Neurotoxicology 68 (2018) 167–176

Contents lists available at ScienceDirect

Neurotoxicology

journal homepage: www.elsevier.com/locate/neuro

Full Length Article

Potentiation of antiseizure and neuroprotective efficacy of standard nerve agent treatment by addition of tariquidar

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ARTICLE INFO

Keywords:
Soman
Seizures
Atropine
Oxime
Diazepam
Tariquidar
p-Glycoprotein

ABSTRACT

Organophosphate (OP) induced seizures are commonly treated with anticholinergics, oximes and anticonvulsants. Inhibition of P-glycoprotein (PgP) has been shown to enhance the efficacy of nerve agent treatment in soman exposed rats. In the present study, the promising effects of the PgP inhibitor tariquidar were investigated in more detail in rats s.c. exposed to 150 µg/kg soman. Treatment with HI-6 and atropine sulfate (125 and 3 mg/kg i.m respectively) was administered 1 min after exposure. Diazepam (0.5 mg/kg i.m.) and/or tariquidar (7.5 mg/kg i.v.) were included either at 1 min or 40 min following onset of seizures.

Animals that received tariquidar, in addition to HI-6 and atropine, at 1 min, displayed a rapid normalization of EEG activity and cessation of seizure-associated behaviour. This improvement by addition of tariquidar was even more substantial in animals that also received diazepam, either immediately or delayed. Animals exhibiting lower intensity seizures displayed less severe neuropathology (neuronal loss, microglia activation and astrogliosis), primarily in the piriform cortex, and to a lesser extent amygdala and entorhinal cortex.

The present findings suggest that the interaction of tariquidar with atropine may be the decisive factor for enhanced treatment efficacy, given that atropine was previously found to be a PgP substrate. A more thorough understanding of the interactions of nerve agent antidotes, in particular the actions of central anticholinergics with benzodiazepines, could contribute to a future optimization of treatment combinations, particularly those aimed at later stage medical interventions.

1. Introduction

Organophoshorus nerve agents are substances that irreversibly inactivate acetylcholinesterase (AChE), leading to accumulation of acetylcholine in the synaptic cleft resulting in epileptic seizures and often death (Shih and McDonough, 1997). Pharmacotherapy of nerve agent poisoning is based on anticholinergic drugs, AChE reactivators and anticonvulsants to prevent lethality. Several agents, in particular soman, result in rapid ageing of AChE, rendering the enzyme resistant to reactivation. Consequently, *de novo* enzyme synthesis in that case is the only mechanism for AChE regeneration. As anticholinergic blockade with atropine is inadequate by itself, oximes are generally employed to aid in AChE reactivation before ageing of the enzyme-nerve agent complex occurs. Due to the low brain penetration of oximes, high doses are currently needed to achieve sufficient AChE reactivation in the brain (Cassel et al., 1997). Minor reactivation is presumably sufficient to be lifesaving, but a higher level of reactivation is assumed to be beneficial to mitigate of initiation and/or reduce or inhibit seizure propagation (Shih et al., 2011; Bueters et al., 2003).

Whereas passive transport through the blood-brain-barrier is largely determined by drug properties like lipophilicity, active drug transport is regulated by specific blood-brain-barrier (BBB) transporters such as the ATP binding cassette (ABC) transporters P-glycoprotein (PgP), and breast cancer resistance protein (BCRP). Both transporters actively lower bioavailability of PgP substrates in the brain. Many drugs, including cytotoxic drugs for cancer treatment, are PgP substrates and may therefore reach an insufficient availability at their site of action inside the brain. It has previously been proposed that a similar active brain efflux of nerve agent antidotes, possibly involving PgP action, also leads to a reduction of their therapeutic potential. Indeed, in a soman exposure model, pretreatment with tariquidar or coadministration of tariquidar, a potent, non-competitive inhibitor of PgP, successfully increased therapeutic efficacy of HI-6 and atropine (Joosen et al., 2011, 2016). In the present study, we set out to investigate in more detail the

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https://doi.org/10.1016/j.neuro.2018.08.005
Received 6 June 2018; Received in revised form 8 August 2018; Accepted 15 August 2018
Available online 18 August 2018
0161-813X/

Table 1
Experimental groups. Soman was administered at t = 0.

		Treatment at 1 min after exposure			*Treatment at 40 min. after seizure onset*		*n per group*	
Group	Soman (150 μg/kg) s.c.	HI-6 (125 mg/kg) AS[1] (3 mg/kg) i.m.	Tariquidar (7.5 mg/kg) i.v.	Diazepam (0.5 mg/kg) i.m.	Tariquidar (7.5 mg/kg) i.v.	Diazepam (0.5 mg/kg) i.m.	ChE activity	immuno
1	•	•	–	–	–	–	6	12
2	•	•	•	–	–	–	6	12
3	•	•	–	•	–	–	–	6
4	•	•	•	•	–	–	–	6
5	•	•	–	–	•	–	–	8
6	•	•	–	–	–	•	–	8
7	•	•	–	–	•	•	–	8
8	–	–	–	–	–	–	–	6

[1] Atropine sulfate.

promising effects of tariquidar on enhancing nerve agent treatment efficacy over the first 24 h after soman exposure in rats. In contrast to earlier studies, the atropine dose was decreased, and the combined treatment of HI-6 and atropine was supplemented with diazepam. Additionally, we investigated whether a delayed treatment with tariquidar could interfere with ongoing seizure activity 40 min after seizure onset, or whether it would augment effects of diazepam administered at the same time. Continuous EEG recordings served as a direct measure of seizure related brain activity and behavioral expressions of intoxication were assessed using a modified Racine scale. The extent of neuropathology, neuronal loss, microglia activation and astrogliosis were studied histologically 24 h after soman exposure in the piriform cortex, entorhinal cortex, amygdala, hilus and presubiculum, the main regions of the brain involved in seizure initiation and propagation.

2. Materials & methods

2.1. Animals

Male Wistar WU rats (240–260 g at arrival, n = 60) were obtained from Charles River Laboratories (Sulzfeld, Germany). The animals were housed 2–3 animals per cage in Macrolon Type 4 cages for at least one week to acclimate to the conditions in the facility. A 12 h light cycle (7 AM–7 PM) was employed and temperature was kept between 19–22 °C with a relative humidity of 55–65%. Standard rodent chow (Teklad Global Diet, Harlan, Horst, The Netherlands) and acidified water were available *ad libitum*. All experiments were in agreement with EU Directive 2010/63/EU for animal experiments and approved by the Ethical Committee on Animal Experimentation of TNO and USAMRMC Animal Care and Use Review Office (ACURO).

2.2. Chemicals

Soman (1,2,2-Trimethylpropyl methylphosphonofluoridate) and HI-6 (1-2-hydroxyiminomethyl-1-pyridino-3-(4-carbamoyl-1-pyridino -2-oxapropane dichloride) were obtained from the stocks of TNO Rijswijk, The Netherlands and were of > 98% purity. Diazepam solution (5 mg/ml) was obtained from AUV Veterinary Service (Cuijck, The Netherlands). Tariquidar (98%) was obtained from MedKoo Biosciences (Chapel Hill, NC, USA), and solubilized by adding 2.2 M equivalents of methanesulphonic acid in milli-Q water/acetonitrile (50/50). The solution was lyophilised overnight, washed with diethyl ether (3 × 2 ml) and lyophilized overnight to produce a final dimethylsulphonate salt that was quantified using NMR and LC/MS. All other chemicals (atropine sulfate salt monohydrate, 97%) and standard chemicals were obtained from Sigma Aldrich and of standard purity.

2.3. Surgery

During cortical EEG probe implantation, rats were anesthetized with 2–3% isoflurane. The EEG electrodes (two stainless steel screws) were placed on the dura mater at A1.0 and P6.0 mm relative to Bregma and at 1 mm from the sagittal suture, and fixed in place with dental cement and connected to an adaptor. In the same surgical procedure, an indwelling jugular vein catheter was routed to the opening for the head stage. The cannula was filled with heparinized glycerol (500 IU/ml) and capped. Subcutaneous analgesia (carprofen, 5 mg/kg) and antibiotics (Borgal® -Trimethoprim 4 mg/kg and Sulfadoxine 20 mg/kg) were given prior to surgery and 24 h post operatively (1 ml/kg). Following surgery, the animals were individually housed and allowed to recover for one week prior to soman exposure.

2.4. Treatments

One week after surgery, baseline EEG values were recorded for at least 30 min before rats were administered soman at a dose of 150 μg/kg (2LD50 s.c. according to Marrs et al., 2007). An overview of the experimental groups is presented in Table 1. One minute after soman exposure, atropine sulfate and HI-6 were given (3 and 125 mg/kg, i.m., respectively 0.5 ml/kg, groups 1–7). In predetermined groups, animals were co-administered diazepam (0.5 mg/kg i.m.) with atropine/ HI-6 in the same injection (groups 3 and 4). For the delayed treatment groups, animals received diazepam 40 min after seizure onset (groups 6 and 7). Tariquidar (7.5 mg/kg i.v.) or its vehicle (propylene glycol/5% sucrose/ethanol 4:5:1) were also administered at 1 ml/kg 1 min after soman (groups 1–4) or 40 min after seizure onset (groups 5–7). Half of the animals were subjected to transcardial perfusion with formaldehyde fixative to allow for brain immunohistochemistry, while cholinesterase activity was assessed in the brains of the remainder of each group. In the groups where tariquidar and diazepam were co-administered 40 min after seizure onset (5–7), only immunohistochemistry was conducted.

2.5. EEG signal acquisition and analysis

Cortical EEG data were acquired using Ponemah software (Data Science Inc, DSI) using the TL11M2-F40-EET transmitter, which was attached to the adapter fixed on the skull. The transmitter data were sampled at 250 Hz. Simultaneously, video capture of the animal's behavior was stored in a synchronized manner with the EEG data. The behavior and EEG data were analyzed offline using Neuroscore software. The onset of seizures was scored by visual inspection of the online signal and was defined as high frequency spiking of at least 3 times elevation from baseline over a period of 20 s. Total EEG power (expressed as EEG-AUC) was defined as the summed power in different frequencies obtained via FFT spectral analysis, which also served as a