ORIGINAL ARTICLE

THE INFLUENCE OF ANTICHOLINERGIC DRUG AND OXIME SELECTION ON THE EFFECTIVENESS OF ANTIDOTAL TREATMENT AGAINST TABUN-INDUCED POISONING IN MICE

Jiří Kassa

Purkyně Military Medical Academy, Hradec Králové: Department of Toxicology

Summary: 1. The influence of oximes (pralidoxime, obidoxime, HI-6) and anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) on the effectiveness of antidotal treatment to eliminate tabun-induced lethal effects was studied in mice. 2. Obidoxime seems to be the most efficacious oxime for the elimination of tabun-induced lethal effects in mice, although the difference in the efficacy of obidoxime and HI-6 is not significant when they are combined with atropine. 3. Obidoxime and HI-6 when combined with centrally acting anticholinergic drugs (benactyzine, biperiden and scopolamine) seem to be more efficacious in the elimination of toxic effects of the lethal dose of tabun than their combination with atropine. 4. The findings support the hypothesis that the choice of acetylcholinesterase reactivators as well as the anticholinergic drug selection are important for the effectiveness of antidotal mixture in the case of antidotal treatment of tabun-induced acute poisoning.

Key words: *Tabun; Oximes; Anticholinergic drugs; Acute toxicity; Mice*

Introduction

Despite the entry into force in April 1997 of the Chemical Weapons Convention forbidding the development, production, stockpiling and use of chemical warfare agents, the world has seen a rapid proliferation of such agents (21). The chemical warfare agents include nerve agents, very dangerous highly toxic organophosphorus compounds (OPs) that exert their toxic effects by phosphonylation and subsequent inactivation of acetylcholinesteraserase (AChE, EC 3.1.1.7). The inactivation of this enzyme allows the accumulation of acetylcholine (ACh) in the synaptic terminals of the central and peripheral nervous systems with subsequent widespread overstimulation of cholinergic receptors (18, 25). Effective management of nerve agent-induced cholinergic overstimulation is critical for immediate casualty treatment and for a rapid and full recovery from the effects of nerve agent exposure. Unfortunately, certain OPs were found to be resistant to the standard antidotal treatment which consists of anticholinergic drugs to counteract the accumulation of ACh and oximes to reactivate nerve agent-inhibited AChE (7,18).

Tabun (O-ethyl-N,N-dimethyl phosphoramidocyanidate) is probably one of the most dangerous compounds among the warfare nerve agents, since its deleterious effects are extraordinarily difficult to counteract because of the existence of a lone electron pair located on an amidic group that makes the nucleophilic attack of oximes almost impossible (4,7,9,11,16).

As the ability of currently used monopyridinium (e.g. pralidoxime) and bispyridinium oximes (e.g. obidoxime) to eliminate toxic effects of nerve agents is generally rather low, the H oximes have been developed to improve the effectiveness of oximes to reactivate nerve agent-inhibited AChE and eliminate nerve agent-induced lethal effects (14,17). Among the series of H oximes, the HI-6 has been the most studied because it seems to be the most efficacious oxime, yet found, against toxic effects of soman (1,12,23). Unfortunately, the reactivating efficacy of HI-6 for tabun-inhibited AChE is not so high as in the case of soman poisoning (5,6,16,22,26,28).

Not only the choice of oximes but also the anticholinergic drug selection could influence the effectiveness of antidotes against OPs. The OP-induced poisoning is usually treated by an oxime in combination with the anticholinergic drug atropine. Nevertheless, there are other anticholinergic drugs that seem to be suitable adjuncts to oxime treatment of nerve agent-induced poisoning, especially in the case of poisonings with centrally acting nerve agents such as soman and tabun (26). It has been described that some centrally acting anticholinergic drugs such as benactyzine are able to increase the ability of HI-6 to eliminate nerve agent-induced lethal toxic effects in comparison with atropine (13).

The present study compares the effects of currently used oximes (pralidoxime, obidoxime) and H oximes (HI-6) in combination with various anticholinergic drugs (atropine, benactyzine, biperiden, scopolamine) against tabun-induced acute poisoning in mice.

Methods

Male mice weighing between 22 and 25 g were obtained from Konárovice. The animals were maintained in an air-conditioned room with light from 07.00 to 19.00 h and were allowed free access to standard food and tap water. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of Medical Faculty of Charles University and Purkyně Military Medical Academy in Hradec Králové.

Tabun of 95% purity was purchased from Military Technical Institute Brno. Its purity was assayed by acidimetric titration. The oxime HI-6 of 98.5% purity was synthesized at the Department of Toxicology of Purkyně Military Medical Academy in Hradec Králové. Its purity was analyzed using HPLC. All other chemicals and drugs of analytical grade were obtained commercially and used without further purification.

In the first part of the experiments, tabun-poisoned mice were treated intramuscularly (i.m.) with one of tested oximes (pralidoxime, obidoxime and the oxime HI-6) at equieffective doses (5% LD_{50}) in combination with atropine (21 mg/kg) one minute after the challenge of tabun. In the second part of the experiments, tabun-poisoned mice were treated i.m. with obidoxime or the oxime HI-6 in combination with one of tested anticholinergic drugs (atropine, benactyzine, biperiden and scopolamine). The oxime as well as anticholinergic drugs were used at equieffective doses (5% LD_{50}).

The effectiveness of tested antidotal mixtures was evaluated by the assessment of the LD_{50} values and their 95% confidence limits using probit-logarithmical analysis of death occuring within 24 h after i.m. administration of tabun at five different doses with six mice per dose (24). The efficacy of tested antidotal mixtures was expressed as protective ratio (LD_{50} value of tabun in protected mice/ LD_{50} value of tabun in unprotected mice).

Statistical significance was determined by the use of Student`s t-test and differences were considered significant when $p < 0.05$.

Results

The therapeutic efficacy of antidotal mixtures consisting of various oximes in combination with atropine is presented in Table 1. These results show that obidoxime seems to be the most efficacious reactivator of tabun-inhibited AChE in the elimination tabun-induced lethal effects in mice, although the difference in the efficacy between obidoxime and the oxime HI-6 is not significant. On the other hand, obidoxime as well as the oxime HI-6 are significantly more efficacious ($p < 0.05$) to protect the mice from the lethal effects of tabun than pralidoxime.

The efficacy of obidoxime or the oxime HI-6 in combination with various anticholinergic drugs is shown in Tables 2 and 3. The data clearly demonstrate the higher effectiveness of centrally acting anticholinergic drugs (benactyzine, biperiden, scopolamine) to eliminate tabun-induced lethal effects in comparison with atropine in the case of the combination of anticholinergic drugs with obidoxime ($p < 0.05$) (Tab. 2). On the other hand, the therapeutic efficacy of the combination of the oxime HI-6 with centrally acting anticholinergic drugs is only slightly higher than the combination of HI-6 with atropine (Tab. 3). In addition, obidoxime appears to be significantly more efficacious than the oxime HI-6 when combined with anticholinergic drugs with pronounced central effects such as benactyzine, biperiden and scopolamine ($p < 0,05$).

Tab. 1: Therapeutic effect of oximes, administered at a dose of their 5% LD_{50} value in combination with atropine (21 mg/kg) 1 min after poisoning, on the LD_{50} value of tabun.
* significantly different from the untreated group at the level of $p < 0.05$, ^{x} significantly different from the group treated with pralidoxime at the level of $p < 0.05$.

Treatment	LD_{50} (μg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
HI-6 + atropine	430.2 (414.0–447.1)^{*x}	1.56
Obidoxime + atropine	454.9 (421.8–490.6)^{*x}	1.65
Pralidoxime + atropine	377.7 (355.8–400.9)*	1.37

Tab. 2: Therapeutic effect of obidoxime in combination with various anticholinergic drugs, administered at a dose of their 5% LD_{50} values 1 min after poisoning, on the LD_{50} value of tabun.
* significantly different from the untreated group at the level of $p < 0.05$, ^{x} significantly different from the group treated with obidoxime and atropine at the level of $p < 0.05$.

Treatment	LD_{50} (μg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
Obidoxime + atropine	454.9 (421.8–490.6)*	1.65
Obidoxime + benactyzine	773.2 (636.7–939.1)^{*x}	2.81
Obidoxime + biperiden	716.9 (685.6–749.6)^{*x}	2.60
Obidoxime + scopolamine	716.9 (657.3–780.0)^{*x}	2.60

Tab. 3: Therapeutic effect of HI-6 in combination with various anticholinergic drugs, administered at a dose of their 5% LD_{50} values 1 min after poisoning, on the LD_{50} value of tabun.
* significantly different from the untreated group at the level of $p < 0.05$, x significantly different from the group treated with HI-6 and atropine at the level of $p < 0.05$.

Treatment	LD_{50} (μg/kg) ± 95% IS	Protective ratio
—	275.4 (269.3–281.6)	—
HI-6 + atropine	430.2 (414.0–447.1)*	1.56
HI-6 + benactyzine	462.1 (432.6–493.5)*	1.68
HI-6 + biperiden	524.5 (503.3–546.6)*x	1.91
HI-6 + scopolamine	460.2 (444.4–476.5)*	1.68

Discussion

Nerve agents are still considered to be the most important chemical warfare agents. With the existing threat of the use of chemical weapons not only in military conflicts but also in terorristic attacks, the search for effective protection is the central concern of different laboratories both civilian and military (7,20).

The effectiveness of antidotal treatment of acute poisoning with tabun is not sufficient regardless of the choice of the oxime because tabun-inhibited AChE is very difficult to reactivate (6,11,22,26,28). The reason for the weak reactivation potency of the oximes is not the rate of aging of phosphonylated AChE that is relatively low (10) but the presence of lone electron pair located on an amidic nitrogen. This lone electron pair makes the nucleophilic attack very difficult (9). Therefore, the oxime HI-6, that is rather effective against soman (12,14), is not too effective against tabun (6,22). According to our results, obidoxime seems to be more effective to eliminate tabun-induced lethal effects in mice than the oxime HI-6. Till now, it is not known what is the reason for higher effectiveness of obidoxime in comparison with the oxime HI-6. Generally, the difference between the stereochemic arrangement of obidoxime and the oxime HI-6 can play a role in the difference in therapeutic efficacy of both oximes.

The effectiveness of oximes against the toxic effects of nerve agents including tabun is usually tested in combination with atropine (6,22,27). Nevertheless, some other anticholinergic drugs (e.g. benactyzine, biperiden) should be more advantageous than atropine for the elimination of toxic effects of nerve agents because of their central antimuscarinic effects (2,13,26). Our results confirm the influence of anticholinergic drug selection on the efficacy of oximes in antagonizing the toxic effects of tabun at the supralethal doses. Both tested oximes, obidoxime and HI-6, were more efficacious in eliminating tabun-induced toxicity when they were combined with centrally acting anticholinergic drugs in comparison with their combination with atropine although the increase in the therapeutic efficacy of the oxime HI-6 was not significant.

Our results can be explained by the difference in the central antimuscarinic effects of anticholinergic drugs studied that are very important for the prevention of tabun-induced central respiratory depression and, thus, the survival of tabun-poisoned mice. Benactyzine, biperiden as well as scopolamine differ from atropine in that they are more potent in the central nervous system as antimuscarinics due to their relatively high affinity to the central muscarinic receptors (15) and their lipophilicity making them possible to readily cross the blood-brain barrier (3,19,29). Atropine is also able to cross the blood-brain barrier but a relatively large dose of atropine is necessary to achieve the central antimuscarinic effects because of its lower lipophilicity and affinity to the central muscarinic receptors when compared to other anticholinergic drugs studied (3,8).

In conclusion, our data indicate that the correct choice of AChE reactivators as well as anticholinergic drugs is important for the survival of tabun-poisoned experimental animals. The most efficacious antidotal mixture against lethal effects of tabun in mice seems to be obidoxime in combination with some of centrally acting anticholinergic drugs.

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Doc. MUDr. Jiří Kassa, CSc.,
P.O. Box 35/T,
Purkyně Military Medical Academy,
500 01 Hradec Králové,
Czech Republic.
e-mail: kassa@pmfhk.cz