# A COMPARISON OF THE EFFICACY OF PHARMACOLOGICAL PRETREATMENTS AGAINST SOMAN IN MICE

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***Summary***

*The ability of Czech original mixed pharmacological pretreatment, designated PANPAL (pyridostigmine in combination with benactyzine and trihexyphenidyle) to increase the resistance of soman-exposed mice in the case of non-treated or treated poisoning was compared to the currently used pharmacological pretreatment involving pyridostigmine alone. A significant decrease in the $LD_{50}$ values of soman in the case of non-treated poisoning of mice was observed when the rats were pretreated with PANPAL. On the other hand, the pharmacological pretreatment with pyridostigmine alone was practically ineffective to protect mice against soman-induced acute toxicity. The pharmacological pretreatment with pyrido- stigmine alone was not able to increase the efficacy of perspective antidotal treatment (the oxime HI-6 in combination with atropine), administered to mice one minute following soman poisoning. In contrast, PANPAL significantly increased the therapeutic effectiveness of antidotes studied in soman-poisoned mice. In addition, PANPAL-induced increase in the efficacy of currently used antidotal mixture (obidoxime in combination with atropine and diazepam) was significantly higher than the increase in the effectiveness of antidotes induced by pyridostigmine alone. These findings confirm that PANPAL pretreatment of soman-poisoned mice seems to be much more suitable pharmacological pretreatment against nerve agents than pyridostigmine alone.*

**Keywords:** Soman; Pyridostigmine; PANPAL; HI-6; Atropine; Obidoxime; Diazepam; Rat.

## Introduction

Despite of the entry into force in April 1997 of the Chemical Weapons Convention forbidding the production, storage and use of chemical warfare agents, the world has seen a rapid proliferation of such agents. Till now, highly toxic organophosphorus (OP) compounds, called nerve agents, are considered to be the most dangerous chemical warfare agents. The toxicity of nerve agents results from the irreversible binding to and inactivation of acetylcholinesterase (AChE, EC 3.1.1.7) and subsequent acetylcholine (ACh) accumulation leading to severe respiratory distress, prolonged limbic seizures, convulsive status and death (13, 16).

The current antidotal treatment of nerve agent-induced poisoning consists of the combined administration of atropine sulphate and AChE reactivators (oximes). Atropine antagonizes ACh-induced overstimulation of muscarinic cholinergic receptors while AChE reactivators repair the biochemical lesion by dephosphonylation of AChE molecule and restore its activity (4, 13, 16). Unfortunately, some nerve agents are resistant to standard antidotal treatment. One of the most resistant OP compounds is soman (pinacolyl methylphosphonofluoridate) that differs from many OPs in the rate of aging of the phosphonylated enzyme that prevents oxime-induced reactivation of AChE. Therefore, the treatment of soman-induced poisoning is so difficult (6, 8, 10, 14). Currently used oximes (obidoxime, pralidoxime) as well as H oximes including HI-6 in combination with anticholinergic drugs are not considered to be sufficiently effective in decreasing the toxicity of soman (6, 8, 10, 12, 14).

The relatively unsatisfactory treatment available for acute soman poisoning has prompted the study of pretreatment possibilities that allow survival and increase resistance of organisms exposed to nerve agents. Currently used method of protection against nerve agent poisoning is the use of pyridostigmine, a reversible carbamate AChE inhibitor (1). The prophylactic 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 the nerve agents (3). However, 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 the dose of pyridostigmine that is otherwise limited by signs and symptoms caused by elevated level of

ACh to be increased and results in higher prophylactic efficacy than that observed for pyridostigmine alone (7, 9). One of the mixtures, pyridostig- mine in combination with benactyzine (BNZ) and trihexyphenidyle (THP), designated PANPAL, has been developed in our laboratory (18).

In the present study, the efficacy of PANPAL (pyridostigmine in combination with BNZ and THP) to increase the resistance of soman-poisoned mice and the therapeutic efficacy of antidotal treatment of soman-induced acute poisoning was compared with the efficacy of pyridostigmine alone.

## Material and methods

Male NMRI mice from Konárovice (Czech Republic), weighing 19–22 g, were kept in an air-conditioned room with light from 07.00 a.m. to 07.00 p.m. and were allowed free access to standard chow and tap water. The rats were divided into groups of six animals each. Handling of experimental animals was under the supervision of the Ethics Committee of the Purkyně Military Medical Academy and the Medical Faculty of Charles University (Hradec Králové, Czech Republic). Soman of 98.5% purity was obtained from Zemianské Kostolany (Slovak Republic). The oxime HI-6 of 99% purity was synthesized at the Department of Toxicology of the Purkyně Military Medical Academy. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Pyridostigmine (5.82 mg/kg of body weight) alone or in combination with BNZ (70 mg/kg of body weight) and THP (16 mg/kg of body weight) was administered perorally (p.o.) as solution in distilled water (0.2 ml/100g of body weight) 60 or 120 min before the soman challenge while antidotal treatment (the oxime HI-6 or obidoxime at equieffective doses - 2% of their $LD_{50}$ in combination with atropine 8.4 mg/kg of body weight) and diazepam (1 mg/kg of body weight) was carried out by intramuscular injection (i.m.) 1 min following soman administration. The dose of pyridostigmine, used in our experiments, causes 40% inhibition of erythrocyte AChE; the doses of anticholinergic drugs, used in our experiments, correspond to common therapeutical doses (5% of their $LD_{50}$) (3). Soman-induced toxicity was evaluated with the help of $LD_{50}$ values and 95% confidence limits. The efficacy of tested pretreatment was expressed as protective ratio A ($LD_{50}$ value of soman in pretreated mice/$LD_{50}$ value of soman in non-pretreated mice without antidotal treatment) and protective ratio B ($LD_{50}$ value of soman in pretreated mice/$LD_{50}$ value of soman in non-pretreated mice with antidotal treatment).

The $LD_{50}$ values and their 95% confidence limits were calculated by probit analysis of deaths occurring within 24 hours after i.m. administration of soman at five different doses with six rats per dose. The differences between $LD_{50}$ values were considered to be significant when $p < 0.05$ (15).

## Results

The prophylactic efficacy of pyridostigmine alone and the prophylactic mixture PANPAL is presented in Table 1. While pyridostigmine alone was practically ineffective to decrease the soman-induced acute toxicity regardless of the time of its administration before the poisoning, PANPAL was able to significantly increase the 24h $LD_{50}$ value of soman in pretreated mice compared to the 24h $LD_{50}$ value in non-pretreated mice ($p < 0.05$).

Table 1

**Prophylactic effect of pyridostigmine alone or PANPAL on the $LD_{50}$ value of soman in mice. Statistical significance: * $p < 0.05$.**

| Pretreatment | Time of pretreatment (min) | $LD_{50}$ of soman (μg/kg) (95% confidence limits) | Protective ratio |
|---|---|---|---|
| - | - | 108.0 (101.7–114.7) | - |
| Pyridostigmine | 60 | 108.6 (92.5–127.5) | 1.01 |
| PANPAL | 60 | 356.1 (301.1–421.0)* | 3.30 |
| Pyridostigmine | 120 | 112.5 (96.5–131.0) | 1.04 |
| PANPAL | 120 | 382.7 (348.4–420.4)* | 3.54 |

Table 2

**The influence of pretreatment on the therapeutic effects of antidotes on the $LD_{50}$ value of soman in mice. Statistical significance: * $p < 0.05$.**

| Pretreatment | Time of pretreatment (min) | Treatment | $LD_{50}$ of soman (μg/kg) (95% confidence limits) | Protective ratio A | Protective ratio B |
|---|---|---|---|---|---|
| - | - | - | 108.0 (101.7–114.7) | - | - |
| - | - | HI-6 + atropine | 218.2 (201.6–236.3)* | 2.02 | - |
| Pyridostigmine | 60 | HI-6 + atropine | 258.1 (237.7–280.2)* | 2.39 | 1.18 |
| PANPAL | 60 | HI-6 + atropine | 449.1 (356.3–566.1)* | 4.16 | 2.06 |
| Pyridostigmine | 120 | HI-6 + atropine | 198.5 (161.9–243.2)* | 1.84 | 0.91 |
| PANPAL | 120 | HI-6 + atropine | 391.0 (336.3–454.6)* | 3.62 | I.80 |

Table 3

**The influence of 2 hour pretreatment on the therapeutic effects of currently used antidotes on the $LD_{50}$ value of soman in mice. Statistical significance: * $p < 0.05$.**

| Pretreatment | Treatment | $LD_{50}$ of soman (μg/kg) (95% confidence limits) | Protective ratio A | Protective ratio B |
|---|---|---|---|---|
| - | - | 108.0 (101.7–114.7) | - | - |
| - | Obidoxime + atropine + diazepam | 179.2 (166.8–192.5)* | 1.66 | - |
| Pyridostigmine | Obidoxime + atropine + diazepam | 420.2 (385.6–456.1)* | 3.89 | 2.34 |
| PANPAL | Obidoxime + atropine + diazepam | 508.6 (458.2–564.8)* | 4.71 | 2.84 |

Pyridostigmine alone did not influence the efficacy of the antidotal treatment of soman-poisoned mice consisting of the oxime HI-6 and atropine either, regardless of the time of pretreatment. On the other hand, the prophylactic mixture PANPAL was able to increase the efficacy of this antidotal mixture approximately two times in comparison with treated soman-poisoned mice without pretreatment (Table 2). PANPAL-induced increase in the effectiveness of the oxime HI-6 in combination with atropine was significant ($p < 0.05$).

In the case of currently used antidotal mixture (obidoxime in combination with atropine and diazepam), pyridostigmine was able to increase the effectiveness of antidotal treatment of soman-poisoned mice when it was administered 2 hours before the poisoning. Nevertheless, the increase in efficacy of antidotes was not so high as in the case of PANPAL pretreatment. When PANPAL was used for the pretreatment of mice, the efficacy of obidoxime in combination with atropine and diazepam was almost six times higher in comparison with the treated soman-poisoned mice without pretreatment (Table 3).

## Discussion

In the case of a threat of soman exposure, it seems to be very important to have sufficiently effective pretreatment because soman-induced deleterious effects are very difficult to counteract (2, 5). Till now, pyridostigmine is stockpiled by various armed forces including the US Army for pretreatment purpose against nerve agent poisoning and has been used by several thousand servicemen during UN operation against Iraq in 1991 (19).

Nevertheless, our results confirm the shortage of effectiveness of pyridostigmine to increase the resistance of soman-exposed mice in the case of treated as well as non-treated acute poisoning. Pyridostigmine is only able to protect peripheral AChE from irreversible soman-induced AChE phosphonylation, while soman can readily cross the blood-brain barrier and, thus, express its deleterious effects through its central toxic effects including centrally mediated seizure activity that can rapidly progress to status epilepticus and contribute to profound brain damage (17). On the other hand, our data show that our pretreatment mixture PANPAL

is able to significantly protect soman-poisoned mice as well as increase the efficacy of antidotal pretreatment regardless of the type of oxime used. The beneficial effect of this prophylactic mixture, developed in our laboratory, is probably caused not only by the protection of AChE from irreversible soman inhibition but also by a decrease in the cholinergic and stressogenic effects of this nerve agent (7). Moreover, PANPAL seems to be very effective in enhancing neuroprotective efficacy of antidotal treatment in the case of soman poisoning (11). The addition of anticholinergic drugs to pyridostigmine is useful not only for the increase in the resistance of soman-exposed animals but also for the elimination of side effects of pyridostigmine caused by accumulated ACh.

In conclusion, our data indicate that pyridostigmine is sufficiently effective in enhancing the survival of mice poisoned by supralethal doses of soman when it is combined with anticholinergic drugs. The combination of pyridostigmine with anticholinergic drugs such as PANPAL has definite advantages over pyridostigmine alone in the pretreatment of soman poisoning and, therefore, it should be considered as replacement for the currently used pretreatment of the nerve agent poisoning.

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