# **ORIGINAL ARTICLE**

# A comparison of protective and anticonvulsive efficacy of two prophylactic mixtures in soman-poisoned rats

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### Summary

The protective and anticonvulsive efficacy of two prophylactic mixtures (PANPAL consisting of pyridostigmine, benactyzine, and trihexyphenidyle and pyridostigmine plus biperiden) administered prior to the administration of soman in a lethal dose  $(1.5 \text{ LD}_{50})$  with or without antidotal treatment (atropine + HI-6) was evaluated using rats as experimental animals. The pretreatment was applied 30 and 60 min before intoxication and the antidotal therapy was administered 1 min after soman poisoning. The anticonvulsive efficacy of both prophylactic combinations was determined using a seven degree scale. Non-treated soman-poisoned rats died within 10 min after soman challenge showing severe tremor, subconvulsions and generalized convulsions. More than 90% of pretreated animals survived for 24 hrs following soman poisoning and they were observed to be free from soman-induced toxic signs 24 hrs after soman administration. Our findings confirm that both prophylactic mixtures are able not only to protect experimental animals from the lethal effects of soman but also to eliminate most soman-induced toxic signs. Our results confirm that both prophylactic mixtures should be considered as a pretreatment for nerve agent poisoning, especially in the case of the threat of exposure to soman.

Keywords: soman - antidotal treatment - convulsion - prophylactic mixture - rat

## INTRODUCTION

Highly toxic organophosphorus compounds called nerve agents (sarin, soman, VX, tabun, cyclosarin) are strongly considered to be a probable chemical threat in war time or terroristic attacks against the civilian population due to their chemical, physical and toxicological properties. Their harmful effect is related to their power to irreversibly inhibit mammalian acetylcholinesterase (AChE, EC 3.1.1.7), the enzyme responsible for the regulation of neurotransmitter acetylcholine (ACh) concentration at the cholinergic synapses (Marrs 1994). Inhibition of AChE induces a major increase in ACh level in the cholinergic nervous system producing muscle fasciculations, respiratory distress and epileptic fits leading to generalized seizures (Petras 1983, Bajgar 1996). Brain seizures and status epilepticus contribute to the profound brain damage that occurs as a consequence of exposure to nerve agents (McLeod at al. 1984).

The current antidotal treatment of nerve agentinduced acute poisoning usually consists of anticholinergic drugs to antagonize the effects of ACh excess at cholinergic receptor sites and oximes to reactivate nerve agent-inhibited AChE (Kassa Unfortunately, 2002, Shih 1993). some organophosphates were found to be resistant to standard antidotal treatment. One of the most resistant organophosphorus compounds is soman (pinacolvl methylphosphonofluoridate). Its deleterious effects are extraordinarily difficult to counteract because of the rapid ageing of the somaninhibited AChE (Bajgar 1996).

The relatively unsatisfactory treatment available for acute nerve agent poisoning has prompted studies of pretreatment possibilities that will allow survival and increase the resistance of organisms exposed to nerve agents. One currently available method of protection against nerve agent poisoning is the use of pyridostigmine bromide, a reversible carbamate AChE inhibitor (Anderson et al. 1992). The pre-treatment effect of pyridostigmine can result from its reversible inhibition of AChE. It binds a small fraction of AChE in the periphery and reversibly shields it from irreversible inhibition by nerve agents (Bajgar et al. 1994). However, pyridostigmine is not able to protect AChE in different brain regions involved in central seizures produced by some nerve agents, primarily soman (McDonough and Shih 1997) due to its peripheral activity. In addition, a pyridostigmine-induced increase in the level of ACh can itself cause signs of poisoning. Therefore, it would be useful to counteract the effects of the accumulated ACh using anticholinergic drugs. In addition, the combination of pyridostigmine with anticholinergic drugs allows an increase in the dose of pyridostigmine because the anticholinergic drugs are able to counteract the cholinergic side effects of pyridostigmine (Kassa and Bajgar 1996, Kassa and Fusek 1998). One of these mixtures, pyridostigmine in combination with benactyzine (BNZ) and trihexyphenidyle (THP), designated PANPAL, has been developed in the Czech Republic and introduced in the Czech Army (Vachek et al. 1993). Another mixture, pyridostigmine in combination with biperiden, a centrally acting anticholinergic drug that also possesses an N-methyl-D-aspartate activity, has been developed in Bulgaria (Samnaliev 2002).

The aim of the current study was to assess the anticonvulsive efficacy of both pharmacological pretreatment mixtures with or without antidotal treatment consisting of HI-6 and atropine in soman-poisoned rats. The soman-induced convulsions were measured using a seven degree scale.



Fig. 1. Anticonvulsive activity of two prophylactic mixtures administered 30 min before soman poisoning; scale score 6=exit, \* statistically significant

## **MATERIAL AND METHODS**

Animals used in our experiments were male albino Wistar rats weighing 180–220 g. They were kept in an air-conditioned room and allowed access to standard food and tap water *ad libitum*. The rats were divided into groups of twelve (n=12). The soman used was of 98.5% purity. Its purity was assayed by acidimetric titration. The oxime HI-6 was synthesised at the Department of Toxicology of

the Military Medical Academy and was 98% pure. Its purity was analysed using HPLC. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Pyridostigmine (0.75 mg/kg of body weight) in combination with BNZ (16 mg/kg of body weight) and THP (6.3 mg/kg of body weight) or pyridostigmine (0.75 mg/kg of body weight) in combination with bideriden (2.5 mg/kg of body weight) was administered intramuscularly (i.m.) as a solution in distilled water (0.1 ml/100g of body weight) 30 and 60 min before i.m. soman challenge at a lethal dose  $(1.5 \text{ LD}_{50})$ . Antidotal treatment (HI-6 in combination with atropine) was carried out by i.m. injection 1 min following soman administration. The doses of HI-6 (15.6 mg/kg of body weight) and anticholinergic drug atropine (10 mg/kg of body weight) correspond to human-relevant doses (2% of their  $LD_{50}$ ) (Bajgar et al. 1994).

The anticonvulsive efficacy of the prophylactic mixtures was determined by using a seven degree scale for assessment of the toxic signs observed after the challenge by soman: 0 - no toxic signs, 1 - hyperactivity, 2 - chewing and/or salivation, 3 - fasciculations and/or tremor, <math>4 - subconvulsions, 5 - convulsions and <math>6 - death. Observations were carried out at 5, 15, 30, 60, 120, 180, 240 and 1440 min after the intoxication (Samnaliev 2001). The survival rate of rats within 24 hours in all experimental groups was also determined.

The evaluated markers of soman-induced convulsive activity in experimental animals were compared to the parameters obtained from control rats, administered with saline instead of pharmacological pretreatment and poisoned with soman at the same dose.

Statistic analyses were performed with Student's t-test at the level  $2\alpha$ =0.05.



Fig. 2. Anticonvulsive activity of two prophylactic mixtures administered 60 min before soman poisoning; \* statistically significant

### RESULTS

The results obtained from the survival study showed that the efficacy of PANPAL does not depend on the time of pretreatment and more than 90% of soman-poisoned animals survived for 24 hrs after the challenge with soman. On the other hand, the protective activity of pyridostigmine plus biperiden decreased in the case of the 60 min pretreatment regimen compared to the 30 min pretreatment. When the pharmacological pretreatment was combined with antidotal treatment, all somanpoisoned animals survived for 24 hrs regardless of the composition of the prophylactic mixtures (Tab. 1).

In a comparison of the anticonvulsive activity of both prophylactic mixtures tested, our results showed that both mixtures are able to protect rats from severe toxic signs caused by  $1.5 \text{ LD}_{50}$  of soman, including subconvulsions and convulsions. Pyridostigmine combined with biperiden, and applied 30 min prior to the soman challenge, seems to be slightly more effective, especially at the beginning of soman poisoning (Fig. 1). On the other hand. PANPAL demonstrated а higher anticonvulsive efficacy compared to the combination of pyridostigmine and biperiden when the time of pretreatment administration was prolonged to 60 min (Fig. 2). Nevertheless, the difference between the anticonvulsive efficacy of both prophylactic mixtures was not significant. There was no marked improvement in soman-induced toxic signs when the pharmacological pretreatment was followed by antidotal treatment consisting of the oxime HI-6 and atropine. Some animals from both experimental groups showed subconvulsions during the first 60 min following soman poisoning. Later, the toxic effects of soman were reduced and only slight tremors and fasciculations were demonstrated 24 hours after the soman challenge (Fig. 3 and 4). Both prophylactic mixtures were a little more effective when administered 30 min before the soman challenge (Fig. 3). All animals survived for 24 hours after soman administration. There were no significant differences between both prophylactic mixtures tested when they are combined with antidotal treatment.

Table. 1. Survival of soman-poisoned rats pretreated with the prophylactic mixtures tested and treated with HI-6 and atropine

Groups/Time	4 hrs after the challenge	24 hrs after the challenge
PANPAL – 30 min	11/12	11/12
Biperiden + Pyr - 30 min	12/12	12/12
PANPAL – 60 min	11/12	11/12
Biperiden + Pyr - 60 min	10/12	10/12
PANPAL – 30 min + Atr + HI-6	12/12	12/12
Biperiden + Pyr $- 30 \text{ min} +$	12/12	12/12
Au + HI-6 PANPAL - 60 min + Atr + HI-6	12/12	12/12
Biperiden + Pyr – 60 min + Atr + HI-6	12/12	12/12

## DISCUSSION

In the case of a threat of soman exposure, it seems to be very important to have a sufficiently effective pretreatment available because soman-induced toxic effects are extraordinarily difficult to counteract due to the very low reactivating efficacy of currently used oximes (Kassa 1995, Kassa 2002, Lallement et al. 1997). Pyridostigmine, that is stockpiled by various armed forces including the US army for pretreatment purposes against nerve agent poisoning, is not sufficiently effective to increase the resistance of soman-exposed experimental animals (Kassa et al. 2001a) because it is only able to protect peripheral AChE from irreversible soman-induced AChE phosphonylation while soman can readily cross the blood-brain barrier and, therefore, exert its deleterious effects through its central toxic effects including centrally mediated seizures (Bajgar 1996). The addition of centrally acting anticholinergic drugs to pyridostigmine for pharmacological pretreatment of acute soman exposures seems to be rational because a mixture of pyridostigmine with anticholinergic drugs should be able to increase the resistance of soman-poisoned animals and eliminate the side effects of pyridostigmine, especially the effects of accumulated ACh (Kassa et al. 2001a).

This prophylactic combination is significantly more efficacious in protecting experimental animals exposed to lethal doses of various nerve agents (e.g. soman, tabun) compared to pyridostigmine alone, as it has been described previously (Kassa et al. 2001b, Kassa and Vachek 2002).

The prophylactic efficacy of two combinations of pyridostigmine with anticholinergic drugs were compared in this study – the combination of pyridostigmine with BNZ and THP, stockpiled by Czech armed forces as PANPAL, and the Bulgarian prophylactic mixture consisting of pyridostigmine and biperiden



Fig. 3. Anticonvulsive activity of two prophylactic mixtures administered 30 min before soman poisoning and antidotal treatment administered 1 min following soman challenge; \* statistically significant



Fig. 4. Anticonvulsive activity of two prophylactic mixtures administered 60 min before soman poisoning and antidotal treatment administered 1 min following soman challenge; \* statistically significant

Both mixtures seem to be useful in increasing the resistance of soman-poisoned rats and in eliminating soman-induced convulsions. The results obtained from the current study showed that both prophylactic mixtures given at 30 or 60 min prior to the administration of  $1.5 \text{ LD}_{50}$  of soman were able

to protect the animals from severe toxic signs such as subconvulsions and convulsions and thus they have sufficient neuroprotective efficacy (Kassa et al. 2003). On the other hand, their anticonvulsive efficacy was not significantly improved when the antidotal treatment was added. Our results confirm that both prophylactic mixtures should be considered as a means of pretreatment of nerve agent poisoning, especially in the case of the threat of exposure to soman.

In conclusion, a combination of the prophylactic antidotal mixture containing pyridostigmine and centrally active anticholinergic drugs with the common antidotal treatment consisting of an anticholinergic drug (mainly atropine) and oxime (mainly HI-6) seems to be till now. the best choice in counteracting the acute toxic effects of soman.

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