Biological monitoring of exposure to nerve agents

J Bajgar

Abstract
Changes in acetylcholinesterase activity in blood and some organs of rats after intoxication with sarin, soman, VX, and 2-dimethylaminoethyl-(dimethylamido)-phosphonofluoridate (GV), in doses of roughly $2 \times LD_{50}$, given intramuscularly, were obtained from published data and by experiment. The time course of inhibition of acetylcholinesterase in blood, regions of brain, and diaphragm and the occurrence of signs and symptoms of poisoning (none, salivation, disturbed ventilation and fasciculations, convulsions, or death) were summarised and compared. When blood enzyme activities were 70-100\% normal, no obvious signs were seen; at 60-70\%, salivation occurred; at less than 30-55\%, disturbed ventilation and fasciculations were seen, and at 15-30\%, convulsions occurred. Less than 10\% was fatal. In experiments with narcotised dogs, the blood acetylcholinesterase activity and the ability to reactivate it with trimedoxime were determined after intoxication by intramuscular administration of the four compounds. It is concluded that acetylcholinesterase activity in the blood corresponds to that in the target organs and can be considered as an appropriate parameter for biological monitoring of exposure to nerve gases. Moreover, determination of reactivation of blood acetylcholinesterase gives more information than simple determination of enzyme activity.

Nerve agents are compounds of high toxicity,\(^1\) one of the many reasons for their possible use as chemical weapons.\(^2\) Others from this class of chemicals

![Figure 1](https://i.imgur.com/3.png)

*Figure 1  Schematic representation of possible complex effects of organophosphate (OP) action. AST, ALT, AcP, AIP, EST, $\gamma$-GT represent transaminases, phosphatases, esterases, and $\gamma$-glutamyl transferase (modified from Bajgar\(^4\)).*
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(organophosphorus compounds) with similar mechanisms of action—but less toxicity—are frequently used as insecticides and medical drugs and they are evaluated by industry. Some of the results and conclusions from this paper could therefore be applied not only to nerve agents but to organophosphates in general.

The basic mechanisms of action of organophosphates (including nerve agents) are well known; they involve cholinesterase inhibition with subsequent accumulation of the neurotransmitter, acetylcholine, changes in membrane permeability, and other metabolic imbalances. Figure 1 shows that these changes are complex. Attempts to correlate action of nerve agents with changes in blood flow, detoxication, and carboxylesterase activity have been made. Cholinesterase activities in the blood (erythrocyte acetylcholinesterase (AChE, EC 3.1.1.7) and plasma butyrylcholinesterase (BuChE, EC 3.1.1.8)) are the most accessible for monitoring the biological effects of nerve agents. The physiological importance of AChE is greater than that of BuChE.

The reaction of organophosphates with cholinesterases has been described (fig 2). It appears that the principal action (in vitro and in vivo) of organophosphates is inhibition of cholinesterases (especially AChE). The site of the inhibition depends on the type of agent examined.

We have used previously published data describing symptoms of poisoning and AChE activity in blood after intoxication with three nerve agents—namely, sarin, soman, and VX. We have also included another anticholinesterase inhibiting substance, 2-dimethylaminoethyl-(dimethylamido)-phosphonofluoridate (GV), with a similar toxicity. Figure 3 gives the formulae of the agents used. The questions arise as to whether and where the degree of AChE activity reflects the actual state of the organism or the degree of poisoning and whether AChE activity in blood is comparable with that in the target organs.

Determination of simple AChE or BuChE activity (or inhibition) is only one indicator for the biological monitoring of organophosphate action. For further information on the poisoned organism and especially for its treatment, it is of interest to know if the enzyme inhibition by organophosphate can be reversed (the enzyme can be reactivated). In some cases, depending on the type of organophosphate and on the duration of its action, the inhibited enzyme is changed into a complex that cannot be reactivated. This has been described as “aging”. The rate of this aging reaction is fast for soman (minutes); AChE activity inhibited by sarin is aged within hours, and aging of AChE inhibited by VX was not found.

No information exists on reactivation of AChE inhibited by GV. Determination of ability to re-activate inhibited AChE offers another possibility for the biological monitoring of exposure to organophosphates.

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**Material and methods**

**EVALUATION OF PUBLISHED DATA**

We used published data summarised in the paper by Baijar describing the inhibition of AChE in the blood and other organs of female rats after intra-

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**Figure 2. Schematic representation of reaction of organophosphate inhibitor (AB) with leaving group (B) and AChE (EH). EHAB, intermediate complex; EA, phosphorylated enzyme; EA', dealkylated enzyme; Kd, dissociation constant, k, bimolecular rate constant; k, k, k, k, and k, rate constants (according to Aldridge and Aldridge).**

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**Figure 3. Structural formulae of the agents used.**
muscular administration of soman, sarin, and VX. The doses were roughly $2 \times \text{LD}_{50}$—namely, 0.36 mg/kg for sarin, 0.154 mg/kg for soman, and 0.030 mg/kg for VX.

INHIBITION OF AChE ACTIVITY IN VIVO BY GV

For compound GV, female Wistar rats (Velaz Praha, Czechoslovakia) weighing 200–220 g were used. The animals were divided into groups of six animals. After intoxication with GV in a dose roughly $2 \times \text{LD}_{50} (0.034 \text{ mg/kg}), AChE$ activity in the blood, regions of brain, and diaphragm proceeded quickly.

REACTIVATION OF AChE ACTIVITY IN VIVO

Reactivation of blood AChE activity in vivo was described previously. The method is based on a duplicate determination of AChE activity with and without reactivator (trimedoxime chloride, Léčiva Praha, Czechoslovakia) at a concentration with negligible effect ($5 \times 10^{-3} \text{M}$) on the hydrolysis of substrate, acetylthiocholine (Lachema Brno, Czechoslovakia). These experiments were performed on beagle dogs (Velaz Praha, Czechoslovakia) of both sexes, weighing 8–12 kg, ($n=3–5$). The dogs were anaesthetised and intoxicated by intramuscular injection of soman (0.007 mg/kg), sarin (0.03 mg/kg), VX (0.003 mg/kg), or GV (0.004 mg/kg). Blood was collected at different time intervals and the percentage of AChE activity or its reactivation was determined. In this case, the symptoms registered were only salivation and fasciculation. Convulsions and death were not seen because of the low doses of the agents used and modification of the symptoms by narcotisation.

STATISTICAL EVALUATION AND CHEMICALS

Statistical evaluation was performed with relevant programmes using a Turbo 12 computer. 5,5-Dithiobis-2-nitrobenzoic acid (Serva Heidelberg, Germany) was used as chromogen for AChE activity determination. Other chemicals of analytical grade were obtained from Lachema Brno.

### Results

Figure 4 shows the changes in AChE activities in rat blood. The decrease in half lives for activity of AChE in the materials examined (table) showed that, after soman intoxication, inhibition of AChE in the pontomedullary area and diaphragm proceeded quickly.

#### Table: Half lives of inhibition of AChE activity in vivo after sarin, soman, VX, or GV poisoning

<table>
<thead>
<tr>
<th>Agent and dose (mg/kg)</th>
<th>Material</th>
<th>Half lives min (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soman: 0.154</td>
<td>Blood</td>
<td>0.7 (0.51–0.90)</td>
</tr>
<tr>
<td></td>
<td>Pontomedullar area</td>
<td>1.5 (0.9–2.1)</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>2.8 (1.8–3.9)</td>
</tr>
<tr>
<td></td>
<td>Diaphragm</td>
<td>1.6 (0.9–2.2)</td>
</tr>
<tr>
<td>Sarin: 0.360</td>
<td>Blood</td>
<td>1.08 (0.8–1.4)</td>
</tr>
<tr>
<td></td>
<td>Pontomedullar area</td>
<td>1.91 (1.5–2.4)</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>2.7 (2.1–3.5)</td>
</tr>
<tr>
<td></td>
<td>Diaphragm</td>
<td>3.1 (2.8–3.4)</td>
</tr>
<tr>
<td>VX: 0.030</td>
<td>Blood</td>
<td>0.8 (0.6–1.0)</td>
</tr>
<tr>
<td></td>
<td>Pontomedullar area</td>
<td>8.5 (7.8–8.2)</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>17.2 (13.7–20.7)</td>
</tr>
<tr>
<td></td>
<td>Diaphragm</td>
<td>3.3 (2.8–3.8)</td>
</tr>
<tr>
<td>GV: 0.034</td>
<td>Blood</td>
<td>1.2 (0.9–1.5)</td>
</tr>
<tr>
<td></td>
<td>Pontomedullar area</td>
<td>5.1 (4.8–5.4)</td>
</tr>
<tr>
<td></td>
<td>Basal ganglia</td>
<td>14.3 (13.7–15.0)</td>
</tr>
<tr>
<td></td>
<td>Diaphragm</td>
<td>1.3 (0.9–1.7)</td>
</tr>
</tbody>
</table>
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Figure 5 The range of AChE activity in rat blood corresponding to different symptoms of poisoning (mean values and SD). The points represent the mean value of blood AChE activity in rats exposed to organophosphorus insecticides according to published data. \( \Delta = \text{Trichlorfon} \), \( \bigcirc = \text{demeton} \), \( \bullet = \text{dichlorvos} \).

and corresponded to that found in the blood. In sarin poisoning, the fastest rate and the greatest degree of AChE inhibition in the pontomedullar area, corresponding to that in the blood was seen. On the other hand, after VX or GV intoxication, the half lives of AChE inhibition were similar in the blood and diaphragm (table). If we summarise the results from different sources on rats, similar relations between enzyme inhibition and clinical state are obtained: near normal activity (70-100%) corresponds to intoxication without symptoms. Salivation is seen when the activity is decreased to 60-70%, fasciculations and dyspnoea are found if the AChE activity is below 55%. When convulsions occurred, AChE activity was 15-30% and in the case of death, a maximum of 10% normal AChE activity was detected. These results were confirmed not only in experiments with the nerve agents but also in cases of intoxication with organophosphate insecticides like trichlorfon, demeton, and dichlorvos (fig 5).

After intoxication of the dogs with soman, a rapid decrease in blood AChE activity was recorded (fig 6). It reached a steady state 10 minutes after the injection of soman. The reactivation potential of blood AChE activity decreased with time and after 100 minutes AChE could not be reactivated at all (fig 6). A mean reactivation in the range of 0-16% was found.

In the case of sarin, a similar decrease in AChE activity was found (fig 6). On the other hand, reactiva-

Discussion
From the results presented, a relation exists between signs of poisoning and changes in AChE activity in the blood for highly toxic organophosphates. These changes are similar to those seen in the target organs. The sites of the toxic effect are different for the different compounds studied. In the case of G-compounds (sarin and soman), the activity of AChE in the brain is a better marker for poisoning than the enzyme activity in peripheral nervous tissues. Soman has a more uniform action than sarin. In the case of VX and GV, their effect on peripheral AChE activity was more pronounced and changes in AChE activity in the blood were similar to those in the diaphragm. This agrees with other results in which the inhibi-

Figure 6 Changes in AChE activity (bottom) and its reactivation (\( R \)) (top) in blood of dogs intoxicated with soman (\( \bigcirc \)) and sarin (\( \bullet \)). Results are means with SD.
an assessment of the extent of intoxication, however, or the prognosis of the repeated administration of reactivators. The test used in our experiments improves the prediction of further prognosis and enables a more rational treatment. Moreover, these results give some pointers as to the likely agent used. Where there is little or no reactivation (up to 10–20%), the poisoning is probably caused by soman or GV: moderate reactivation (more than 50%), would tend to indicate sarin intoxication. An AChE activity reactivated to 80% or more suggests that the toxic agent might be VX. These findings have obvious importance for cases of intoxication with organophosphorus insecticides; in most cases aging of AChE activity did not occur. Thus in instances of decreased AChE activity for reasons other than inhibition by organophosphorus insecticides (carbamates, metabolic or genetic diminution of activity etc) no reactivation will be found.22 We can assume that these two methods could serve as basic tests in the diagnosis of organophosphate poisoning.

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