

22. SIMPLE METHOD FOR MORE PRECISE DIAGNOSIS AND TREATMENT OF NERVE AGENT POISONING

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INTRODUCTION

In the treatment of nerve agents (NA) poisoning, two therapeutic principles are used. Paralysis of accumulated acetylcholine is due through the action of anticholinergic drugs, most frequently atropine, cholinesterase reactivators are used for so called causal therapy (1,2). The stabile phosphorylated enzyme is possible to return to normal activity by these reactivators (3,4), under condition that the inhibited cholinesterase is not converted to non-reactivable complex. It is known that NA produced this so called dealkylation or aging at different times after the some reaction of enzyme with inhibitor. For soman, the half time for dealkylation was determined to be about 5-10 min., for sarin about 10-15 hours and for VX the dealkylation was not possible to determine more than 24 hours (5).

These findings can be applied for the both cholinesterases (acetylcholinesterase, AChE, EC 3.1.1.7. and butyrylcholinesterase, BuChE, EC 3.1.1.8.) in the nervous system and in the blood. Knowledge of state of AChE (reactivable of unreactivable complex) would of interest for diagnosis, treatment and prognosis of NA poisoning.

Description of simple test for these purposes is the aim of this study.

MATERIALS AND METHODS

In experiments *in vitro*, the blood from 55 years-old healthy man was haemolyzed by distilled water (1:20) or by solutions of soman, sarin and VX compound, respectively. The concentrations of inhibitors caused high (80-90 %), middle (50 %) or low (10-20 %) inhibition. Following 30 min., 5 hours or 24 hours, AChE activity in these samples was determined in the presence or the absence of trimedoxime ($5 \cdot 10^{-4}$ M).

In experiments *in vivo*, the dogs of both sexes, weighing 6,75 - 14 kg, were used. The animals were *i.m.* intoxicated with soman (0,007 mg/kg), sarin (0,03 mg/kg) or VX compound (0,003 mg/kg), respectively. The AChE activity and reactivation efficacy in the blood samples was determined at different time intervals after the intoxication. AChE activity was expressed as μmol of substrate hydrolyzed per ml of the blood per min, or as per cent of controls, the reactivation potency was expressed as per cent of reactivation.

The homogeneity of both control and experimental groups was evaluated by Bartlett's test. For these experiments, 5-9 measurements were used for experiments *in vitro* (each time interval and every concentration) and 3-6 animals for experiments *in vivo*. The differences between groups were determined by analysis of variance, using Hewlett Packard computer 9830 A.

RESULTS

Very low reactivation was demonstrated for soman inhibited AChE. AChE reactivation from 15 to 63 % for the blood AChE inhibited by sarin was observed and AChE reactivation higher than 55 % was determined for AChE inhibited by VX.

Following intoxication with soman, fast decrease of the blood AChE activity was determined. It reaches to steady state 10 min after the injection of soman. The reactivation ability of the blood AChE was decreased in time and following 50 min the blood AChE in soman intoxicated dogs was not able to reactivate. The mean reactivability in the range of 0 - 16 % for soman intoxication was observed.

In case of sarin, similar decrease of the blood AChE activity was demonstrated. On the other hand, reactivability 100 min after the injection of sarin was observed, the mean reactivation for sarin inhibited AChE was determined to be approximately 60 %.

The prolonged AChE inhibition was demonstrated following VX intoxication: the steady state was achieved 30min after the injection of VX. On the other hand, practically 100 % reactivation *in vivo* was obtained 120 min after intoxication with VX. The results *in vitro* and *in vivo* are summarized in Table I.

Reactivation (%) of the human blood AChE *in vitro* and the dog AChE *in vivo* following exposition by sarin, soman and VX

Compound	% of reactivation	
	(AChE, human blood, in vitro, variation limits for all intervals studied)	(AChE, dog blood, in vivo, means with their confidence limits, $p \leq 0.05$)
Soman	0 - 10	16,06 ± 6,06
Sarin	15 - 63	62,67 ± 5,79
VX	55 - 85	87,61 ± 8,79

DISCUSSION

Our results dealing with reactivatability of the blood AChE following soman, sarin and VX intoxication showed that this approach is suitable as that used for insecticides. Our results in vitro and in vivo are in good correlation. The reactivation test would be useful also for OP in forensic chemistry. If we compare the results in vivo, it can be concluded that the test can be used not only for determination of reactivatability but also for differential diagnosis of intoxications with soman: reactivation about 50 % demonstrates intoxication with sarin and higher reactivation than 80 % might be typical for VX intoxication.

From the results presented, it can be assumed halftime of dealkylation, for soman it lies in the range of 5-10 min, for sarin it is more than 2 hours and for VX dealkylation was not observed within 2 hours. This is in excellent agreement with literature data (2,3,5).

We developed method for double AChE determination in the blood - the first one without and the second one with reactivator. When the activity in the second determination is the same or lower than 0 - 10%, then soman intoxication is very suspect and repeated administration of reactivators will be ineffective. When reactivation is observed in the range of 30 - 50 %, very probably, organism was poisoned with sarin. In case of 80 % and higher reactivation, VX is probably the toxic agent. In these cases, administration of reactivators is indicated.

SUMMARY

Nerve agents inhibit acetylcholinesterase (AChE, EC 3.1.1.7.) in the erythrocytes and cholinergic nervous system. The basic therapeutic interventions comprise administration of atropine (paralyzing effects of accumulated neuromediator acetylcholine) and reactivators of AChE (reactivating by nerve agent inhibited AChE). However, in some cases, depending on the structure of nerve agent, inhibited AChE is changed to complex resistant to reactivators. It is unreactivable. In these cases, repeated administration of reactivators is not effective. This reaction called aging or dealkylation is very fast for soman ($t_{0,5}$ approximately 10 - 12 hours) and for VX, the $t_{0,5}$ was more than 24 hours.

We developed method for double AChE determination in the blood - the first one without and the second one with reactivator. When the activity in the second determination is the same or lower than 0 - 10 %, then soman intoxication is very suspect and repeated administration of reactivators will be ineffective, when reactivation is observed in the range of 30 - 50 %, very probably, organism was poisoned with sarin. In case of 80 % and higher reactivation, VX is probably the toxic agent. Reactivators are indicated. The test was verified by experiments in vitro (human blood) and in vivo on dogs using sarin, soman and VX.

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KEYWORDS

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