ORIGINAL ARTICLE

EFFECT OF METHOXIME COMBINED WITH ANTICHOLINERGIC, ANTICONVULSANT OR ANTI-HCN DRUGS IN TABUN-POISONED MICE

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Summary: The effect of methoxime combined with a) atropine, b) benactyzine, c) atropine and natrium thiosulphate, d) atropine and diazepam on antidotal treatment effectiveness was studied in tabun-poisoned mice. In addition, the influence of pretreatment consisiting of pyridostigmine, benactyzine and trihexyphenidyle (PANPAL) administered 2 hours before tabun intoxication on the treatment effectivity of methoxime combined with e) atropine or f) benactyzine was tested. The most efficacious therapeutic mixture in non-pretreated mice was methoxime, atropine and diazepam. Natrium thiosulphate did not significantly increase neither decrease the antidotal treatment efficacy in comparison with methoxime and atropine alone. Pretreatment with PANPAL significantly decreased tabun toxicity (nearly 4 times in methoxime and benactyzine combination and more than 4 times in atropine and methoxime mixture). The present study demonstrates that the tabun toxicity in mice is more effectively reduced when PANPAL prophylactically is administered than in case of treatment with methoxime and cholinergic drug alone. We established that anticholinergic drug option in the therapeutic mixture of methoxime and anticholinergic drug did not cause the difference in the antidotal treatment effectivities.

Key words: *Tabun; Methoxime; Atropine; Benactyzine; Natrium Thiosulphate; Diazepam; PANPAL; Acute Toxicity; Mice*

Introduction

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is the earliest synthetised compound from the G-series of nerve agents. It is one of the most persistant organophosphorus compound (OPC), but its hydrolytic stability is low. This physical property markedly limits its use in „wet" areas with high density of streams and rivers, high frequency and intensity of precipitation. Politic changes in Europe in recent 12 years have caused a moving of localization of risk areas to the south (e.g. Persian Gulf). The tabun handicap is supressed in dry regions respectively and it was already used in those climatic conditions – by the Iraqis during the Iran-Iraq war (3).

OPCs cause hyperactivity of the cholinergic system as a result of inhibition of cholinesterases (ChE), in particular, acetycholinesterase (AChE), and the subsequent increase in the concentration of the neurotransmitter acetylcholine (ACh) at the central and peripheral sites (13).

The usual pharmacotherapy of poisoning by OPC anticholinesterases (insecticides or chemical warfare agents) is based on antimuscarinics, reactivators (oximes), and anticonvulsants. Oximes function by reacting with OPC at the active site of AChE, thereby displacing the phosphonyl residue from the enzyme. Although oximes have been designed to reactivate the inhibited AChE, clinical experience has indicated that they are not always very effective as reactivators and at this very moment none of them can be regarded as a broadspectrum antidote (2). Tabun is quite resistant to oxime therapy because of the existence of a free electron pair located on amidic nitrogen which makes the nucleophilic attack of oximes almost impossible (9). Reactivating effect of oximes is probably one of group of oxime effects in organism. Especially bis-pyrridinium oximes can influence:

- acetylcholine releasing in central and peripheral cholinergic synapses
- allosteric modulation of muscarinic receptors
- nicotinic receptors connected with ion channels
- neuromuscular transmission totally (7).

The new series of oximes – the H oximes, has been developed to improve the effectiveness of oximes to reactivate nerve agent-inhibited AChE and eliminate nerve agent-induced lethal effects (8). HI-6, the most efficacious oxime against toxic effects of soman has not as high reactivating efficacy for tabun –inhibited AChE as in the case of soman poisoning (10).

Accumulation of ACh in the synaptic cleft as a result of ChE inhibition is considered as the main cause of the symptoms that ultimately lead to death in OP-poisoning. Secondly, if there is accumulation of ACh, the postsynaptic cell must be protected against overstimulation in a use-dependent way, by blocking of open nicotinic ion-channels, mainly with anticholinergics (7). So not only the choice of oximes

but also the anticholinergic drug selection could influence the effectiveness of antidotes against OPC (8).

The relatively unsatisfactory treatment available for acute tabun 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 AChE inhibitor. 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 accumulated ACh using anticholinergic drugs. One of these mixtures, pyridostigmine in combination with benactyzine (BNZ) and trihexyphenidyle (THP), designated PANPAL, has been developed in the Czech Republic and introduced to the Czech Army (9).

When tabun decomposes in the blood, it produces hydrogen cyanide (HCN). After tabun intoxication we administered together with oxime therapy HCN antidotum – natrium thiosulphate in one group of animals.

The aim of this study was to compare the therapeutic effectiveness of methoxime alone and its combination with atropin or BNZ or atropine and natrium thiosulphate or atropine and diazepam in tabun-poisoned mice. Methoxime belongs to the conventional oximes. Czech Army is equipped with special preparation with methoxime called Renol, which is determined for specialised medical care in case of nerve agent intoxication.

We have also studied efficacy of pretreatment (pyridostigmin, THP and BNZ) and following treatment with methoxime and atropine or methoxime and BNZ against supralethal poisoning with tabun.

Material and Methods

Animals

Female mice, weighing 21–27 g from Konárovice (Czech Republic) were kept in air-conditioned room with light from 07:00 to 19:00 h and were allowed to free access to standard chow and tap water. The mice were divided into groups of six 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).

Material

Tabun of 95% purity was obtained from Military Technical Institute in Brno (Czech Republic). Its purity was determined by acidimetric titration. The methoxime of 98.1 % purity was synthetized in the Department of Toxicology of Purkyne Military Medical Academy in Hradec Králové (Czech Republic). All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

Animals experiment

In our experiment methoxime (13.8 $mg.kg^{-1}$ of body weight ≈ 2% LD_{50}) in combination with atropine (8.4 $mg.kg^{-1}$ of body weight ≈ 2% LD_{50}) or BNZ (3.4 $mg.kg^{-1}$ of body weight ≈ 2% LD_{50}) or atropine and natrium thiosulphate (50 $mg.kg^{-1}$ of body weight ≈ 2% LD_{50}) or atropine and diazepam (1 $mg.kg^{-1}$ of body weight) was used as an antidotal treatment of tabun intoxication. This oxime therapy combined with anticholinergic and optionally anticonvulsive or anti-cyanide drug was administered intramusculary (i.m.) 1 min after tabun application (i.m.). As a prophylactic pretreatment pyridostigmine (5.82 $mg.kg^{-1}$ of body weight) in combination with BNZ (70.0 $mg.kg^{-1}$ of body weight) and THP (16.0 $mg.kg^{-1}$ of body weight) was administered perorally as solution in distilled water (0.2 ml/ 10 g of body weight). The dose of pyridostigmine used in our experiment, causes 40% inhibition of erythrocyte AChE activity determined by the spectrophotometric method of Ellman et al. (4). The experimental doses of BNZ (10% LD_{50}) and THP (2% LD_{50}) for pretreatment were chosen according to results obtained in previous experiments. The doses of methoxime and anticholinergic drugs (atropine and BNZ) used for the antidotal treatment correspond to human relevant doses (2% of LD_{50}) (1).

Data analysis

Tabun-induced toxicity was evaluated by the assessment of LD_{50} values and their 95% confidence limits within 1 week after administration of tabun at five different doses with six mice per dose (14). The efficacy of tested treatment was expressed as a protective ratio (LD_{50} value of tabun in treated mice/ LD_{50} value of tabun in non-pretreated mice without antidotal treatment). The differences between LD_{50} values were considered to be significant when $P< 0.05$ (11).

Results

The therapeutic efficacy of antidotal mixtures consisiting of various anticholinergic drugs in combination with methoxime is presented in Table 1. Atropine and BNZ were comparably effective in combination with methoxime as antidotal treatment of tabun poisoned mice. The difference in protective ratios was not significant. The combination of methoxime and atropine or BNZ decreased tabun-induced acute toxicity more than 1.5 times ($P< 0.05$). Natrium thiosulphate did not significantly increase neither decrease the antidotal treatment efficacy in comparison with methoxime and atropine alone ($P< 0.05$). Diazepam administered as an anticonvulsive drug together with methoxime and atropine decreased tabun-induced acute toxicity 1.8 times, more than methoxime and atropine alone, but the difference in protective ratios was not significant.

The prophylactic mixture PANPAL significantly increased the efficacy of antidotal treatment consisting of methoxime and atropine in tabun-poisoned mice ($P< 0.05$). When PANPAL was used for pretreatment, tabun-induced toxicity was almost 4.5 times reduced in comparison with the mice

Tab. 1: The influence of different anticholinergics, HCN antidotum and pretreatment on the therapeutic effect of antidotal treatment with methoxime in tabun-poisoned mice.

Pretreatment	Treatment	LD_{50} (μg/kg) with 95% confidence limits	Protective ratio
-	-	290 (279–302)	-
-	Methoxime, Atropine	446 (397–502)*	1.54
-	Methoxime, Benactyzine	464 (438–492)*	1.60
-	Methoxime, Atropine, Natrium Thiosulphate	423 (394–454)*	1.46
-	Methoxime, Atropine, Diazepam	523 (474–580) *	1.81
PANPAL	Methoxime, Atropine	1873 (1560–2250)*	6.46
PANPAL	Methoxime, Benactyzine	1788 (1351–2369) *	6.17

* significantly different from the untreated group at the level of $P< 0.05$

treated with methoxime and atropine without pretreatment. In case of combination of PANPAL as pretreatment and atropine and BNZ as an antidotal treatment the tabun-induced toxicity decreased more than 6 times. The difference in protective ratios 6.46 for PANPAL, methoxime and atropine and 6.17 for PANPAL, methoxime and BNZ was not significant.

Discussion

The threat of use of chemical warfare agents is still existing either in military or local conflicts including terroristic attacks, that is why search for effective protection is the central concern of different laboratories both civilian and military (6).

In the case of a threat of tabun exposure, it seems to be very important to have sufficiently effective pretreatment because tabun-induced deleterious effects are extraordinaly difficult to counteract due to the very low reactivating efficacy of currently used oximes (10). The reason for the weak reactivating potency of the oximes is not rate of ageing of phosphorylated AChE that is relatively low but the presence of free electron pair makes the nucleophilic attack very difficult (5).

In our study we established that pretreatment consisting of pyridostigmine, BNZ and THP significantly increases the treatment effectivity. The combination of pyridostigmine with anticholinergic drugs such as PANPAL has definite advantages over pyridostigmine alone. The anticholinergic drug option in the therapeutic mixture of methoxime and anticholinergic drug did not cause the difference in the antidotal treatment effectivities. Anticholinergic drugs are very important components of antidotal therapy against OP poisoning. The anticonvulsant effects of atropine sulphate in larger doses as well as other anticholinergic compounds, such as biperiden, scopolamine, and THP, in much smaller doses, have been recognized and documented against seizures elicited by the nerve agents soman, sarin, tabun, VX, GF and VR. Especially atropine sulphate in combination with an oxime has traditionally been utilized as the mainstay of therapy against lethal effects of OPC, including commercial pesticides as well as nerve agents (12).

Data about obidoxime and HI-6 used for treatment of tabun-poisoned mice have been already published (8,9). Both oximes were used in combination with atropine and diazepam, the protective ratios were 1.63 for obidoxime and 1.93 for HI-6, with PANPAL pretreatment 7.64 and 9.16 respectively (9). These results show that obidoxime and methoxime are comparably effective in the treatment of tabun-poisoned mice and HI-6 seems to be more effective than two previous tested oximes.

In conclusion, the present study demonstrates that the tabun toxicity in mice is more effectively reduced when pretreatment PANPAL is administered than in case of antidotal treatment with methoxime and anticholinergic drug alone. Both tested anticholinergic drugs atropine as well as BNZ are equieffective in reducing of toxic reaction in tabun-poisoned mice when used as antidotal treatment in combination with methoxime.

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