Success of pyridostigmine, physostigmine, eptastigmine and phosphotriesterase treatments in acute sarin intoxication

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Abstract

The acute toxicity of organophosphorus (OP) compounds in mammals is due to their irreversible inhibition of acetylcholinesterase (AChE) in the nervous system, which leads to increased synaptic acetylcholine levels. The protective actions of intravenously (i.v.) administered pyridostigmine, physostigmine, eptastigmine, and an organophosphate hydrolase, phosphotriesterase, in acute sarin intoxication were studied in mice. The acute intragastric (i.g.) toxicity (LD50) of sarin with and without the pretreatments was tested by the up-and-down method. The mice received pyridostigmine (0.06 mg/kg body weight), physostigmine (0.09 mg/kg body weight), the physostigmine derivative eptastigmine (0.90 mg/kg body weight) or phosphotriesterase (104 U/g, 10.7 μg/g body weight) 10 min prior to the i.g. administration of sarin. Physostigmine was also administered with phosphotriesterase. Phosphotriesterase was the most effective antidote in sarin intoxication. The LD50 value for sarin increased 3.4-fold in mice receiving phosphotriesterase. Physostigmine was the most effective carbamate in sarin exposure. The protective ratios of physostigmine and pyridostigmine were 1.5- and 1.2–1.3-fold, respectively. Eptastigmine did not give any protection against sarin toxicity. Both the phosphotriesterase and physostigmine treatments protected the brain AChE activities measured 24 h after sarin exposure. In phosphotriesterase and physostigmine-treated mice, a 4- and 2-fold higher sarin dose, respectively, was needed to cause a 50% inhibition of brain AChE activity. Moreover, the combination of phosphotriesterase-physostigmine increased the LD50 value for sarin 4.3-fold. The animals pretreated with phosphotriesterase–ephysostigmine tolerated four times the lethal dose in control animals, furthermore their survival time was 2–3 h in comparison to 20 min in controls. In conclusion, phosphotriesterase and physostigmine were the most effective treatments against sarin intoxication. However, eptastigmine did not provide any protection against sarin toxicity. © 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Pyridostigmine; Physostigmine; Eptastigmine; Phosphotriesterase; Sarin; Mice
1. Introduction

The acute toxicity of organophosphorus (OP) compounds in mammals is primarily due to their irreversible binding to the esteratic site of acetylcholinesterase (AChE, EC 3.1.1.7) in the nervous system and a subsequent accumulation of acetylcholine in the nervous system, in the synaptic cleft and at the myoneural junctions. The inhibition of AChE in the diaphragm and brain is important due to the fact that death is attributed to respiratory failure. The inhibition of AChE causes a cholinergic crisis producing several symptoms of poisoning, e.g. salivation, sweating, tearing, miosis, diarrhea, CNS depression, cardiovascular effects such as bradycardia, decreasing cardiac output, cyanosis, muscle twitches followed by muscular weakness, convulsions and respiratory failure which may lead to death (Ecobichon, 1991).

Several different approaches have been tested in the therapy of OP poisoning. The conventional treatment of OP poisonings includes the muscarinic antagonist atropine, and a cholinesterase reactivator oxime. Anticonvulsants such as benzodiazepines (diazepam), barbiturates (phenobarbitone) and Ca\(^{2+}\) channel blockers are also used. The cholinolytic drug atropine relieves symptoms of the intoxication. Oximes release the enzyme AChE from OP compounds and thus restore the normal enzyme function. None of the oximes can be regarded as a broad spectrum antidote. Both pralidoxime-2-chloride (2-PAM) and obidoxime (Toxogonin\(^{®}\)) are quite effective against OP pesticides (Worek et al., 1996). However, the cholinesterases (ChEs) inhibited by some OP nerve agents like soman appear to be quite resistant to oxime-induced reactivation. Traditional oximes such as 2-PAM fail to reactivate the inhibited ChE activity due to rapid aging of the soman-inhibited and phosphorylated enzyme. Bispyridinium Hagedorn oximes (HLö-7, HI-6) are the most effective oximes against these nerve agents (Ligtenstein and Kossen, 1983; Lundy et al., 1992). Moreover, high doses of 2-PAM may also inhibit ChEs (Holmes and Robins, 1955). Rapid i.v. injection of 2-PAM iodide leads to several adverse reactions, e.g. direct depression of the respiratory center, transient mild weakness, blurred vision, dizziness and impairment of accommodation, headache, nausea and tachycardia (Jager and Stagg, 1958; Jager et al., 1958). One problem is also the short half-life of the hydrophilic oximes.

A potentially novel alternative nerve agent antidote is to use OP hydrolases (aryldialkylphosphatase, EC 3.1.8.1 or arylester hydrolase, EC 3.1.1.2) (Raveh et al., 1992; Tuovinen et al., 1994, 1996a,b,c). OP hydrolases (A-esterases) such as phosphotriesterase can actively hydrolyze OPs. Paraoxon can be hydrolyzed by phosphotriesterase very rapidly (Dumas et al., 1989a,b). This reduces the amount of OP in the body and prevents the inhibition of esterases. Phosphotriesterase hydrolyzes several other OPs, e.g. DFP, sarin, soman and tabun (Dumas et al. 1989a; Dumas et al., 1989b, 1990; Ashani et al., 1991; Raveh et al., 1992).

In previous studies we have shown that phosphotriesterase-treated mice tolerated 33–50-fold higher paraoxon doses than controls (Tuovinen et al., 1994). Moreover, we found that recovery of non-aged paraoxon-inhibited ChEs was faster in phosphotriesterase-treated mice (Tuovinen et al., 1994). The i.v. injected phosphotriesterase protected the body ChE activities in mice, when it was administered before or after paraoxon (Tuovinen et al., 1994, 1996a). It also prevented ChE inhibition following DFP, sarin or soman exposures (Tuovinen et al., 1994, 1996a,b,c).

One protection method in OP warfare nerve agent poisoning is the use of reversible AChE inhibitors such as carbamates (pyridostigmine and its derivatives) and acridines (tacrine and its derivatives). The carbamates act by forming a relatively unstable bond, which serves to protect AChEs. Carbamates temporarily occupy the catalytic site of AChE and thus prevent phosphorylation of the enzyme. In combination with atropine plus oxime, the centrally-active carbamate, physostigmine, and the peripherally active pyridostigmine have been shown to protect against multiple LD\(_{50}\)s of soman (Leadbeater et al., 1985). Pyridostigmine was used by various armed forces of several western countries for pretreatment against nerve agent poisoning in the war against...
Iraq in 1991. However, pyridostigmine as a quaternary compound cannot penetrate the blood–brain barrier. Thus, the brain AChE is not protected, and nerve agent poisoning may lead to convulsions causing brain damage.

The objective of the present study was to find out the best prophylactic carbamate to alleviate, together with phosphotriesterase, the toxicity of sarin. We compared the traditional and peripherally acting pyridostigmine to the centrally acting carbamates physostigmine and its derivative eptastigmine. Eptastigmine was chosen for this study because it is more lipophilic, less toxic, and has a longer duration of action than physostigmine in rodents (Marta et al., 1988; Unni et al., 1994; Sramek et al., 1995).

2. Materials and methods

2.1. Chemicals and materials

Pyridostigmine bromide (C₉H₁₃BrN₂O₂; molecular weight 261) and physostigmine sulfate (C₁₅H₂₁N₃O₂; molecular weight 275) were purchased from Sigma (St. Louis, MO). The derivative of physostigmine sulfate, eptastigmine (MF-201 tartrate; heptylphysostigmine; C₂₁H₃₃N₃O₂; molecular weight 359), was kindly supplied by Mediolanum (Milano, Italy). The carbamates were dissolved in 0.9% NaCl (saline) solution. The phosphotriesterase coding gene from *Pseudomonas diminuta* was transferred into *Escherichia coli* and a protein preparation was purified according to the method of Omburo et al. (1992) at Texas A&M University. The enzyme was dissolved in 50 mM Hepes buffer, pH 8.5, 1.34 mg/ml with 13 000 U/ml (9700 U/mg protein) of paraoxon hydrolyzing activity. In the purification system the naturally occurring Zn²⁺ was replaced with Co²⁺ (Omburo et al., 1992). Sarin (isopropyl methylphosphonofluoridate) was obtained from the Research Centre of The Finnish Defense Forces (Lakiala, Finland), stored in ethylacetate solution (1 mg/ml) at −20°C, and dissolved in olive oil. Acetylthiocholine and butyrylthiocholine, the substrates for ChEs, and other reagents such as bovine albumin, 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB), 4,4dithiopyridine (PDS), Triton X-100, iso-OMPA (tetraisopropyl pyrophosphoramide), the specific inhibitor for BChE, were purchased from Sigma. Paraoxon (diethyl-p-nitrophenylphosphate), the substrate for paraoxonase and phosphotriesterase, was purchased from Ehrenstorfer (Augsburg, Germany).

2.2. Animals

The animals used were 8–10 weeks old CD2F1 male mice (20–23 g) (National Laboratory Animal Center, Kuopio, Finland). They were housed in groups of six in stainless-steel cages with aspen bedding (Tapvei, Kaavi, Finland), in a controlled environment: temperature 22 ± 1°C, the relative humidity 50 ± 10%, and a light period of 12 h (07:00–19:00). The mice were acclimatized to their new environment for at least 1 week before the start of experiments. The mice were fasted for 20 h before the OP dosing, but were allowed free access to water. After this, the mice were allowed to eat pellets of mouse diet (R36, Lactamin AB, Sodertalje, Sweden) and drink water ad libitum. This study was approved by the Ethical Committee for Animal Experiments of the University of Kuopio.

2.3. Protection experiments

Pyridostigmine (0.06 mg/kg body weight), physostigmine (0.09 mg/kg body weight), eptastigmine (0.90 mg/kg body weight), and phosphotriesterase (104 U/g and 10.7 μg/g body weight) were injected into the tail vein 10 min prior to i.g. administration of sarin doses. Doses of carbamates were selected to cause a clear inhibition (approximately 40%) of AChE activities in the brain or in the muscle. The doses of carbamates were chosen on the basis of the results of other studies and data from our previous studies (e.g. Harris et al., 1984; Tuovinen et al., 1996b,c). The antidotes were all injected in volumes of approximately 0.17 ml of saline. Sarin was given in a volume of 0.5–1.0 ml olive oil. The combination of phosphotriesterase–physostigmine was also studied. The control animals received i.v. saline and/or i.g. olive oil.
2.4. Acute toxicity testing

The acute toxicity testing was done by the up-and-down method (Bruce, 1985, 1987). According to this, sarin was i.g. administered as a single dose for one animal, pretreated or not with antidotes. Depending on the lethality of the dose intervals during the next 24 h, the next animal was given a higher or lower dose. When the dose level was found to be near the LD$_{50}$, the testing was continued until 3–5 animals died at the upper dose level (100% mortality), and the same number of animals survived (no lethality) at the lower dose. The LD$_{50}$ was calculated as the mean of the dose with 100% mortality and the highest dose with 0% mortality.

The protection ratios for antidotes were calculated as the ratio of the LD$_{50}$ in pretreated mice to the LD$_{50}$ in untreated mice.

2.5. Sampling

Twenty-four hour after sarin exposure, the surviving mice were sacrificed by carbon dioxide, and blood samples (approximately 1.0 ml) were drawn by cardiac puncture into heparinized tubes. Samples (25 μl) of heparinized blood were diluted with 20 volumes of 0.1% Triton in 50 mM sodium phosphate buffer (Augustinsson et al., 1978). After that brain, lung, and gastrocnemius muscle were quickly dissected, and immediately frozen on dry ice. Plasma was separated with a hematocrit centrifuge at 1000 x g for 10 min at 4°C. All samples were kept at −75°C until analysed.

2.6. Enzyme assays

ChE activities were measured spectrophotometrically at 410 nm at 37°C by the method of Ellman et al. (1961) using acetylthiocholine iodide as the substrate for the brain, lung, muscle and butyrylthiocholine iodide for the plasma. Whole brain samples were homogenized with a Heidolph homogenizer in 10 volumes of ice-cold 50 mM sodium phosphate buffer, pH 7.7. The brain homogenates were further diluted with 4 volumes of 0.2% Triton in the same buffer and solubilized for 1 min. Then the homogenates were centrifuged at 1500 x g for 30 min at 4°C. AChE activity in the blood was measured using acetylthiocholine iodide as the substrate according to the method of Augustinsson et al. (1978). AChE activity was measured after the inhibition of BChE with a specific inhibitor, iso-OMPA (0.23 mM). The blood samples were preincubated with iso-OMPA for 2 min before addition of the substrate. Paraoxon hydrolyzing activity was measured from plasma samples using paraoxon as the substrate. In this assay, the enzyme activity was measured in 0.0125 M borate buffer (pH 7.5) containing 50 μM cobalt (Tuovinen et al., 1996c). The activity was spectrophotometrically monitored at 405 nm at 37°C.

2.7. Protein

Protein content of the samples was determined by the dye-binding method of Bradford (1976).

2.8. Statistical analysis

The data are presented as means ± S.D. Statistical significance between groups was determined using one-way analysis of variance followed by Fisher’s post hoc test. The P-value 0.05 or less was used as the level of statistical significance.

3. Results and discussion

The given amount of phosphotriesterase (approximately 2240 U/mouse) increased the paraoxon hydrolyzing activity in mouse plasma by up to 2-fold (320 ± 98 versus 163 ± 38 nmol/min/ml) compared to the control level, measured 24 h after the administration of the enzyme.

The effects of different antidotes against sarin toxicity are summarized in Tables 1 and 2. The LD$_{50}$ value of i.g. administered sarin in mice was approximately 1.4 mg/kg (Table 1). According to Inns et al. (1990), the LD$_{50}$ value of subcutaneously-administered sarin in mice was 0.172 mg/kg. Presumably, the difference between these values may be due to the administration route of sarin. In this study, phosphotriesterase was the best antidote against the sarin toxicity, with the
protective ratio of 3.4. Concerning the carbamates, physostigmine and pyridostigmine slightly decreased sarin toxicity with the protective ratios of 1.5 and 1.2–1.3, respectively. In contrast, eptastigmine did not protect against sarin intoxication. The LD_{50} values were quite similar at 30 min and 24 h post exposure, indicating that the lethality occurs during the first few minutes. However, the phosphotriesterase–physostigmine combination was an exception at a sarin dose level of 6.0 mg/kg. Generally, phosphotriesterase and physostigmine given alone or together decreased the occurrence of serious signs of poisoning such as tremors/convulsions and respiratory failure (data not shown). With the phosphotriesterase–physostigmine combination, the LD_{50} value of sarin was increased to 6.0 mg/kg; the value was 4.3-fold compared to the unpretreated animals (Table 1.), and their survival time being 2–3 h but in controls only 20 min (Table 2.). In spite of this, however, all the phosphotriesterase–physostigmine treated animals died over a period of 24 h (Tables 1 and 2.), and asphyxiation was the cause of death. This could be due to the short elimination half-life of physostigmine (and/or phosphotriesterase). Presumably physostigmine had prevented the irreversible inhibition of an essential amount of ChE by sarin during the first hours. Gordon et al. (1978) has also reported, that in soman poisoning the duration of action of the protective effect of physostigmine was 2–4 hours in guinea pigs.

The ChE activity profiles with phosphotriesterase, physostigmine, pyridostigmine, and eptastigmine treatments after sarin exposure are shown in Fig. 1. After the sarin dose of 0.75 mg/kg, the brain and lung ChE activities were inhibited by 50% without pretreatments. Only phosphotriesterase and physostigmine protected the brain AChE activity within 24 h. In phosphotriesterase- and physostigmine-treated mice, a 4- and 2-fold higher sarin dose was needed to cause 50% inhibition of brain AChE activity (Fig. 1A). In phosphotriesterase-treated mice approximately 50% of the lung AChE activity was inhibited at the 1.5-fold sarin dose level, whereas in physostigmine-treated animals the activities were only inhibited by 40% (Fig. 1B). A 3-fold (approximately) sarin dose was needed in phosphotriesterase-treated mice before the lung BChE activity reduced to 50% of the control (Fig. 1C). Phosphotriesterase also protected the peripheral muscle AChE activities (Fig. 1D). Only in pyridostigmine-treated animals was the AChE activity in the peripheral muscles inhibited by 50%. In circulation, the inhibition effect of sarin was focused on the erythrocyte AChE (Fig. 1E and F).

### Table 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LD_{50} at 30 min (protective ratio^{a})</th>
<th>LD_{50} at 24 h (protective ratio^{b})</th>
</tr>
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<tbody>
<tr>
<td>No pretreatment</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.2</td>
</tr>
<tr>
<td>PTE + Phy</td>
<td>&gt; 6.0 ± 0.0 (&gt; 4.29)</td>
<td>-</td>
</tr>
<tr>
<td>PTE</td>
<td>4.7 ± 0.2 (3.36)</td>
<td>4.4 ± 0.1 (3.38)</td>
</tr>
<tr>
<td>Phy</td>
<td>2.1 ± 0.0 (1.50)</td>
<td>2.0 ± 0.2 (1.54)</td>
</tr>
<tr>
<td>Pyr</td>
<td>1.7 ± 0.2 (1.21)</td>
<td>1.7 ± 0.2 (1.31)</td>
</tr>
<tr>
<td>Epta</td>
<td>1.5 ± 0.0 (1.07)</td>
<td>1.3 ± 0.2 (1.00)</td>
</tr>
</tbody>
</table>

^{a} Eptastigmine (Epta; 0.90 mg/kg body weight), physostigmine (Phy; 0.09 mg/kg body weight), pyridostigmine (Pyr; 0.06 mg/kg body weight) and phosphotriesterase (PTE; approximately 104 U/g body weight), or a combination of phosphotriesterase–physostigmine were administered i.v. 10 min before i.g. administration of sarin. All phosphotriesterase–physostigmine administered animals died within 24 h.

^{b} Protective ratio = LD_{50} with pretreatment/LD_{50} without pretreatment.

### Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival time (min)^{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarin (1.49 mg/kg)</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Epta + Sarin (1.49 mg/kg)</td>
<td>22 ± 12</td>
</tr>
<tr>
<td>Pyr + Sarin (1.87 mg/kg)</td>
<td>36 ± 17</td>
</tr>
<tr>
<td>Phy + Sarin (2.21 mg/kg)</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>PTE + Sarin (4.52 mg/kg)</td>
<td>51 ± 44</td>
</tr>
<tr>
<td>PTE + Phy + Sarin (5.70 mg/kg)</td>
<td>177 ± 4*</td>
</tr>
<tr>
<td>PTE + Phy + Sarin (6.00 mg/kg)</td>
<td>120 ± 48*</td>
</tr>
</tbody>
</table>

^{a} Eptastigmine (Epta; 0.90 mg/kg body weight), physostigmine (Phy; 0.09 mg/kg body weight), pyridostigmine (Pyr; 0.06 mg/kg body weight) and phosphotriesterase (PTE; approximately 104 U/g body weight), or a combination of phosphotriesterase–physostigmine were injected i.v. 10 min before i.g. administration of sarin.

^{b} Means ± S.D. are given.

* P < 0.05 compared to the sarin-treated mice.
Fig. 1. The effect of eptastigmine (Epta; 0.90 mg/kg body weight), physostigmine (Phy; 0.09 mg/kg body weight), pyridostigmine (Pyr; 0.06 mg/kg body weight) and phosphotriesterase (PTE; approximately 104 U/g body weight) on brain AChE (A), lung AChE (B), lung BChE (C), muscle AChE (D), blood AChE (E), and plasma BChE (F) activities in mice 24 h after the sarin exposure. The antidotes were injected i.v. 10 min prior to the i.g. administration of sarin. All phosphotriesterase–physostigmine administered animals died within 24 h. Control activities and means ± S.D. are given, n = 3–5; a, P < 0.05 compared with the control; b, P < 0.05 compared with the sarin-treated mice; c, P < 0.05 compared with the epta-sarin-treated mice; d, P < 0.05 compared with the phy-sarin-treated mice; e, P < 0.05 compared with the pyr-sarin-treated mice; and f, P < 0.05 compared with the PTE-sarin-treated mice.
Phosphotriesterase has been shown to hydrolyze sarin with $k_{\text{cat}} = 56/s$ (Dumas et al., 1990), and the elimination half-life of phosphotriesterase is approximately 4–5 h (Tuovinen et al., 1994, 1996a). Due to these facts repeated phosphotriesterase administrations might have offered better
protection against sarin intoxication. However, in this study with one phosphotriesterase treatment, the animals survived several hours after a single injection. In addition, the present results were obtained without atropine. The efficacy of carbamates is greatly amplified by atropine (Gordon et al., 1978). The atropine treatment would have improved the protective effect of carbamates, because sarin depresses the respiratory centres in the central nervous system. According to Deshpande et al. (1986), the combination of physostigmine–atropine was effective against lethal doses of sarin. Also the combination of pyridostigmine–atropine protected animals against tabun and soman poisoning and gave marginal protection against sarin or VX (Gordon et al., 1978).

Carbamates compete with OPs for the binding sites of ChE. They did not, however, additively potentiate the ChE inactivating effect of OPs. Both physostigmine and pyridostigmine reversibly inhibit ChE but not CaE activity (Junge and Krisch, 1975). The antidotal action of carbamates as irreversible inhibitors is attributed to their ability either to prevent irreversible phosphorylation of ChE by OPs (Green, 1983; Lennox et al., 1985), or to temporarily block the ion channels associated with nicotinic cholinergic receptors or glutamatergic receptors in the neuromuscular junction (Karlsson et al., 1984; Aracava et al., 1987; Kawabuchi et al., 1988). In this study, physostigmine as a short acting carbamate was the most effective carbamate as an antidote against sarin intoxication. Physostigmine also delayed the appearance of serious signs of poisoning such as convulsions and tremors which take place prior to permanent damage of nervous system. The protective effect of physostigmine may be due to the ability of the carbamate to penetrate the blood–brain barrier and cause reversible inhibition of brain AChE. In this study, physostigmine protected the brain AChE activity, and this would explain the milder signs of poisoning in mice treated with physostigmine. One explanation for the decreased signs of intoxication of physostigmine could also be the direct or secondary effects of carbamates on the nicotinic acetylcholine receptor and on the glutamatergic neuromuscular junction (Albuquerque et al. 1985; Aracava et al. 1987). Physostigmine is also known to reduce the lesions of the end-plates in muscle in sarin exposure (Kawabuchi et al., 1985).

Additional protection by pyridostigmine was quite poor in sarin exposure. This may be due to the fact that pyridostigmine can not cross the blood–brain barrier and has only peripheral action. Pyridostigmine has been observed to delay the inhibition of AChE, but the diaphragm, extensor digitorum longus and soleus muscles displayed different sensitivities to pyridostigmine (Lintern et al., 1997). Pyridostigmine was chosen for this study because it is known to be used presently in pretreatment in the armed forces. The duration of action of pyridostigmine is also known to be longer (approximately 4 h) than physostigmine (Gordon et al., 1978). These results suggest that there are other factors than the temporary inhibition of the brain AChE determining the protective effect of carbamates in protection against OP poisoning. In vivo, factors such as the rate of absorption, distribution, metabolism, and excretion of OPs must be considered.

In the present study, eptastigmine did not give any protection against sarin. However, eptastigmine, due to its long lasting effect, might be a more effective carbamate than physostigmine against potent OPs such as soman, because the permanent phosphorylation of soman-ChE complex is very rapid, occurring within a few minutes (Dunn and Sidell, 1989). In previous studies, phosphotriesterase–eptastigmine treatment protected the ChE activities in the plasma and in the lung (Tuovinen et al., 1996b) and also in the brain (Tuovinen et al., 1996c) during DFP exposure. In addition, our previous results showed that eptastigmine causes only minimal changes in the motor activity of mice, whereas the physostigmine treatment clearly decreased the activity of the animals (Tuovinen et al., 1996b). The decreased behavioral activity did not clearly correlate with the inhibition of AChE in the brain or in other tissues. In all tissues the dose of eptastigmine which caused only minor behavioral changes induced greater inhibition of the enzyme activities than physostigmine. Hence, the decreased behavioral activity of mice was not due to the AChE inhibition.
In conclusion, phosphotriesterase and physostigmine were the most effective antidotes against sarin intoxication. The LD$_{50}$ value for sarin was increased by 3.4- and 1.5-fold in mice receiving phosphotriesterase and physostigmine, respectively. With sarin exposure, physostigmine also decreased the signs of poisoning. Eptastigmine did not provide any protection against sarin toxicity.

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References


