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Supralethal poisoning by any of the classical nerve agents is effectively counteracted by procyclidine regimens in rats

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ABSTRACT

A treatment regimen consisting of HI-6, levetiracetam, and procyclidine (termed the triple regimen) has previously been shown to work as a universal therapy against soman poisoning in rats, since it has capacities to function as both prophylactic and therapeutic measure. The purpose of the present study was to examine whether the triple regimen may have antidotal efficacy against intoxication by other classical nerve agents than soman. The treatment was given 1 and 5 min after exposure to a supralethal dose of nerve agents, and the results showed that the triple regimen successfully prevented or terminated seizures and preserved the lives of rats exposed to 5 × LD_{50} of soman, sarin, cyclosarin, or VX, but solely 3 × LD_{50} of tabun was managed by this regimen. To meet the particular antidotal requirements of tabun, the triple regimen was reinforced with obidoxime and was made to a quadruple regimen that effectively treated rats intoxicated by 5 × LD_{50} of tabun. The rats recovered very well and the majority gained pre-exposure body weight within 7 days. Neuropathology was seen in all groups regardless of whether the rats seized or not. The most extensive damage was produced by sarin and cyclosarin. Differentiation between the nerve agents' potency to cause lesions was probably seen because the efficacious treatments ensured survival of supralethal poisoning. A combination of 2 oximes and 2 anticonvulsants may be a prerequisite to counteract effectively high levels of poisoning by any classical nerve agent.

1. Introduction

Organophosphorus nerve agents, such as tabun, sarin, soman, cyclosarin, and VX, are categorized as the most toxic of chemical warfare means. Exposure to nerve agent can induce increased salivation, respiratory distress, tremor, seizures/convulsions, coma, and death. The destructive effects of nerve agents have repeatedly been demonstrated in recent years. Sarin was used twice in Japan by terrorists (Okumura et al., 2003, 2005), and sarin was dispersed in the suburbs of Damascus in Syria in 2013 resulting in massive casualty (Rosman et al., 2014).

The primary mechanism of action of nerve agents is inhibition of acetylcholinesterase (AChE), the enzyme that hydrolyzes acetylcholine. Accumulation of acetylcholine results in excessive stimulation of muscarinic and nicotinic receptors. Increased cholinergic activity in the brain is probably related to the initial phase of seizures, whereas sustained seizures are probably associated with increased glutamatergic activity leading to neuronal damage (McDonough and Shih, 1997).

Exposure to nerve agent requires immediate medical treatment. For this purpose, military personnel are issued with autoinjectors containing countermeasures for self-administration or "buddy aid". Antidotes against nerve agents are based on drugs acting at the muscarinic receptors and $GABA_A$ receptors (McDonough and Shih, 1997). In addition, partial protection against nerve agents can be obtained by the use of reversible (carbamate) AChE inhibitors shielding a portion of AChE from irreversible inhibition by nerve agents prior to nerve agent exposure. Furthermore, reactivation of any unaged AChE by an oxime is regarded as important immediate treatment after nerve agent exposure.

A number of armed forces have based their therapy against nerve agent intoxication on an oxime (obidoxime, 2-PAM, HI-6), an anticholinergic agent (atropine) combined with carbamate (pyridostigmine) pretreatment (Aas, 2003). Such treatment regimens can, however, reduce immediate lethality, but they do not attenuate the occurrence of nerve agent-induced seizure activity and concomitant convulsions, unless atropine is given early and at a high dose (McDonough and Shih, 1997). To overcome this shortcoming, several nations have provided their personnel with autoinjectors containing diazepam or avizafone (both benzodiazepine analogues with similar anticonvulsant action).

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The choice of oxime in autoinjector therapy differs among countries. Currently, there is no universal oxime that effectively can reactivate AChE inhibited by any known nerve agent. This oxime-nerve agent specificity makes the choice of a single oxime difficult; alternatively plural oximes have to be considered. Most studies indicate that obidoxime is more efficient than HI-6 (1-[([4-(aminocarbonyl)pyridinio]methoxy)methyl]-2-[(hydroxyimino)methyl]pyridinium) against tabun, whereas HI-6 is a better drug than obidoxime against soman. However, HI-6 is assessed by many as the most promising broad spectrum oxime against nerve agent intoxication (Aas, 2003; Kassa, 2002), although in a recent comprehensive study examining a variety of oximes against all classical nerve agents and a number of organophosphorus pesticides in guinea pigs it was concluded that MMB-4 and HLö-7 are the most efficacious oximes (Wilhelm et al., 2014). Some countries use atropine along with obidoxime (e.g., Germany, Norway, The Netherlands, and Finland). A future autoinjector regimen containing atropine, HI-6, and diazepam (presently used by Canada) is proposed by several nations within NATO (Aas, 2003).

After the Gulf War, the prevalent opinion has been to reduce reliance on pretreatment against nerve agent. However, in the present situation, an adequate stand-alone post-poisoning treatment does not exist. As a substitute, it has been claimed that it is reassuring and necessary to have an adequate pretreatment in place (Van Helden et al., 2011). In the search for an effective stand-alone therapy, we have performed a series of lesion and microinfusion studies to identify critical control sites for soman-induced seizure activity in the forebrain of rats. Subsequent specification of important pharmacological subreceptors in the sites identified can provide information for the selection of drugs affecting the control sites in the forebrain and avoiding the brainstem with the control center for respiration (Myhrer, 2010). Through this process, procyclidine turned out to have the highest impact of the drugs tested in seizure controlling sites in the forebrain of rats (Table 4 in Myhrer, 2010). Enhancement of procyclidine's outstanding antidotal properties (anticholinergic and antiglutamatergic) would make up a novel and interesting approach. Levetiracetam with a unique profile in preclinical models of epilepsy has been shown to increase the potency of other antiepileptic drugs up to 19-fold (Kaminski et al., 2009).

In a recent study, it was demonstrated that a triple regimen consisting of HI-6, levetiracetam, and procyclidine is able to prevent or terminate seizures and conserve lives when given 1 and 5 min after poisoning with supralethal doses of soman (3, 4, and 5 × LD_{50}) (Myhrer et al., 2013b). In order to investigate whether the triple regimen may have a more extensive applicability, the present study was designed to test the anticonvulsant and life-saving properties of the regimen in rats challenged with supralethal doses of tabun, sarin, soman, cyclosarin, or VX. Results from pilot experimentation showed that a quadruple regimen consisting of HI-6, obidoxime, levetiracetam, and procyclidine was required to effectively manage intoxication by 5 × LD_{50} of tabun, but for the other nerve agents (5 × LD_{50}) the triple regimen was effective (Fig. 1).

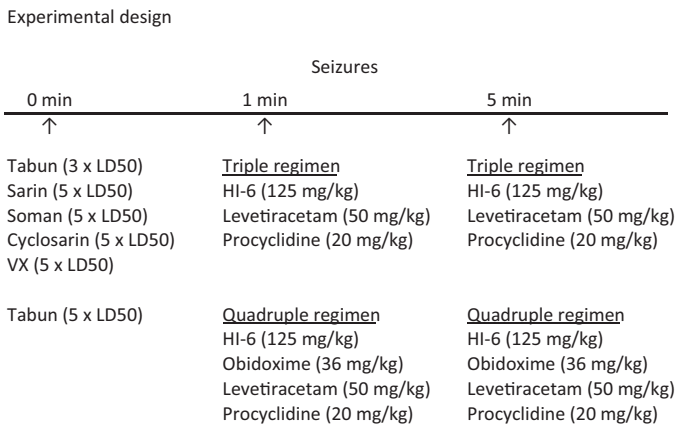

Fig. 1. Schematic overview of the experimental design. Because the triple regimen was only able to counteract poisoning by a tabun dose of 3 xLD_{50}, obidoxime was added to make up a quadruple regimen that effectively managed survival following a tabun dose of 5 × LD_{50}.

2. Materials and methods

2.1. Animals

Male Wistar rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 300–330 g served as subjects. The experiments were approved by the National Animal Research Authority. The animals were housed individually and had free access to commercial rat pellets and water. The rats were handled individually 3 days preoperatively and 3 days postoperatively, being allowed to explore a table top (80 × 60 cm) for 3 min a day. The climatized vivarium (21 °C) was illuminated from 0700 to 1900 h.

2.2. Surgery

The rats were anesthetized ip with diazepam (4.5 mg/kg) and fentanyl fluanisone (2 mg/kg). Stainless screws were implanted bilaterally in the parietal cortex (1 mm behind bregma, 3 mm lateral to midline); one served as recording electrode and the other one served as ground. The screws were fixed with dental cement (Durelon; ESPE, Seefeldt, Germany). The rats were given a recovery period of 7 days.

2.3. Drugs

The drug doses chosen were derived from previous studies of anticonvulsant effects against soman-evoked seizures in rats; HI-6 dimethanesulphonate 125 mg/kg, obidoxime 36 mg/kg, levetiracetam 50 mg/kg, procyclidine hydrochloride 20 mg/kg (Kassa and Koupilová, 2000; Myhrer et al., 2011, 2013b). The drugs were dissolved in 0.9% saline and were administered intramuscularly. The injection site alternated between the left and right muscle in the hind leg. The drugs were given successively in the following order: oxime(s), levetiracetam, and procyclidine. Because only LD_{50} doses of soman are known for our strain of male Wistar rats, we calculated the conversion factor for the toxicity relation between soman and each of the other nerve agents to reach at the exact doses for our rats. The relevant information is available in previous studies in which soman was used in addition to 1 or several of the other nerve agents (Bajgar, 1992; Hamilton and Lundy, 1989; Hoskins et al., 1986; Kassa et al., 2012; Shih and McDonough, 1999; Sivam et al., 1984). The mean conversion factor based on 2–3 studies was used for the toxicity between soman and each of the other nerve agents. According to the calculations, soman is 2.3, 1.32, 1.95, and 0.17 times more toxic than tabun, sarin, cyclosarin, and VX, respectively. The nerve agent dose of 5 × LD_{50} was for tabun 920 μg/kg, sarin 528 μg/kg, cyclosarin 780 μg/kg, soman 400 μg/kg, and VX 68 μg/kg. The 3 and 4 × LD_{50} doses for tabun were 552 μg/kg and 736 μg/kg. The nerve agents were injected subcutaneously. The treatment regimens were given 1 min (injections started at 50 s) and 5 min (injections started at 4.7 min) after exposure to nerve agent. Levetiracetam is commercially available (Keppra®). Procyclidine was purchased from Sigma (St Louis, MO, USA), and obidoxime was purchased from Merck (Darmstadt, Germany). HI-6 dimethanesulphonate was a gift from