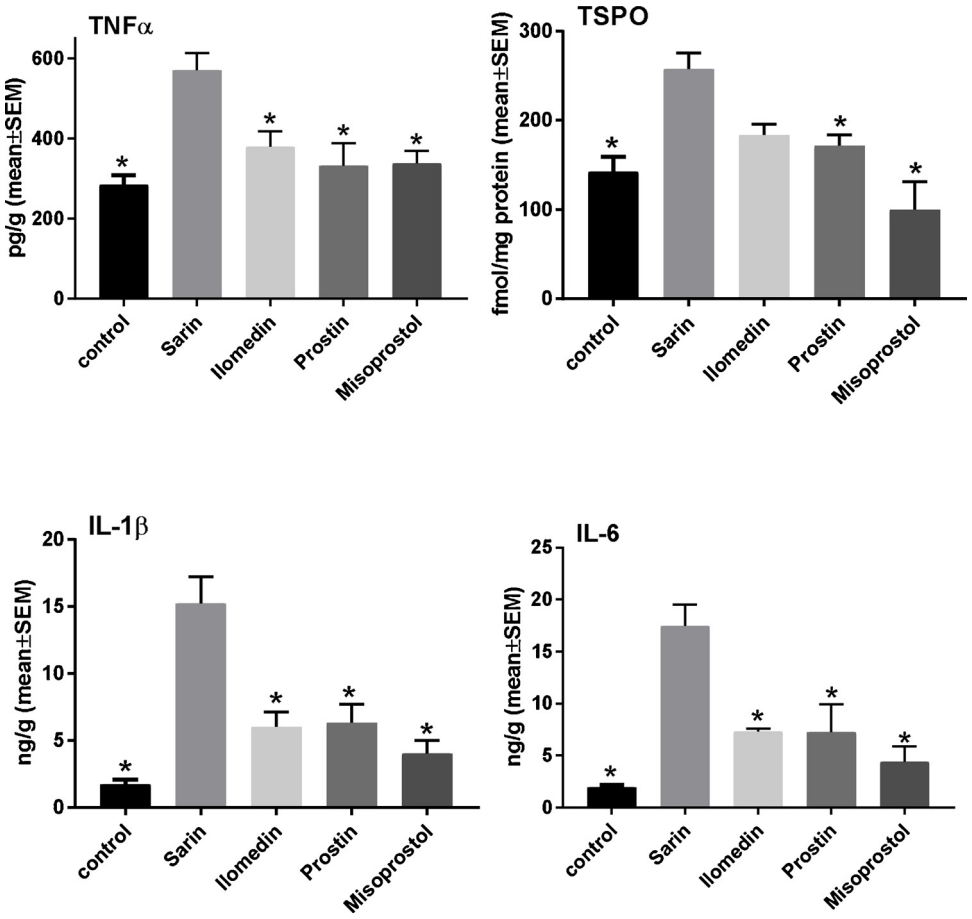

Fig. 7. TNFα, TSPO, IL-1β, and IL-6 in brain of rats 24 h after exposure to sarin (1LD50), treated only 2 min and 2 h later with the PGE2 analogs ilomedin (5 μg/kg i.p.), prostin (0.5 mg/kg i/p/) or misoprostol (1 mg/kg i.p.). n = 6/group, *vs. sarin, p < .04–.0001.

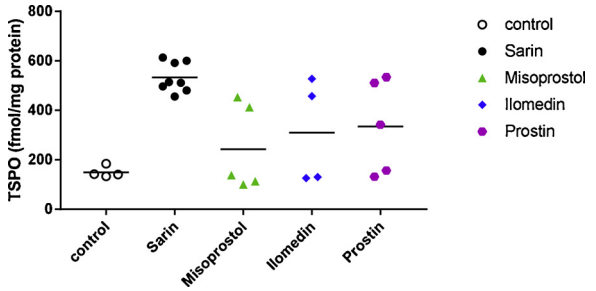

Fig. 8. TSPO in brain of rats one week after exposure to sarin (1LD50), treated 2 min and 2 h later with the PGE2 analogs ilomedin (5 μg/kg), prostin (0.5 mg/kg) or misoprostol (1 mg/kg). Data presented for individual animals to demonstrate within group variance. Horizontal lines are group means.

markers as well.

Others have shown that manipulation of PGE2 receptor pathways triggered both toxic and paradoxical protective effects after brain ischemia (Taniguchi et al., 2011, 2014). There are several possible mechanisms to explain early PGE2 neuroprotective activity which are not mutually exclusive:

- Early after exposure, high PGE2 may cause inhibition of COX-2 induction, which may explain the beneficial effect of immediate treatment with the PGE2 analogs at seizure onset, as early activation of PGE2 receptors might inhibit this inducible enzyme.
- Similar to the endogenous PGE2, the analogs themselves may have a beneficial effect in reducing seizure activity. This is construed from the aggravation of convulsions that were detected following the inhibition of PGE2 synthesis both in our study and in other studies following steroids (Supko and Johnston, 1994; Lee et al., 1989; Duffy et al., 2014) and NSAIDs (Régnier et al., 2010).
- PGE2 analogs may act as neuroprotective agents on PGE2-receptors and their downstream pathways in the brain and on the endothelium of brain blood vessels (Li et al., 2008; Tian et al., 2016; McCollough et al., 2004).
- Sarin is known to inhibit thrombin (Shuster et al., 1959; Thompson, 1970; Quistad and Casida, 2000) and interfere with the clotting cascade thus inducing brain and lungs hemorrhages (Chapman et al., 2019). Since PGE2 promotes blood clotting, it may reduce leakage of blood from brain capillaries to the brain parenchyma, thus preventing hemorrhage-induced neuronal insults. On the other hand, COX inhibitors act as anticoagulants and may exacerbate brain bleeding and the ensuing neurotoxic effect following sarin exposure. Furthermore, oximes may add to this effect by enhancing the anticoagulation cascade (Golderman et al., 2016).

The present sets of experiments supports all possible mechanisms. EEG studies following sarin exposure treated with PGE2 analogs may enable better differentiation between these mechanisms. Measuring clotting time following sarin exposure with the various treatments may help in supporting the thrombin hypothesis (Golderman et al., 2016).

The partial effect of PGE2 analogs on TSPO levels at 24 h and one week, suggest a reduction in activated glia following this treatment. This may be further investigated using other specific glia markers such as GFAP (Lazar et al., 2016) in order to delineate this specific effect.

In conclusion, although major inflammation is detected in the brain following sarin induced seizures, early treatment with steroids or with

NSAIDs is contraindicated, since the acute rise in PGE2 following sarin exposure may have an ameliorating effect on the consequent inflammation and brain injury. The delayed anti-inflammatory treatment administered following prolonged seizures, that did improve inflammatory markers, did not reverse the specific brain pathology that followed sarin induced prolonged seizures.

Transparency document

The Transparency document associated with this article can be found in the online version.

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