

THE EFFICACY OF THE CZECH ORIGINAL PROPHYLACTIC MIXTURE, CALLED PANPAL, AS PHARMACOLOGICAL PRETREATMENT OF TABUN OR SOMAN-POISONED RATS AND MICE

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ABSTRACT

The potency of the Czech original pharmacological pretreatment consisting of pyridostigmine, benactyzine and trihexyphenidyle, designated PANPAL, to increase the resistance of rats and mice against tabun or soman and to increase the therapeutic efficacy of standard antidotal treatment of tabun or soman-poisoned experimental animals was studied and compared with commonly used pyridostigmine alone. While PANPAL significantly increased the resistance of animals against tabun or soman and increased the efficacy of currently used antidotal treatment of tabun or soman poisoning, pyridostigmine alone was not able to sufficiently protect experimental animals against tabun or soman-induced acute toxicity. Our findings confirm that PANPAL, licensed and introduced to the Czech Army, seems to be promising and beneficial pharmacological pretreatment in the case of the threat of exposure to nerve agents, especially tabun or soman. It appears to be more suitable than currently used pyridostigmine alone.

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 compounds (OPs), called nerve agents, are considered to be the most dangerous chemical warfare agents. They pose potential neurotoxic threats to both military and civilian populations, as evidenced in recent terroristic attacks (1) as well as occupational hazard to individuals exposed to certain organophosphorus insecticides. OPs toxicity 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 (2,3). The current standard treatment for poisoning by OPs consists of the combined administration of atropine sulfate and AChE reactivators (oximes). Atropine blocks the effects of overstimulation by accumulated ACh at muscarinic receptor sites while AChE reactivators (generally nucleophilic compounds with high affinity for phosphorus) repair the biochemical lesion by dephosphonylation of AChE molecule and restore its activity (2-4). Unfortunately, some OP compounds, especially soman and tabun, were found to be resistant to standard antidotal treatment. The relatively unsatisfactory treatment available for acute tabun and soman poisoning has prompted 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 bromide, a reversible carbamate AChE inhibitor (5). 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 nerve agents (6). However, pyridostigmine-induced increase in the level of ACh can itself cause symptoms of poisoning. Therefore, it would be useful to counteract the effects of the accumulated ACh by using anticholinergic drugs. In addition, the combination of pyridostigmine with anticholinergic drugs allows the dose of pyridostigmine that is otherwise limited by symptoms cause by elevated level of ACh to be increased and results in higher prophylactic efficacy than that observed for pyridostigmine alone (7,8). One of these mixtures, pyridostigmine in combination with benactyzine (BNZ) and

Report Documentation Page			Form Approved OMB No. 0704-0188		
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1. REPORT DATE 01 OCT 2005		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE The Efficacy Of The Czech Original Prophylactic Mixture, Called Panpal, As Pharmacological Pretreatment Of Tabun Or Soman-Poisoned Rats And Mice			5a. CONTRACT NUMBER		
			5b. GRANT NUMBER		
			5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S)			5d. PROJECT NUMBER		
			5e. TASK NUMBER		
			5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Department of Toxicology, Purkyne Military Medical Academy, Hradec Králové, Czech Republic			8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)			10. SPONSOR/MONITOR'S ACRONYM(S)		
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

trihexyphenidyle (THP), designated PANPAL, has been developed in the Czech Republic and introduced into the Czech Army (9). In the present study, the influence of pyridostigmine alone or in combination with BNZ and THP on the resistance of soman or tabun-exposed mice and rats and on the neuroprotective and therapeutic efficacy of currently used antidotal treatment (obidoxime in combination with atropine and diazepam or the oxime HI-6 in combination with atropine) of soman or tabun-induced acute poisoning was compared.

EXPERIMENTAL METHODS

Male NMRI mice, weighing 24-26g, and male Wistar rats, weighing 190-210g, from Konárovice (Czech Republic) 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 animals were divided into groups of eight animals each. Handling of experimental animals was under the supervision of the Ethics Committee of the Purkyne Military Medical Academy and the Medical Faculty of Charles University (Hradec Králové, Czech Republic). 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 orally as solution in distilled water (0.2 ml/100g of body weight of rats or 10 g of body weight of mice) 60 or 120 min before intramuscular (i.m.) soman or tabun challenge while antidotal treatment (the oxime HI-6 or obidoxime at equieffective doses – 2% of their LD₅₀ in combination with atropine at 2% of its LD₅₀ and diazepam at the dose of 1 mg/kg of body weight) was carried out by i.m. injection 1 min following soman or tabun administration. The dose of pyridostigmine, used in our experiments, causes 40% inhibition of erythrocyte AChE activity determined by the spectrophotometric method of Ellman et al. (10) using acetylthiocholine as substrate and 5,5'-dithio-bis(2-nitrobenzoic) acid as chromogen. The experimental doses of BNZ (10% LD₅₀) and THP (2% LD₅₀) were chosen according to results obtained in our previous experiments (11). The used doses of oximes (HI-6 at 13.8 mg/kg of body weight for mice and 15.6 mg/kg of body weight for rats, obidoxime at 3.8 mg/kg of body weight for mice and 3.2 mg/kg of body weight for rats) and anticholinergic drug (atropine at 8.4 mg/kg of body weight for mice and 25.2 mg/kg of body weight for rats) for the antidotal treatment correspond to human-relevant doses (2% of their LD₅₀) (6). Soman or tabun-induced toxicity was evaluated by the assessment of LD₅₀ values and their 95% confidence limits within 24 h after administration of soman or tabun at five different doses with eight animals per dose (12). The efficacy of tested pretreatment was expressed as protective ratio A (LD₅₀ value of soman or tabun in pretreated mice/ LD₅₀ value of soman or tabun in non-pretreated mice without antidotal treatment) and protective ratio B (LD₅₀ value of soman or tabun in pretreated mice/ LD₅₀ value of soman or tabun in non-pretreated mice with antidotal treatment). Soman or tabun-induced neurotoxicity was evaluated using Functional observational battery (FOB) that consists of 41 measures of sensory, motor and autonomic nervous functions (13-14) in rats at 24 hours following soman or tabun challenge. The LD₅₀ values and their 95% confidence limits were calculated by probit analysis of deaths occurring within 24 hours after i.m. administration of soman or tabun at five different doses with eight rats per dose. The differences between LD₅₀ values were considered to be significant when $p < 0.05$ (12).

RESULTS

A comparison of the prophylactic efficacy of pyridostigmine alone and the prophylactic mixture PANPAL is presented in Table 1-3. Pyridostigmine alone was not able to decrease soman or tabun-induced acute toxicity in mice (Table 1-2), but it decreased tabun-induced acute toxicity almost two times ($p < 0.05$) when rats were used as experimental animals (Table 3). Nevertheless, the efficacy of PANPAL to decrease the acute toxicity of soman or tabun seems to be significantly higher in comparison with pyridostigmine alone ($p < 0.05$) regardless of the choice of experimental animals (Table 1-3). PANPAL increased the 24h LD₅₀ value of soman or tabun in pretreated mice or rats more than three times in the case of soman and more than two times in the case of tabun in comparison with the 24h LD₅₀ value of soman or tabun in non-pretreated experimental animals ($p < 0.05$).

TABLE 1 Prophylactic effect of pyridostigmine alone and PANPAL on the LD₅₀ value of soman in mice. Statistical significance: * p < 0.05 (between non-pretreated and pretreated mice), ^x p < 0.05 (between pyridostigmine-pretreated and PANPAL-pretreated mice).

Pretreatment	Time of pretreatment (min)	LD ₅₀ of soman (µg/kg) ± 95% IS	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)* ^x	3.30
Pyridostigmine	120	112.5 (96.5-131.0)	1.04
PANPAL	120	382.7 (348.4-420.4)* ^x	3.54

TABLE 2 Prophylactic effect pyridostigmine alone and PANPAL on the LD₅₀ value of tabun in mice. Statistical significance: * p < 0.05 (between non-pretreated and pretreated mice), ^x p < 0.05 (between pyridostigmine-pretreated and PANPAL-pretreated mice).

Pretreatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio
----	275 (269 – 281)	----
Pyridostigmine alone	277 (261 – 295)	1.01
PANPAL	701 (655 – 789)* ^x	2.55

TABLE 3 Prophylactic effect pyridostigmine alone and PANPAL on the LD₅₀ value of tabun in rats. Statistical significance: * p < 0.05 (between non-pretreated and pretreated rats), ^x p < 0.05 (between pyridostigmine-pretreated and PANPAL-pretreated rats).

Pretreatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio
----	128 (121 – 136)	----
Pyridostigmine alone	240 (220 – 262)*	1.88
PANPAL	285 (270 – 300)* ^x	2.23

Pyridostigmine alone was not able to increase the efficacy of antidotal treatment of soman-poisoned mice consisting of the oxime HI-6 and atropine regardless of the time of its administration while PANPAL significantly increased the efficacy of the same antidotal mixture approximately two times (p < 0.05) (Table 4). On the other hand, both pharmacological pretreatments studied significantly increased the efficacy of antidotal treatment of soman-poisoned mice consisting of obidoxime, atropine and diazepam (p < 0.05) although the potency of PANPAL to increase the efficacy of this antidotal mixture was higher compared to pyridostigmine alone (p < 0.05) (Table 5).

TABLE 4 The influence of pharmacological pretreatment on the potency of antidotal treatment to increase the LD₅₀ value of soman in mice. Statistical significance: * p < 0.05 (between non-pretreated and non-treated mice and pretreated and/or treated mice), ^x p < 0.05 (between treated mice with pretreatment and treated mice without pretreatment)

Pretreatment	Time of pretreatment	Treatment	LD ₅₀ of soman (µg/kg) ± 95% IS	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 (235.7-280.2)*	2.39	1.18
PANPAL	60	HI-6 + atropine	449.1 (356.3-566.1)* ^x	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)* ^x	3.62	1.80

TABLE 5 The influence of pharmacological pretreatment on the potency of antidotal treatment to increase the LD₅₀ value of soman in mice. Statistical significance: * $p < 0.05$ (between non-pretreated and non-treated mice and pretreated and/or treated mice), ^x $p < 0.05$ (between treated mice with pretreatment and treated mice without pretreatment).

Pretreatment	Treatment	LD ₅₀ of soman (µg/kg) ± 95% IS	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)* ^x	3.89	2.34
PANPAL	Obidoxime + atropine + diazepam	508.6 (458.2-564.8)* ^x	4.71	2.84

Pyridostigmine alone did not increase the efficacy of the antidotal treatment of tabun-poisoned rats consisting of obidoxime, atropine and diazepam, while the prophylactic mixture PANPAL significantly increased the efficacy of the same antidotal mixture ($p < 0.05$). It increased the 24h LD₅₀ value of tabun in pretreated and treated rats more than two times in comparison with treated tabun-poisoned rats without pretreatment ($p < 0.05$) (Table 6). PANPAL was also able to significantly increase the therapeutic efficacy of both tested antidotal mixtures in tabun-poisoned mice. When PANPAL was used for the pretreatment of mice, the efficacy of both tested antidotal mixtures was almost five times higher in comparison with the treated tabun-poisoned mice without pretreatment ($p < 0.05$) (Table 7).

TABLE 6 The influence of pharmacological pretreatment on the potency of antidotal treatment to increase the LD₅₀ value of tabun in rats. Statistical significance: * $p < 0.05$ (between non-pretreated and non-treated rats and pretreated and/or treated rats), ^x $p < 0.05$ (between treated rats with pretreatment and treated rats without pretreatment).

Pretreatment	Treatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio A	Protective ratio B
---	---	128 (121 – 136)	---	---
---	Obidoxime, atropine, diazepam	382 (340 – 430)*	2.98	---
Pyridostigmine alone	Obidoxime, atropine, diazepam	412 (350 – 487)*	3.22	1.08
PANPAL	Obidoxime, atropine, diazepam	990 (820 – 1180)* ^x	7.73	2.59

TABLE 7 The influence of pharmacological pretreatment with PANPAL on the potency of antidotal treatment to increase the LD₅₀ value of tabun in mice. Statistical significance: * p < 0.05 (between non-pretreated and non-treated mice and pretreated and/or treated mice), ^x p < 0.05 (between treated mice with pretreatment and treated mice without pretreatment).

Pretreatment	Treatment	LD ₅₀ (µg/kg) ± 95% IS	Protective ratio A	Protective ratio B
---	---	275 (260 – 281)	---	---
---	Obidoxime, atropine, diazepam	449 (423 – 477)*	1.63	---
PANPAL	Obidoxime, atropine, diazepam	2100 (1010 – 2440)* ^x	7.64	4.68
---	HI-6, atropine, diazepam	531 (444 – 635)*	1.93	---
PANPAL	HI-6, atropine, diazepam	2520 (2310 – 2740)* ^x	9.16	4.79

PANPAL was also able to eliminate many of soman or tabun-induced neurotoxic signs observed at 24 hours following soman or tabun poisoning of rats. To compare soman or tabun-induced neurotoxic signs in pretreated or non-pretreated rats with or without antidotal treatment consisting of obidoxime and atropine, the potency of PANPAL to increase the neuroprotective efficacy of antidotal treatment of soman or tabun poisoning was also demonstrated.

DISCUSSION

In the case of a threat of soman or tabun exposure, it seems to be very important to have sufficiently effective pretreatment because soman or tabun-induced deleterious effects are extraordinarily difficult to counteract due to the very low reactivating efficacy of currently used oximes (15-17). 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(18). Nevertheless, our results confirm the shortage of effectiveness of pyridostigmine alone to increase the resistance of soman or tabun-exposed experimental animals. Pyridostigmine is only able to protect peripheral AChE from irreversible soman or tabun-induced AChE phosphorylation, while soman as well as tabun can 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 brain damage (19-21). On the contrary of pyridostigmine alone, PANPAL seems to be a sufficiently effective pretreatment of soman or tabun-exposed experimental animals. This effect of PANPAL is probably caused not only by pyridostigmine-induced protection of peripheral AChE from irreversible inhibition by nerve agents but also by anticholinergic drug-induced protection of central muscarinic cholinergic receptors from their overstimulation by accumulated ACh (11). Moreover, PANPAL seems to be very effective in enhancing the efficacy of antidotal treatment to protect experimental animals poisoned

with lethal doses of soman or tabun. Pyridostigmine is also able to increase the therapeutic efficacy of currently used antidotal mixtures (22-23) but pyridostigmine-induced increase in the therapeutical efficacy of antidotal treatment is significantly lower in comparison with PANPAL because pyridostigmine alone is not able to prevent the central effects of nerve agents (11). The addition of anticholinergic drugs to pyridostigmine is useful not only for enhancing of the efficacy of pretreatment to increase the resistance of soman or tabun-exposed animals but also in eliminating the side effects of pyridostigmine, especially the effects of accumulated ACh. Generally, pyridostigmine at commonly used dose (30 mg pyridostigmine tablet three times a day) is thought to be without significant side effects but when it was taken by 10 asthmatic soldiers during Operation Desert Storm the exacerbation of asthma symptoms in seven of the asthmatics was observed (18,24). It was demonstrated that exposure to physiologically relevant doses of pyridostigmine leads to neurobehavioral deficits and region-specific alterations in AChE and ACh receptors (25). On the other hand, a peripherally acting carbamate with centrally acting anticholinergics may also results in severe side effects. Nevertheless, the combination of pyridostigmine with two centrally acting anticholinergics - BNZ and THP, designated as PANPAL, was clinically examined at doses recommended for humans and no health problems or side effects were found during clinical and laboratory observation of the volunteers following usage of PANPAL. Therefore, PANPAL was licensed and introduced to the Czech Army (11).

CONCLUSIONS

Our data indicate that pyridostigmine bromide is sufficiently effective in enhancing the survival of experimental animals poisoned by supralethal doses of soman or tabun 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 or tabun poisoning and, therefore, it could be considered as replacement for the currently used pretreatment of the nerve agent poisoning, especially in the case of the threat of exposure to soman or tabun.

ACKNOWLEDGEMENTS

The authors express their appreciation to Mrs J. Uhlirova, Mrs. E. Vodakova and Mrs. E. Reslova for their skill technical assistance and to Mr. V. Blaha for the statistical evaluation. The study was supported by the grant of Ministry of Defence, OBVLAJEP20032.

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