

21. THE RELATIONSHIP BETWEEN THE DOSE OF SARIN AND CHOLINESTERASE INHIBITION IN THE BLOOD FOLLOWING INHALATION INTOXICATION

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SUMMARY

Inhalation exposure is one of the important routes of administration of nerve agents. There are scarce data dealing with the effect of low concentrations of these agents. We studied effects of sublethal concentrations of sarin administered by inhalation on acetylcholinesterase (AChE, EC 3.1.1.7) activity in the blood of rats. The aim of this study was to determine if it is possible to monitor exposures to low concentrations of sarin using current methods of determining blood cholinesterases. We used a dynamic inhalation chamber of our own construction. AChE was determined according to modified Ellman's method. The concentrations of sarin were the following: 0.5, 0.67, 0.8 and 1.0 mg.m⁻³, 4 hrs exposure. At the concentration of 0.5 mg.m⁻³, the effects of duration of exposure were studied in intervals 3, 4 and 6 hrs, respectively. Different concentrations of sarin (4 hrs exposure) caused decreases of AChE activity from 71.9% (the lowest concentration of sarin) to 40.4% (the highest concentration of sarin). Increased duration of exposure has led to increased AChE inhibition, dependent on the time of exposure. At the last interval studied (6 hrs), AChE activity was 60%. It appears from these results that AChE in the blood is very sensitive marker for diagnosis of inhalation exposure to low concentrations of sarin.

INTRODUCTION

Intoxication with organophosphorus cholinesterase inhibitors constitutes a problem in industrial and agriculture toxicology. Similar compounds, e.g., sarin are considered as potential chemical warfare agents due to their toxicity and physicochemical properties. The main route of toxic compounds to the body is through the respiratory or gastrointestinal tract and the skin. A large number of results concerning parenteral administration has been published (1), however, few experiments have been published on inhalation intoxication, especially for very low concentrations (2-4).

MATERIAL AND METHODS

Animals

Female Wistar rats (VELAZ Prague), weighing 180-200 g, were used. The animals were maintained in an air-conditioned room (22°C and 50% relative humidity, with light from 7:00 to 19:00 h, and were allowed free access to standard chow and tap water. The Ethics Committee of Charles University at Hradec Králové supervised the handling of experimental animals.

Inhalation intoxication

A dynamic inhalation chamber of our own construction was used. It was composed from the chamber (total volume 30 L) with active exchange of air (30 L/min) with suction of air through the filter, i.e., the chamber of dynamic character was used. The formation of sarin vapors was based on evaporation of sarin in a rotating bar. The concentration of sarin in the chamber was measured using detection equipment based on determination of cholinesterase inhibition in the alcoholic extract of air and calibrated for sarin. This allowed us to monitor sarin concentration discontinuously. Concentrations of sarin were as follows: 0.5, 0.67, 0.8 and 1.0 mg m⁻³ for 4 hrs exposure. At the concentration of 0.5 mg m⁻³, the effects of exposure duration were studied in intervals of 3, 4 and 6 hrs, respectively.

AChE activity determination

AChE activity of erythrocytes was determined with the modified method of Ellman et al., (5) as described elsewhere. Acetylthiocholine was used as substrate and activity was expressed as (cat/L of the whole blood).

Statistical evaluation

Statistical evaluation was made using the method of linear regression analysis calculated by means of minimal squares. The equations with relevant correlation coefficients were calculated. For each concentration and interval, six animals in each group were used.

RESULTS AND DISCUSSION

Exposure to different sarin concentrations caused decreases in AChE activity from 71.9% (concentration 0.5 mg m⁻³) to 40.4% (the highest concentration). The relationship between AChE activity and concentration of sarin is shown in Table 1. At the highest concentration of sarin, with AChE activity at 40.4% of the uninhibited AChE, marked clinical symptoms of intoxication were not observed. AChE activity is dependent on the duration of exposure as it is demonstrated for concentration 0.5 mg m⁻³ in Table 2.

In all cases, both relationships (i.e., sarin concentration vs. AChE activity and duration of exposure vs AChE activity) were linear. These results suggest that at the low concentrations, AChE in the blood is "titrated" by sarin and that AChE in the blood is very sensitive marker for diagnosis of inhalation exposure to low concentrations of sarin.

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REFERENCES

1. Bajgar, J. (1998) Voj. zdrav Listy Suppl. 2, 67, 12-15.
2. Moore D.H. Low dose exposure to nerve agent. 2nd Chemical Medical Defence Conference, 23-24 April 1997, Munich, Germany.
3. Moore D.H. (1998) J. Physiol, 92,325-328, 1998.
4. Kassa, J. et al. (2000) Homeostasis, in press.
5. Ellman G.L. et al. (1961) Biochem Pharmacol. 7, 88-95.

KEY WORDS

sarin, inhalation, acetylcholinesterase, rat, blood

TABLES AND FIGURES

Table 1. Dependence of sarin concentration and AChE activity in the blood

Sarin Concentration (mg m ⁻³)	AChE activity (% of controls, mean±SD)
0	100±12.1
0.5	71.9±13.1
0.67	61.4±10.2
0.8	50.7±9.1
1.0	40.4±9.2

Equation: $y = 100 - 59.1x$, $r_{xy} = -0.99844$

Table 2. Relationship between duration of sarin exposure and AChE activity in the blood

Exposure Time (hrs)	AChE activity (% of controls, mean±SD)
0	100±12.1
3	78.2±10.2
4	71.9±13.1
6	60.0±10.2

Equation: $y = 100 - 7.33x$, $r_{xy} = -0.99842$