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Tabun-inhibited rat tissue and blood cholinesterases and their reactivation with the combination of trimedoxime and HI-6 in vivo

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ABSTRACT

Up to now, intensive attempts to synthesize a universal reactivator able to reactivate cholinesterases inhibited by all types of nerve agents/organophosphates were not successful. Therefore, another approach using a combination of two reactivators differently reactivating enzyme was used: in rats poisoned with tabun and treated with combination of atropine (fixed dose) and different doses of trimedoxime and HI-6, changes of acetylcholinesterase activities (blood, diaphragm and different parts of the brain) were studied. An increase of AChE activity was observed following trimedoxime treatment depending on its dose; HI-6 had very low effect. Combination of both oximes showed potentiation of their reactivation efficacy; this potentiation was expressed for peripheral AChE (blood, diaphragm) and some parts of the brain (pontomedullar area, frontal cortex); AChE in the basal ganglia was relatively resistant. These observations suggest that the action of combination of oximes in vivo is different from that observed in vitro.

1. Introduction

The treatment of OP/nerve agent poisoning comprises administration of anticholinergics, cholinesterase reactivators and anticonvulsants. While the use of atropine as anticholinergic drug and diazepam as anticonvulsant is usually accepted, there are contraversies in the use of reactivators [1–5]. Some of them are effective against nerve agents, some of them are more effective against OP insecticides and up to now, a universal reactivator against all these agents is not available. There were (and are) some attempts to synthesize a universal reactivator able to reactivate cholinesterases inhibited by all types of nerve agents/OP but without practical results [6–8].

Therefore another approach was described using a combination of two reactivators differently reactivating enzyme inhibited by different inhibitors. This approach was tested in vitro with obidoxime and HI-6 on acetylcholinesterase (AChE, EC 3.1.1.7) inhibited by tabun; it was demonstrated that reactivating effect is due to the effective oxime (i.e. obidoxime in the case of tabun-inhibited AChE) and combination of obidoxime with HI-6 copied the effect of obidoxime alone [9]. This combination was tested in experiments with therapeutic efficacy [10,11].

We tried to know if this effect will be observed in vivo. For better distinguishing of different oxime's action, we used trimedoxime. Trimedoxime is a very effective reactivator of tabun-inhibited AChE in vitro and when combined with atropine, it is one of the best therapies against tabun poisoning [12–14]. The percentages of reactivation of tabun-inhibited blood and tissue AChE in poisoned rats showed trimedoxime to be the most efficacious reactivator [13,14]. Testing the extent of reactivation by trimedoxime and HI-6 in vivo of tabun-inhibited cholinesterases, especially in the different parts of the central nervous system was the aim of this study.

2. Materials and methods

2.1. Animals

Female Wistar rats (VELAZ Prague), weighing 200–220 g, were used in this study. The animals were divided into groups of 6 animals each. Housing of rats was realized in the Central Vivarium of the Faculty of Military Health Sciences under veterinary control. All experiments were performed under permission and supervision of the Ethics Committee of the Faculty of Military Health Sciences, Hradec Kralove (permission no. 153/06) according to §17 of the Czech law no. 207/2004.

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Table 1
AChE activity in biological samples following treated and untreated tabun poisoning.

Group	Blood	Diaphragm	Frontal cortex	Basal ganglia	Pontomedullar AREA
Control	30.1 + 4.2 nc	70.4 + 7.2 nc	247.4 + 15.4 nc	1103 + 132 nc	380.1 + 20.3 nc
Tabun	**6.13 + 0.3** nc	**1.52 + 0.4** nc	**3.29 + 0.24** nc	**824 + 91** nc	**5.93 + 0.9** nc
5TR	***9.53 + 0.4*** 14.2	***8.96 + 1.5*** 10.5	***11.1 + 0.8*** 3.2	934 + 93 nc	***14.18 + 1.2*** 2.2
5H	**6.78 + 0.7** 2.7	***5.17 + 2.2*** 5.3	**3.93 + 0.8** 0.3	**764 + 121** nc	**9.78 + 4.1** 1.0
5H + 5TR	***24.55 + 4.0*** 76.8	***23.28 + 2.4*** 31.6	***51.64 + 2.4*** 19.8	1141 + 94 nc	***98.75 + 4.0*** 24.8
5H + 2.5TR	***17.37 + 4.9*** 46.9	***19.68 + 4.2*** 26.4	***31.53 + 19.4*** 11.6	1090 + 89 nc	***69.28 + 32.4*** 16.9
2.5H + 2.5TR	***11.20 + 1.3*** 31.2	***5.82 + 1.0*** 6.3	***10.27 + 1.8*** 2.9	1123 + 98 nc	***12.13 + 1.6*** 1.7
2.5H + 5TR	***21.54 + 1.2*** 64.3	***18.8 + 4.4*** 25.2	***53.52 + 19.2*** 20.6	1060 + 102 nc	***99.24 + 23.2*** 24.9

Doses of oximes. 5TR: 5% of the lethal dose of trimedoxime, i.e. 3.75 mg/kg; 5H: 5% of the lethal dose of HI-6, i.e. 19.5 mg/kg; the numbers (5 and 2.5) in other groups indicated the dose ratio of oximes.
The first line: AChE activity in μkat/l (blood) or μkat/kg wet weight tissue (mean ± SED); the second line: % of reactivation; nc: not calculated.
In bold – statistically significant difference ($p < 0.05$) from control group – control (for AChE activity).
In bold italics – statistically significant difference ($p < 0.05$) from group with tabun treated with atropine only – Tabun.
Reactivation was not statistically evaluated.

2.2. Chemicals

Tabun was obtained from Military Technical Institute of Protection (Brno, Czech Republic). It was of minimally 95% purity and stored in glass ampullas (1 ml). The solutions of the agents for experiments were prepared before use. The oximes (trimedoxime dibromide, m.w. 443.98 and HI-6 dichloride monohydrate, m.w. 377.22) were synthesized at the Department of Toxicology of the Faculty of Military Health Sciences (Hradec Kralove, Czech Republic). Their purities were analyzed using HPLC technique. All other chemicals of analytical purity were obtained commercially and used without further purification. All substances were administered i.m. at a volume of 1 ml/kg body weight.

2.3. Intoxication and treatment

Control group: The animals were injected with saline i.m. and 1 min later, they were injected once again with saline i.m. (0.1 ml/100 g). The decapitation and sampling was realized 30 min after the last saline injection.

Tabun group: The animals were injected with tabun (i.m.) in a dose of $1.5 \times LD_{50}$, i.e. 300 μg/kg; 1 min later, the animals were injected with atropine (i.m., 21 mg/kg). 31 min after the intoxication, the animals were decapitated and the blood and tissues were collected for biochemical examinations.

Treated groups: The animals received tabun (i.m.) at a dose of $1.5 \times LD_{50}$, i.e. 300 μg/kg, followed by 21 mg/kg atropine sulfate (i.m.) without or with an oxime (i.m.), 1 min later. Animals were decapitated and tissues were immediately collected 31 min after tabun. Oxime doses are given in Table 1.

Six animals were used for each group.

2.4. Biochemical determination of AChE

The blood was obtained by bleeding from the carotid artery. The brain and diaphragm were prepared. The tissues were frozen and the brain parts (frontal cortex—FC; pontomedullar area—PM, and basal ganglia—BG) were prepared. After thawing, tissues were homogenized (1:10, distilled water, Ultra Turrax homogenizer) and homogenates were used for enzymatic analysis. Concentration of the wet weight tissue was 2 mg per cuvette (2 ml). AChE activity was determined according the method of Ellman et al. [15] as described elsewhere [16]. Acetylthiocholine iodide (0.5 mM) was used as substrate (Tris–HCl buffer pH 7.6) and 5,5′-dithiobis-2-nitrobenzoic acid (0.5 mM) as chromogen. UVIKON 752 spectrophotometer was used for the determination of absorbancy at 412 nm. The activity was expressed as μkat/kg wet weight tissue or as % of control values. The blood was haemolysed with distilled water and in haemolysates, activity of AChE was determined. The activity was expressed as μkat/l or as % of control values.

2.5. Statistical evaluation

Enzyme activities determined by biochemical method were expressed as a mean ± SD or % of control values and statistical differences were tested by *t*-test. The reactivation (%) was determined using the AChE activity values:

$$\left[1 - \frac{a_o - a_r}{a_o - a_i}\right] \times 100$$

where a_o is the activity in control group (with administration of saline), a_r is the activity in tabun-intoxicated group treated with atropine and reactivator, a_i is the activity in tabun-intoxicated group treated with atropine only.

3. Results

Normal AChE activity varied from high (BG) to low (FC, blood and diaphragm) values. The AChE activity in the brain areas, blood and diaphragm were inhibited following tabun untreated intoxication and treated with atropine only (Table 1). Percentual inhibition was highest in PM, FC and diaphragm, containing about 2% of the control activity. Residual activity of about 20% was observed in the blood, and AChE activity in BG was relatively resistant preserving 75% of control values. Following tabun intoxication, in groups treated with atropine and different doses of reactivators, AChE reactivation was the lowest in group treated with atropine and HI-6, varying from 0.3 to 5.3%. Treatment with atropine and trimedoxime had higher effect—reactivation from 2 to 14%. Combination of trimedoxime and HI-6 in highest doses caused the highest percentage of reactivation. Relative resistance towards reactivation of tabun-inhibited brain AChE is apparent. HI-6 augments the reactivation brought by