BIOCHEMICAL EFFECTS OF LOW LEVEL EXPOSURE TO SOMAN VAPOUR

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SUMMARY

The aim of this study was to demonstrate changes in acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities, tyrosine aminotransferase activity (TAT) and plasma corticosterone level, neuroexcitability and behavior following 24 hours and 4 weeks of soman sublethal inhalation exposure at low level. AChE activity in erythrocytes and BuChE activity in plasma was decreased (dependent on the concentration of soman) 24 h and 4 weeks after the exposure. Similar decrease in AChE activity in different brain parts was observed. One of stressogenic parameters (TAT) was changed after 24 h exposure only. 4 weeks after the exposure, these parameters (corticosterone and TAT) were in the range of normal values. Behaviour of experimental animals was changed 24 h after the exposure persisting 4 weeks after the exposure as well as neuroexcitability.

Key words: cholinesterases, guinea pig, soman, inhalation, stressogenic markers

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INTRODUCTION

Soman belongs to the most dangerous chemical warfare agents. Soman-induced effects like central, muscarinic and nicotinic symptoms including cholinesterase inhibition are usually manifested immediately after high-level or intermediate-level exposures to these chemical warfare agents (1, 2). However, there are numerous studies in both human beings and animals showing that survivors of acute poisoning by nerve agents can experience subtle but significant long-term neurological and neuropsychological outcomes that are detectable months even years after the recovery from acute poisoning. From scarce data published in literature (3-6) it can be concluded that exposure to nerve agents leading to acute effects or chronic exposure to nerve agents may lead to delayed and persistent adverse effects. The aim of this study was to demonstrate changes in acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) activities, stressogenic markers (i.e. tyrosine aminotransferase activity, TAT, EC 2.1.6.5, and plasma corticosterone level), neuroexcitability and behaviour following soman inhalation exposure at low level.

MATERIALS AND METHODS

Experimental Animals

Female guinea pigs (Tricolor, BIO.TEST.s.r.o., Konarovice, Czech Republic), weighing 350 g ± 30 g were used in groups of 6 animals.

Female guinea pigs were exposed to soman vapour (60 min) in three different concentrations: below threshold dose, representing 0.3xLD50 (1.2 mg/m^{-3}), causing no erythrocyte AChE and low plasma butyrylcholinesterase (BuChE) inhibition without clinical symptoms of intoxication, threshold dose, representing 0.4xLD50 (1.5 mg/m^{-3}) causing decrease of AChE and BuChE activities without clinical symptoms and above threshold dose, corresponding to 0.7xLD50 (2.7 mg/m^{-3}) causing AChE and BuChE inhibition with clinical symptoms of intoxication. The animals were sacrificed 24 h or 4 weeks after the exposure and the blood and organs were prepared. Before obtaining the samples, the animals were tested for neuroexcitability (pentamethylenetetrazole test) and behaviour using Functional Observational Battery (FOB).

Blood was collected after killing the guinea pigs by decapitation. Subsequently, brain, diaphragm and liver were removed and frozen at –40 °C. Before use, the following parts of the brain were prepared: frontal cortex, basal ganglia, hippocampus and pontomedullar area, and homogenates (in distilled water) of the brain parts, diaphragm and liver were made 10%, 10% and 2%, respectively.

Determination of Cholinesterase Activity

Blood samples were centrifuged and erythrocytes and plasma were isolated for determination of AChE and BuChE activities, respectively. Cholinesterase activity was determined according to Ellman et al. (7) using acetylthiocholine or butyrylthiocholine as a substrate in case of AChE or BuChE, respectively. The activities determined were expressed as ncat/l blood or ncat/g wet tissue or as % of controls.

Determination of Other Biochemical Parameters

Corticosterone in plasma was determined by using a fluorimetric method according to Mattingly (8) and expressed as µg/ml of

plasma. TAT activity in liver homogenate was determined by using the method described by Diamondstone (9) and expressed as nmol p-hydroxypyruvate transaminated (min × g wet tissue) or as % of controls.

Neuroexcitability

Determination of neuroexcitability was based on the observation of an enhancement of the activity induced by intraperitoneal administration of 1,5-pentamethylenetetrazole at a subconvulsive dose (25 mg/kg) after 1 day and 4 weeks following soman intoxication and registered 30 min after the administration of 1,5-pentamethylenetetrazole (10).

Behaviour

Behaviour of the experimental animals was observed 1 day and 4 weeks after exposure by using a FOB of 36 characteristics. After behavioural examination, neuroexcitability was tested.

Apparatus for Inhalatory Exposure

An exposure chamber was designed and constructed in Purkyně Military Medical Academy for exposure of guinea pigs, mice and rats to sarin and soman vapours (11). Using this apparatus, the LCt50 of soman (60 min exposure) was calculated to be 3.8 mg/m^3 (95% confidence limits 2.8-5.1 mg/m^3) by using probit analysis of the mortality data (12).

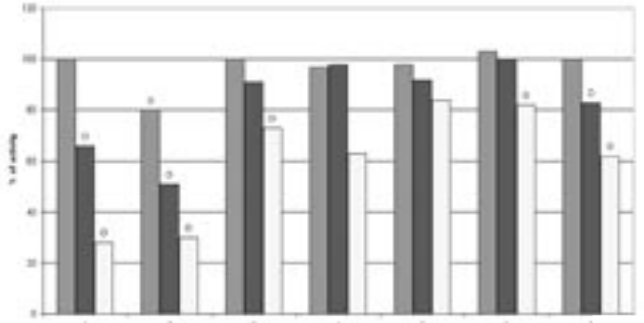

Fig. 1. *Plasma BuChE and AChE activities following inhalation of three concentrations of soman (24 hours). o - statistically significant (p<0.05) changes from controls (100 %). For explanation of numbers on axis x see Fig. 2.*

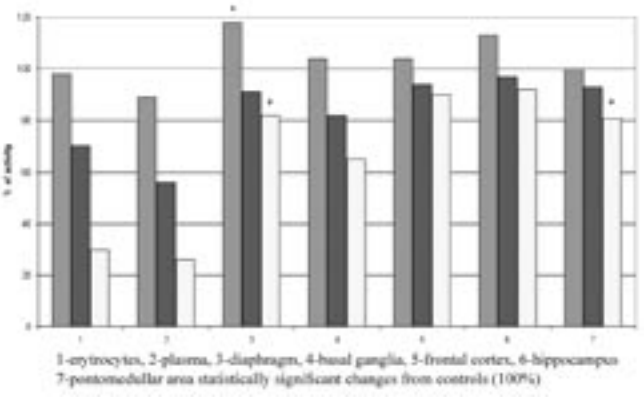

Fig. 2. *Plasma BuChE and AChE activities following inhalation of three concentrations of soman (4 weeks). * statistically significant (p< 0.05) changes (24 hours – 4 weeks).*

RESULTS

The results obtained on plasma BuChE activity and on AChE activity in the various tissues are shown in Fig. 1 and 2. The lowest dose did not have an effect on the erythrocyte AChE activity, as anticipated, nor was the AChE activity affected by this dose in the other tissues studied. Remarkably, no recovery of activity or a very small increase in activity was found for all cholinesterases after 4 weeks.

The results obtained due to the effect of soman intoxication on neuroexcitability and on stressogenic markers, i.e., corticosterone levels in plasma and TAT activity in liver, are given in Fig. 3. Neuroexcitability was slightly increased 1 day after intoxication by the three doses, but had become about normal after 4 weeks. The corticosterone levels were not affected by intoxication with the three doses of soman. TAT activity in liver was increased to a small extent 1 day after intoxication by the two highest doses. The activities were back to normal after 4 weeks.

A total number of 36 characteristics were scored in order to study the effect of soman intoxication on the behaviour of the animals. The scores of the characteristics that were significantly affected by exposure to at least one of the three doses are given in Tables 1 and 2 for the observations made 1 day and 4 weeks after intoxication, respectively. The effects, although significant, are always small. The symptoms for four characteristics (ease of handling, approach response, touch response and click response) were similar 1 day and 4 weeks after intoxication. The score for vocalism was slightly higher than that of the control animals 1 day after exposure to the highest dose, but slightly lower 4 weeks after exposure to the two highest doses. Horizontal activity and total motor activity were increased for the animals intoxicated with the highest dose with respect to the activities in the control animals and these activities were lower than those of the control and treated animals observed 1 day after exposure.

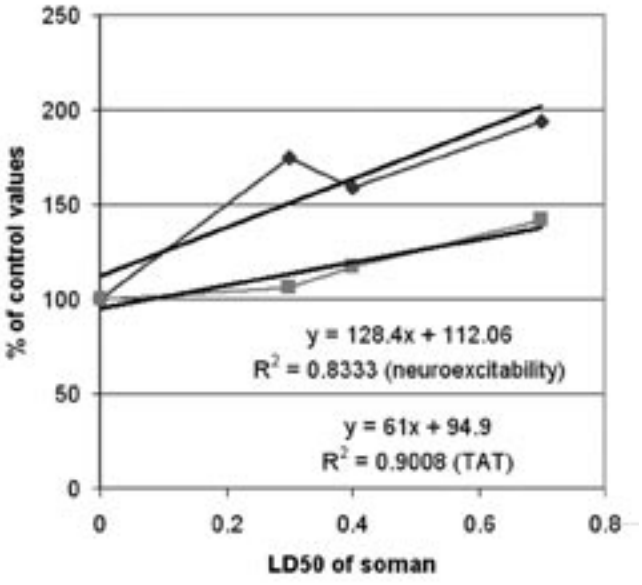

Fig. 3. *Correlation between dose of soman (% of LD50) and neuroexcitability and tyrosine aminotransferase (% of controls).*

Table 1. *Behavioural characteristics out of a total number of 36 which are significantly affected by inhalatory exposure of guinea pigs to at least one of three concentrations of soman vapour in air for 60 min as observed 1 day after exposure. Scores for the extent of severity of the symptoms are given as well as values for non-treated animals.*

Characteristics	Scores given after exposure to a soman concentration of			
	0 mg/m³	1.2 mg/m³	1.5 mg/m³	2.7 mg/m³
♦ease of handling	2 ± 0	2 ± 0	2 ± 0	**1.5 ± 0.6***
tonic movements	0 ± 0	**0.5 ± 0.6***	**0.5 ± 0.6***	**0.8 ± 0.4****
gait	0 ± 0	0.8 ± 2.0	**1.2 ± 1.2***	**0.8 ± 0.4****
gait score	1 ± 0	**1.5 ± 0.6***	**1.8 ± 0.4****	**2.0 ± 0****
mobility score	1 ± 0	1 ± 0	1.3 ± 0.5	**1.7 ± 0.5***
arousal	4 ± 0	4.2 ± 0.4	3.5 ± 1.1	**4.7 ± 0.5***
♦vocalism	1.7 ± 0.8	2 ± 1	1.2 ± 1.3	**2.7 ± 0.5***
♦approach response	2 ± 0	**2.5 ± 0.6***	2.5 ± 0.8	**3.0 ± 0.9***
♦touch response	2 ± 0	2.3 ± 0.8	2 ± 0	**3.0 ± 0.9***
♦click response	2 ± 0	2 ± 0	2 ± 0	**3.3 ± 1.0***
pupil size	0 ± 0	**–1.0 ± 0****	**–0.8 ± 0.4****	–0.3 ± 0.5

Mean values ± SD, ∗0.01<p<0.05, ∗∗p< 0.001,
♦changes after 24 hours and 4 weeks

Table 2. *Behavioural characteristics out of a total number of 36 which are significantly affected by inhalatory exposure of guinea pigs to at least one of three concentrations of soman vapour in air for 60 min as observed 4 weeks after exposure. Scores for the extent of severity of the symptoms are given as well as values for non-treated animals.*

Characteristics	Scores given after exposure to a soman concentration of			
	0 mg/m³	1.2 mg/m³	1.5 mg/m³	2.7 mg/m³
catch difficulty	3.8 ± 0.5	2.7 ± 0.5	**2.5 ± 0.8***	3.3 ± 0.5
♦ease of handling	2.8 ± 0.5	2.5 ± 0.6	2.5 ± 0.8	**1.3 ± 0.8***
muscular tonus	0 ± 0	0 ± 0	0.3 ± 0.5	**0.5 ± 0.6***
♦vocalism	1.8 ± 0.5	1.0 ± 1.1	**0.5 ± 0.8***	**0.5 ± 0.8***
♦approach response	2 ± 0	2.2 ± 0.4	2.5 ± 0.8	**3.3 ± 1.0***
♦touch response	2 ± 0	2 ± 0	2 ± 0	**3.2 ± 0.8***
♦click response	2 ± 0	2 ± 0	2 ± 0	**3.0 ± 0.9***
horizontal activity	52 ± 44	61 ± 33	75 ± 65	**160 ± 23***
total motor activity	54 ± 46	62 ± 33	76 ± 65	**170 ± 29***

Mean values ±SD, ∗0.01<p< 0.05,
♦changes after 24 hours and 4 weeks

DISCUSSION

According to our experiences (13), there exist four basic reactions of nerve agents in the organism: resorption – the agent is resorbed in dependence on the route of administration - from the skin, lung, other tissues to the transport system (blood stream). In the blood, according to the principle "first come, first served" (14) it reacts with AChE and BuChE (erythrocytes and plasma), esterases etc. and this part of organophosphate represents the losses (it does not penetrate to the target sites). The function of these enzymes in the blood is not known but they are good diagnostic markers of exposure to nerve agents and organophosphates (15). Inhibition of AChE and BuChE following inhalation intoxication with sarin and soman in low doses is well comparable. The highest AChE activity in basal ganglia corresponds to results demonstrated previously in rats and other species (13, 16). The high activity in this structure and relative resistance to soman is explainable by the high AChE activity in the basal ganglia which is (in very low doses of soman) comparable with molar concentrations of enzyme and soman and the effect (inhibition) is the result of "titration" of the soman and AChE activity. From the results demonstrated it can be concluded that AChE is inhibited by these concentrations and persisted for at last 4 weeks after the exposure**.**

Morita et al. (17) showed that severaly poisoned patients during the terroristic attack in Matsumoto also suffered from changes in biochemical and haematological parameters for a relatively short time. Erythrocyte AChE level of these patients returned to normal values within 3 months (17). On the contrary, Scremin et al. (16) observed similar cholinesterase inhibition following s.c. administration of sarin to rats in sublethal doses, however, the activity in the blood and brain parts returned to normal values within 2-16 weeks of post-treatment. This discrepancy could be explained by different way of administration and different organophosphate used.

Stressogenic effects of nerve agents and organophosphates involve general stressogenic reaction of poisoned organism characterized by the activation of sympathoadrenal and hypothalamic pituitary adrenocortical system (18). For manifestation of stressogenic effect, low doses of nerve agents / organophosphates are used for excluding acute lethal effect. At these doses, corticosterone is increased during first hours after the exposure followed by TAT increase (18, 19).

This situation corresponds to our results: corticosterone level was unchanged during 24 h after the exposure and increased TAT activity persisted 24 h. However, all these two parameters were in the range of normal values after 4 weeks. These results support an idea that low level inhalation exposure to soman vapours have stressogenic effect in short time interval but this effect lasts only 4 weeks.

Influencing of behavioral manifestation is observed also for lethal compounds but in lower doses than those causing death.

There are some indications for long term effect of low level inhalation exposure to sarin 3 months after the intoxication (4-6). This is slightly different from results of experiments with low doses of sarin administered subcutaneously (16). They observed changes in behaviour following 2 weeks after the exposure but these changes were not present 4 weeks after the intoxication. It can be connected with changes in AChE activity in the brain parts following soman inhalation exposure.

Neuroexcitability was determined through the action of pentamethylenetetrazole. It seems to be an appropriate parameter as it

was demonstrated for different compounds previously (10). The increased excitability following sarin inhalation exposure was demonstrated, too (6). However, the score of excitability in our experiments was only slightly increased following highest dose of soman and there were observed low numbers of petit mal and not grand mal.

CONCLUSIONS

Female guinea pigs were exposed to soman vapours 60 min in doses representing 0.3, 0.4 and 0.7 LD50 and some biochemical parameters were determined 24 h and 4 weeks after the exposure.

AChE activity in erythrocytes and BuChE activity in plasma was decreased (dependent on the dose of soman) 24 h and 4 weeks after the exposure.

One of stressogenic parameters (tyrosine aminotransferase) was changed after 24 h exposure only. 4 weeks after the exposure, these parameters (corticosterone and tyrosine aminotransferase) were in the range of normal values.

Some characteristics of behaviour of experimental animals were changed 24 h after the exposure and persisted 4 weeks after the exposure.

Neuroexcitability of the nervous system was increased 24 h after the exposure.

Good correlation for erythrocyte AChE and plasma BuChE activities and the dose of soman inhaled was demonstrated.

AChE (erythrocyte) and BuChE (plasma) activities are in good correlation with AChE inhibition in diaphragm, frontal cortex and pontomedullar area of the brain.

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