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Sarin-induced brain damage in rats is attenuated by delayed administration of midazolam

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ABSTRACT

Sarin poisoned rats display a hyper-cholinergic activity including hypersalivation, tremors, seizures and death. Here we studied the time and dose effects of midazolam treatment following nerve agent exposure. Rats were exposed to sarin (1.2 LD50, 108 μg/kg, im), and treated 1 min later with TMB4 and atropine (TA 7.5 and 5 mg/kg, im, respectively). Midazolam was injected either at 1 min (1 mg/kg, im), or 1 h later (1 or 5 mg/kg i.m.). Cortical seizures were monitored by electrocorticogram (ECoG). At 5 weeks, rats were assessed in a water maze task, and then their brains were extracted for biochemical analysis and histological evaluation. Results revealed a time and dose dependent effects of midazolam treatment. Rats treated with TA only displayed acute signs of sarin intoxication, 29% died within 24 h and the ECoG showed seizures for several hours. Animals that received midazolam within 1 min survived with only minor clinical signs but with no biochemical, behavioral, or histological sequel. Animals that lived to receive midazolam at 1 h (87%) survived and the effects of the delayed administration were dose dependent. Midazolam 5 mg/kg significantly counteracted the acute signs of intoxication and the impaired behavioral performance, attenuated some of the inflammatory response with no effect on morphological damage. Midazolam 1 mg/kg showed only a slight tendency to modulate the cognitive function. In addition, the delayed administration of both midazolam doses significantly attenuated ECoG compared to TA treatment only. These results suggest that following prolonged seizure, high dose midazolam is beneficial in counteracting adverse effects of sarin poisoning.

1. Introduction

Sarin poisoned rats display hyper-cholinergic activity that includes seizures, tremors, hyper-salivation and death. In surviving animals the intensity and duration of seizures were associated with the extent of neuronal degeneration and cognitive impairment (McDonough et al., 1995, 1999, 2000). Anticonvulsants were long being considered as a supplement for antidotal treatment against organophosphate poisoning (Domino, 1987). Midazolam is a commonly used anticonvulsant that was previously shown to be effective against convulsions in experimental animals. Midazolam was the most potent and rapidly acting compound out of six benzodiazepines tested in guinea-pigs exposed to 2LD50 of soman, the ED50 was 0.77 (0.30–1.64) and 2.24 (1.08–4.21) mg/kg when administered 5 and 40 min post exposure respectively (McDonough et al., 1999). Midazolam was similarly effective against sarin and VX toxicity in guinea-pigs (Shih et al., 2003). Since rapid seizure control minimizes the development of brain damage, the use of midazolam as an anticonvulsant is expected to improve clinical outcome in the treatment of nerve agent exposure. In a study of the long term consequence of untreated sarin inhalation, prolonged behavioral and histological deficits were demonstrated in surviving rats. The deficits were associated with long term elevation of PGE_2 and with glial activation measured by the increased TSPO (translocator protein) levels (Allon et al., 2011; Grauer et al., 2008). We have previously shown that rats treated with only TMB4 and atropine (TA) 1 min following 1.2 LD50 of sarin, developed long term seizure activity that can be terminated by midazolam (1 mg/kg) administered within 5 min after the onset of seizures. The termination of seizures inhibited the development of neuroinflammation and the long term behavioral and histological consequence of the seizures induced by sarin poisoning (Chapman et al., 2006). However, delaying this treatment for 30 min following the onset of seizures only ameliorated the seizures while brain damage and long-term neuronal inflammation still occurred (Chapman et al., 2006). Since

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in most cases of OP exposure, treatment is expected to be administered at a substantial delay, we studied the efficacy of midazolam administered either immediately (1 mg/kg, 1 min) or delayed (1 or 5 mg/kg, 1 h), to rats exposed to sarin (1.2 LD50) and treated with TA. Extensive behavioral, electrophysiological, biochemical and histological tests were employed to evaluate the ensuing brain deficits. Thus, the aim of the present study was to evaluate the potential contribution of delayed midazolam administration to animals exposed to sarin and treated sub-optimally with immediate TA.

2. Methods

2.1. Animals

Male albino Sprague-Dawley rats weighing 300–350 g at the beginning of the experiment were purchased from Charles River (England). All procedures involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals, National Academy Press, Washington, DC, 1996, and were approved by the Institutional Animal Care and Use Committee. Animals were housed three per cage, in a controlled environment with a temperature of 21 ± 2 °C and a 12-h light/dark cycle with lights on at 6 am. Food and water were available ad lib.

2.2. Materials

Sarin (isopropyl methylphosphono-fluoridate) was supplied by the department of Organic Chemistry of IIBR, dissolved in propylene glycol and kept frozen. Fresh dilute solutions in saline were prepared for each experiment. Atropine sulfate, TMB4, purchased from Sigma Chemical (Poole, Dorset, UK). Midazolam (Dormicum, 5 mg/ml solution) was purchased from Hoffman-La Roche (Nutley, NJ).

2.3. Experimental outline

Rats were exposed to sarin (108 μg/kg i.m., 1.2LD50), and 1 min later were treated with TMB4 and atropine (7.5 and 5 mg/kg i.m. respectively, TA). Midazolam was injected either 1 min after exposure (1 mg/kg i.m.), or 1 h following the onset of seizures (1 or 5 mg/kg i.m.). The doses were determined in previous studies and were selected to ensure prolonged seizure activity and long term survival. Midazolam 1 mg/kg was selected based on its ability to terminate seizures if administered up to 5 min post sarin. The higher dose (5 mg/kg) was selected in an attempt to terminate the seizures at 60 min. Rats were observed, and their signs scored at 2, 5 and 24 h post exposure based on a standard severity scale ranging from 0 (no signs) to 17. Rats were weighed once a week for up to 56 days. Cortical activity was sampled in some of the animals for up to 48 h post exposure. At 5 weeks, rats were assessed in a water maze task, at the end of which the animals were decapitated and their brains removed and divided into two hemispheres; one for biochemical analysis of inflammatory markers and the other for morphometric histological evaluation. Due to technical limitations, the study was divided into 2 separate experiments performed successively.

2.4. Electrocorticograms (ECoG)

Animals were anaesthetized (Equithesin, 0.3 mg/kg, i.p.) and electrode holes were drilled into the skull, approximately half way between Bregma and Lamda, and bilaterally at −4.0 mm. Chronic cortical stainless steel electrodes (PlasticOne, USA) were implanted and anchored to the skull with dental acrylic cement and stainless steel screws. Animals were allowed at least 1 week of recovery prior to sarin exposure. Electrophysiological recordings and Power analysis were conducted using the Acknowledge software and the MP-150 hardware system (Biopak, USA).

2.5. Water maze, working memory task

The water maze was a black pool, 140 cm in diameter and 50 cm deep, filled with tap water to a height of 25 cm. A square top wire mesh platform (12 cm × 12 cm) was placed 2 cm below water surface at one of four possible locations. The locations were the centers of the 4 quadrants formed by 2 crossing lines dividing the pool into 4 equal size quarters. At the beginning of each trial the rat was placed on the platform for 50 s and then gently placed in the water at one of four possible exit locations formed by the 2 crossing lines and the pool wall. If the rat failed to reach the platform within 120 s, it was pushed gently to the platform and left resting there for 30 s after which the rat was returned to its home cage and left under a heating lamp for at least 10 min. Each animal was tested 2 trials per day, 1 h apart. The platform and the exit locations were kept constant within days but were semi randomly changed between days. The decrease in time to reach the platform over days was indicative of intact reference memory, while the decrease in time from trial 1 to trial 2 within each day was indicative of intact working memory (Morris, 1984). Animal activity was monitored by a visual tracking system (HVS image, UK).

2.6. Inflammation markers and Microglia activation

Five weeks post exposure rats were decapitated and their brains rapidly removed and immediately frozen in liquid N2 and kept at −80 °C until used. Brain tissue was homogenized in 10 volumes of 50 mM Tris–HCl buffer (pH 7.4) using Polytron (Ultra Turrax, 15 s, setting 5–6). Translocater protein (TSPO, formerly termed PBR) was assayed using labeled PK-11195 ([3H]PK-11195, specific activity 83.5 Ci/mmol, purchased from Perkin Elmer, USA). Binding of [3H]PK-11195 to rat brain membranes was performed with minor modifications as previously described (Benavides 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 [3H]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). Nonspecific 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). For PGE_2 and cytokines 1 ml from the brain homogenate was used. Protease inhibitor cocktail (diluted 1:300, Sigma, P 8340) and indomethacin 2 mg/ml were added. Samples were centrifuged for 15 min at 15,000 rpm and the supernatant was frozen in aliquots for the biochemical assays. PGE_2 was assayed according to Sigma's procedure, supplied with its anti-prostaglandin E2 antiserum (P 5164) and used tritiated PGE_2 ([3H]PGE_2, specific activity 180 Ci/mmol, purchased from Amersham, England). The homogenates were then assayed as described. Cytokines were assayed by DuoSet ELISA development systems for rats, IL-1β (DY501), IL-4 (DY504), IL-6 (DY506) and IL-10 (DY522), R&D Systems, according to the protocols provided with the kits.

2.7. Histological evaluation

Two months post exposure, rats were decapitated, their brains rapidly but carefully removed and cerebellum discarded. Brains were fixed in 4% neutral buffered paraformaldehyde and processed routinely for paraffin embedding. Six micron coronal paraffin sections were cut serially and stained with hematoxylin and eosin