

RELATIONSHIP BETWEEN PLASMATIC LEVELS OF SARIN AND CHOLINESTERASE INHIBITION IN RATS EXPOSED TO SARIN VAPORS

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SUMMARY

In our study we monitored plasmatic levels of sarin and changes in cholinesterase activities in rats after exposure to sarin vapors at low concentration. Rats were exposed to sarin in inhalation chamber at concentration 1.25 or 2.5 µg/l for 60 minutes. The acetylcholinesterase activity was measured in erythrocytes and in different brain regions (frontal cortex – FC, pontomedullar area – PM, basal ganglia – BG). Butyrylcholinesterase activity and sarin levels were measured in plasma. Acetylcholinesterase activity in erythrocytes as well as butyrylcholinesterase activity in plasma were significantly decreased in both groups of animals after intoxication with sarin. In brain, the significant decrease in acetylcholinesterase activity was measured in FC and PM for both groups. In group exposed to higher concentration of sarin vapors, the plasmatic level of sarin was nearly 2x higher than in group exposed to sarin at concentration 1.25 µg/l. The linear dependence between plasmatic levels of sarin and AChE activity was assayed in FC region of brain and in erythrocytes, exponential relationship in PM region of brain and for BuChE activity in plasma.

Key words: sarin, rats, inhalation intoxication, plasmatic levels, acetylcholinesterase, butyrylcholinesterase, blood, brain

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INTRODUCTION

Inhalation and skin penetration are the most threatening routes of intoxication with highly toxic organophosphorus compounds (OPC). In case of the most volatile nerve agent from G-series - sarin (O-isopropyl methylphosphonofluoridate), the inhalation intoxication is more realistic than intravenous or intramuscular administration. In most experiments with nerve agents realized on laboratory animals, the OPC are administered parenterally. This route of administration is not ideal, because it does not mimic well the course of the most presumable way of intoxication. During inhalation intoxication, the dose of OPC is distributed for longer time and in smaller amounts than in case of parenteral application of the same total dose of OPC (1).

OPC cause hyperactivity of the cholinergic system as a result of inhibition of cholinesterases (ChE), in particular acetylcholinesterase (EC 3.1.1.7; AChE) and subsequent increase in the concentration of neurotransmitter acetylcholine (ACh) at the central and peripheral sites (2).

The fact that the highest concentration of the OPC as well as the highest inhibition of ChE are in transport system (blood), is clear from the metabolic model of intoxication with OPC. In blood there are two types of ChE – AChE in erythrocytes and butyrylcholinesterase (EC 3.1.1.8; BuChE) in plasma (3). The activity of BuChE in blood is 1000 times higher compared to blood AChE activity. However the function of erythrocyte AChE

as well as plasma BuChE is not clear. So these peripheral ChE are serving during intoxication with OPC like buffer, because they decrease the effective concentration of OPC in target organs (brain, diaphragm etc.) (4).

Presently used methods for detection and diagnosis of severity of intoxication by OPC are mostly those that quantify inhibition of blood ChE. These methods do not allow identification of OPC to which people were exposed and when inhibition of AChE activity in the red blood cells is less than 20% they are not reliable evidence for OPC exposure. Even if intoxication is not treated, the activity of blood ChE increases because of de novo synthesis of enzymes, so these methods are not suitable for retrospective analysis of exposures to OPC (5). The intact compound and its metabolites can only be measured shortly after the exposure (6).

It was found that when plasma inhibited with OPC is incubated in the presence of a high concentration of fluoride ions, the organophosphate is released from the enzyme thus yielding a phosphofluoridate, which can be analyzed with gas chromatography and NP detection or mass selective detection. Sample cleanup through solid phase extraction and solvent transfer to ethylacetate is necessary to enable GC analysis (7).

In our study we have monitored plasmatic levels of sarin and changes in blood and brain cholinesterase activities in rats after exposure to sarin vapors at low concentration. We have correlated these markers of sarin intoxication.

MATERIALS AND METHODS

Chemicals

Sarin and diisopropylfluorophosphate (DFP) were obtained from Zemianské Kostolany, Slovak Republic. All other chemicals used were obtained commercially and were of p.a. purity. SepPak C₁₈ cartridges type „Classic“ 360 mg/cartridge were from Waters Associates (Millipore Corporation, Milford, MA, U.S.A.).

Animals

Female albino Wistar rats weighing 170-190 g were purchased from Konárovice (Czech Republic). They were kept in an air-conditioned room with lights on from 7 a.m. to 7 p.m. and were allowed access to standard food and tap water ad libitum. The rats were divided into groups of five animals. Handling of the experimental animals was done under supervision of the Ethics Committee of the Military Medical Academy, Czech Republic.

Inhalation Exposure to Sarin

Each group of rats was exposed to sarin vapors in inhalation chamber for 60 min. In inhalation chamber the sarin concentrations were 1.25 µg.l⁻¹ for E1 group and 2.5 µg.l⁻¹ for E2 group. Control group was exposed to „clean“ air without sarin for 60 min in inhalation chamber.

Determination of Sarin in Plasma

Rat plasma was obtained by centrifugation (2,000 g, 10 min) of fresh heparinized blood of rats which was collected 30 min after exposure to sarin vapours and then quickly frozen. Then fluoride-induced reactivation of inhibited BuChE by adding a solution (80 µl) of 12.5 M kalium fluoride in water was performed and the mixture was incubated at 25 °C for 15 min. Then an aliquot (10 µl) of the standard solution of DFP (10 µg.ml⁻¹) was added as an internal standard (ISTD) and the mixture was well shaken. After this procedure, solid phase extraction of sarin using SepPak C18 column and ethylacetate for elution followed.

Gas chromatography analyses were performed by using an HP 6890 equipped with an CP-Sil 8CB column (length 10 m, i.d. 0.32 mm, film thickness 1.2 µm), an alkali flame ionization (NP) detector and an HP ChemStation, Rev. A.06.01.(403) (Hewlett Packard, Wilmington, DE, U.S.A.). Quantitative analysis of sarin was based on comparison of the peak area ratio of sarin and the internal standard DFP with those obtained for mixtures of standard solutions of the two organophosphates.

Cholinesterase Activities

After withdrawal of the blood, the centrifugation was performed to obtain plasma and erythrocytes. Different brain regions - FC, BG and PA were prepared. The erythrocytes were haemolyzed 1:10 with distilled water, the brain parts were homogenized 1:10 in distilled water. AChE activity in haemolysates of erythrocytes and homogenates of different brain regions was determined by the method of Ellman (7) using acetylthiocholine as substrate. Plasma BuChE activity was measured by the same method using butyrylthiocholine as a substrate. The activities were expressed as ncat/l blood and ncat/g of wet tissue respectively (cat/l = mol of substrate hydrolysed/s/l). Results were presented as percents of controls. Statistical significance was determined by the use of Student t-test and differences were considered significant when $p < 0.05$.

RESULTS

Following inhalation intoxication with sarin, decrease in erythrocyte and brain AChE activities as well as in plasma BuChE activities was observed. The decrease of ChE activity in blood expressed in percents of controls, was greater in AChE activity (38.9%) than in BuChE activity (53.0%) (both $p < 0.005$). In groups exposed to different concentrations of sarin vapors (E1 and E2), the difference in ChE activities was significant only for BuChE activity.

In brain, the decrease in AChE activity after inhalation intoxication with sarin, was significant for all regions in both experimental groups (E1, E2). The greatest decrease in AChE activity was in PM (41.3%) ($p < 0.005$), the smallest in BG (90.5%) ($p < 0.05$). Only in FC and PM the difference in AChE activities of E1 and E2 group was significant (FC $p < 0.01$; PM $p < 0.05$).

The mean concentration of sarin in plasma of animals from E1 group was determined to be 262 ng.ml⁻¹ (SD = 12 ng.ml⁻¹) and for E2 group 454 ng.ml⁻¹ (SD = 77 ng.ml⁻¹). The ratio of these values (1.73) is not so different from the ratio of sarin concentrations to which the animals were exposed (2.0).

We assume that a relation of ChE activity on plasmatic level of sarin is linear till value of sarin concentration in plasma achieves a limit concentration. When the plasmatic level of sarin is higher than this limit concentration, the relationship is exponential, because even if concentration of sarin in plasma increases the cholinesterase activity remains on zero level. Correlations between plasmatic levels of sarin and ChE activities in blood are presented in Fig. 1, in brain in Fig. 2. We found that in range of sarin concentrations used in

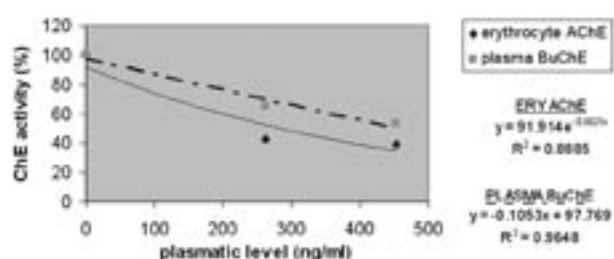


Fig. 1. Relationship between ChE activities in blood and plasmatic levels of sarin in rats after inhalation exposure to sarin vapors.

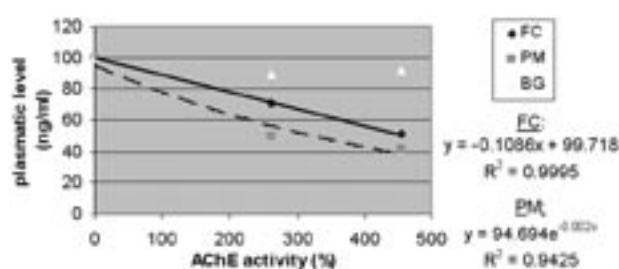


Fig. 2. Relationship between AChE activities in brain and plasmatic levels of sarin in rats after inhalation exposure to sarin vapors.

our study, the relationship for erythrocyte AChE activity was exponential, whereas for plasma BuChE activity linear. In brain the linear dependence between plasmatic levels of sarin and AChE activity was assayed in FC region, exponential relationship in PM region. In BG no correlation was found.

DISCUSSION

The possibility that military personnel in a conflict situation or first responders and medical personnel in case of a terrorist attack may be exposed to low-level OPC makes it necessary to ascertain that exposure has or has not taken place based on detection and reliable diagnosis of trace exposure (8). Exactly method of fluoride-induced reactivation is suitable for monitoring of exposures without clinical symptoms of intoxication when decrease in blood ChE activities is less than 20% and is not measurable with Ellman method (9). It is estimated that 0.01% inhibition of BuChE can be measured with fluoride-induced reactivation, which is an improvement by approximately three orders of magnitude over Ellman methodology (3). Methods used in our experiment differs in principle of determination, while fluoride-induced reactivation is based on measurement of amount of sarin released from inhibited BuChE, Ellman methodology measures ChE activity, which was not inhibited by sarin. Also the method of fluoride-induced reactivation has some limitations, because it can not be used when some reactivators of ChE were applied and in case of soman-poisoned organisms, the reactivation of BuChE with fluoride ions is feasible only until dealkylation process was realized.

Fluoride-induced reactivation method was successfully applied in analysis of blood of victims from terrorist attack by the Aum Shinrikyo sect in Tokyo (1995) (7) and in experiments with laboratory animals for monitoring of sarin in plasma of guinea-pigs and marmosets after inhalation exposure to sarin low concentration (9). Neither in guinea-pigs, nor in marmosets ChE activities were monitored.

In our study we analysed relationships between quantified amounts of sarin released from BuChE in plasma and ChE ac-

tivities in chosen regions. Our results confirmed that erythrocyte AChE is more sensitive for OPC than plasma BuChE, because in the range of sarin concentrations generated in inhalation chamber, the dependence of BuChE activity was still linear, while in case of AChE activity it turned to be exponential. In brain, PM was found to be more sensitive than FC. AChE in BG is inhibited up to final stage of intoxication with sarin, that is why no correlation was found in our study.

In conclusion, the present study demonstrates that ChE activities as well as sarin amounts in plasma are very useful markers of sarin intoxication and a relationship between them can show the sensitivity of the ChE depending on its location. For detailed analysis of relationships, further measurements with different sarin concentration are planned to be realized.

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