Subchronic Physostigmine Pretreatment in Marmosets: Absence of Side Effects and Effectiveness against Soman Poisoning with Negligible Postintoxication Incapacitation

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Subchronic pretreatment with physostigmine (PHY) (0.0125 mg/kg/h) leading to a blood acetylcholinesterase inhibition of about 30% caused no side effects when applied to marmoset monkeys. This was evident on behavioral parameters and on EEG and cortical visual evoked response. Furthermore, this treatment regime, followed by atropine as postintoxication therapy, protected the marmosets against lethality after a 2 × LD50 dose of soman with negligible postintoxication incapacitation. These findings suggest that a symptom-free pretreatment with subchronic PHY could protect man sufficiently against severe soman intoxication.

Key Words: organophosphate; physostigmine; soman; AChE; ACh; marmoset monkeys

Nerve agents of the organophosphorus type inhibit irreversibly the enzyme acetylcholinesterase (AChE). This inhibition leads to accumulation of acetylcholine (ACh), resulting in central and peripheral cholinergic effects. Protection against organophosphate (OP) intoxication may be achieved by an effective treatment regime. Pretreatment with a carbamate that protects the AChE from attack by the OP compound, followed by a therapy with an anticholinergic drug, seems to be most effective against the OP soman (1,2,2-trimethylpropyl methylphosphonofluoridate) in different animal species (Berry and Davies, 1970; Dirnhuber et al., 1979; Gordon et al., 1978, Heyl et al., 1980; Inns and Leadbeater, 1983). The generally accepted treatment upon acute OP intoxication is to apply several autoinjectors containing an oxime, atropine, and diazepam. The current concept of pretreatment is the oral administration of pyridostigmine (PYR). A major drawback of the quaternary carbamate PYR is that it is unable to penetrate the central nervous system (CNS) to any significant extent and therefore cannot protect the brain against the intoxicating effects of OPs. Based on the lack of penetration of PYR in the brain, experimental animals take many hours to recover from an OP intoxication (Inns and Leadbeater, 1983). Therefore, it is essential that a combination of pretreatment and treatment should prevent, or at least markedly reduce, nerve agent-induced decrements in human performance. For this reason physostigmine (PHY) has been proposed as an alternative for PYR (Leadbeater et al., 1985). This compound possesses a tertiary nitrogen atom, penetrates the brain, and has been shown to be effective against OP intoxication. A significant protection against lethality after sarin or soman intoxication was reported (Leadbeater et al., 1985). However, a pretreatment not only must be effective against lethality and postintoxication incapacitation, but also be devoid of side effects, especially when given for a long period of time. Because PHY has a short plasma half-life and a narrow therapeutic index (Somani and Khalique, 1986), a bolus injection of PHY is not a very realistic pretreatment procedure. A more chronic application should be considered and evaluated. In a previous study, the presence of side effects after subchronically administered PHY were determined in guinea pigs (Philippens et al., 1998). Subchronic application by osmotic minipumps of PHY caused no behavioral or neurophysiologic side effects and was effective against a 3× LD50 dose of soman. In order to provide a firmer basis for this pretreatment in man, a similar type of study as in the guinea pig was undertaken in the marmoset, a nonhuman primate. The duration of the subchronic administration in this study, as well as in the previous studies, was maximal 11–12 days. Behavioral and neurophysiologic test methods were used to determine the side effects of PHY and postintoxication incapacitating effects after soman poisoning with 2 × LD50.

MATERIALS AND METHODS

Animals
Adul t marmoset monkeys (Callithrix jacchus) of both sexes bred and raised at the Biomedical Primate Research Centre (BPRC), Rijswijk, The Netherlands, were used. The animals were housed separately in cages (61 × 61 × 41 cm) in a room kept at 23–25°C and at a relative humidity > 60%. In this room a 12-h day and night cycle was maintained. Daily they were fed with rice, peanuts, fruit, boiled egg, baby biscuit, sunflower seeds, honey, broad bean, Karvan cevitam1, and pellet chow after training or testing. Water was available ad libitum. The experiments described here received prior approval by an independent ethical committee.

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Drug Solutions and Implantation of Osmotic Minipumps

PHY (eserine) was obtained from Sigma (St.Louis, MO); atropine sulphate was obtained from ACF (Amsterdam, The Netherlands); soman (O-picolinyl methylphosphonofluoridate) was synthesized at the Prins Maurits Laboratory TNO (Dr H.P. Benschop). Alzet® Osmotic Minipumps with a constant delivery rate of 0.5 μl/hr (Model 2002, Alza Corp., Palo Alto, CA) were used to deliver PHY solved in a vehicle. The osmotic minipump was implanted subcutaneously on the back between the shoulders of the animal under ketamine anaesthesia (20 mg/kg/animal). The wounds were sutured with silk. The dose of PHY was used 0.0125 mg/kg/hr. In a pilot study this dose offered a therapeutically relevant AChE inhibition (0.0025 mg/kg/hr: 1.2 ± 0.46 %, n = 5; 0.0075 mg/kg/hr: 20.3 ± 6.2; n = 2; 0.0125 mg/kg/hr: 34.4 ± 0.68 %, n = 3). The PHY concentration was based on the body weight of the animals before implantation. The vehicle consisted of 20% propylene glycol, 10% ethanol, and 70% water (1 part glacial acetic acid in 2000 parts distilled water).

Study Design

The study described herein was performed in two different treatment groups of animals. One group received subchronic pretreatment with PHY (0.0125 mg/kg/h for 12 days) before soman exposure (2 × LD50) intoxication (n = 6; monkeys CJ, GW, DP, GT, GU, and GV). The other group served as a control group not receiving PHY pretreatment (n = 3; monkeys FM, DJ, and FH). Both groups received atropine sulphate (5 mg/kg im) therapy 1 min after soman intoxication. The LD50 dose of soman (applied subcutaneously) used was 9 mg/kg (Dirnhuber et al., 1979). The appearance of side effects after PHY pretreatment and the protection of this pretreatment against lethality and postintoxication incapacitation after soman poisoning were tested.

Training of the animals in the hand-eye coordination task started 5 weeks before the start of this study. Electrodes for the measurement of EEG and visual evoked response (VER) were fitted 4 days before implantation of the Alzet® minipumps. In order to obtain control values all the parameters (blood AChE activity, body weight and temperature, hand-eye coordination, binaural performance, startle reflex, EEG, and VER) were registered/determined before implantation of the Alzet® minipumps. The hand-eye coordination was performed in only five monkeys. (Monkey GW did not reach the critical performance of the animal.)

The day Alzet® minipumps were implanted was called day 0. Three days after implantation, behavioral and neurophysiologic tests were started; these were repeated at days 6 and 10. Body weight and temperature were measured and blood samples were collected at days 3, 5, 7, 10, and 12. At day 12, the animals were intoxicated with 2 × LD50 soman (sc) 30 min after removal of the pumps, followed by atropine sulphate therapy. Twenty minutes after removal of the Alzet® minipump, all animals had a blood sample drawn for the determination of blood AChE. After soman intoxication, the animals were observed for symptoms such as ataxia, salivation, tremors, and convulsions. One hour after soman poisoning, the body temperature was registered, and a blood sample was taken to measure the AChE inhibition. After the intoxication symptoms had disappeared, the same behavioral and neurophysiologic tests were carried out. After the first two monkeys were intoxicated with soman, it appeared that they were in such good condition that we decided to perform the behavioral and neurophysiologic tests 1 h after intoxication in the remaining four animals. The tests were repeated at days 13, 17, and 19. In the animals given soman but not PHY, the clinical symptoms of intoxication became progressively worse until the animals died. These animals could not be tested in any of the tests.

Behavioral Tests in Marmosets

Hand-eye coordination test. The hand-eye coordination was performed with an automated robot-guided apparatus according to Wolthuis et al. (1995) using positive reinforcement (small pieces of marshmallow) as a motivating stimulus. A robot is situated behind a test panel with two windows (8 cm wide and 5 cm high). These windows can open and close through a pneumatically driven and vertically sliding door. For the hand-eye coordination task, only the left window is used. Situated in front of the test panel is the test cage (32.5 × 24 × 24 cm) in which the marmoset is placed. The side of the test cage directly in front of the panel consists of horizontal stainless steel rods, spaced far enough apart to allow the animal to reach its arm at full length through the window. The robot holds an 8.5 cm long suction tube. For each trial, the robot turns to a plateau containing the rewards, sucks one reward onto the tip of the tube, then moves it into the starting position behind the test panel. The presence of the reward at the tube is checked by a pressure detector that also registers the time needed for removal during the trial. A photocell-monitored trough on the inner side of the test panel registers the rewards that are not properly retrieved by the animal through the window into the test cage. Infrared detectors within the windows allow the registration of successful attempts of the animal to grasp a reward. With this system, three types of trials are performed: one using a nonmoving reward in the middle of the window, one using a slow horizontally moving reward (0.04 m/s), and one using a fast horizontally moving (0.08 m/s) reward from the left to the right side of the window. The animal is allowed 1 min to grasp the nonmoving reward. Each type of trial is performed 14 times in one session. At the beginning of each trial, a sound signal intended to alert the animal is presented. Immediately thereafter the window opens. At that point, the suction tube is in the ready position in the nonmoving trials and starts in the moving trials to move to the other position. After a hit is registered when the animal successfully retrieves the reward from the suction tube. The number of attempts and failures are also registered. The percentage of correct hits is used as criterion to judge the performance of the animal.

Bungalow test. The bungalow test is an automated test system that allows the registration of the activity/exploration of the marmoset. This equipment consists of four equal compartments 23 × 23 × 23 cm connected with each other by six photocell-guarded PVC tubes. The animals can freely move and change from one compartment to the other during a 20-min session (for details, see Wolthuis et al., 1994). The motor activity is expressed as the number of compartment changes in this time period.

Auditory startle response. In this test, the stretching movement of the legs is used to reflect the reaction of the animal to a startle signal (Davis et al., 1982). Animals are exposed to 20 auditory startle pulses (120 dB, pink noise, 20 ms) while standing on a platform in a PVC tube (diameter, 17.5 cm; length, 26 cm). The startle response of 200-ms duration is measured by a transducer connected with the platform, registering the force exerted by the animal upon presentation of the stimulus. The AD converter of an IBM-compatible PC digitizes the responses. The amplitude and latency of the startle response are registered and used to measure the motor force of the startle reflex.

Neurophysiologic Measurements

Under ketamine anaesthesia, a silver electrode is placed using stereotactic techniques into a small hole in the skull above the visual area (3 mm lateral to the sutura sagitialis and 5 mm caudal from intra-aural), leaving the dura mater intact. A reference electrode is placed over the sinus. Both electrodes are connected with a plug and fixed on the skull with dental cement. During the test a transmitter is connected to the plug for telemetric registration of the EEG and VER. All EEG signals are amplified (50,000 ×), filtered (between 0–30 Hz for EEG and 0–500 Hz for VER), and fed into the AD converter of an IBM-compatible PC. Sampling frequency is 50 Hz for EEG and 1 kHz for VER.

EEG registration. Fast Fourier transformation (FFT) to obtain power spectra is performed from five randomly chosen EEG epochs of 12 s out of a total recording time of 3 min. The obtained power spectra are subdivided into eight frequency classes (0.8–2; 2–3.5; 3.5–5.5; 5.5–7.5; 7.5–10; 10–12.5; 12.5–18; and 18–25 Hz). The total power (Vf) of the different frequency classes is used for the evaluation of the brain activity.

VER registration. For registration of the VER, the animals receive 30 light stimuli with a time interval of 2 sec ± 20%. Following the stimuli, EEGs
are registered for 250 ms and the responses averaged. For evaluation of effects, the latencies and amplitudes of the positive (P1, P2, P3) and negative (N1, N2, N3) peaks are measured and compared with the baseline values.

**Determination of AChE Activity**

The sole of the foot of the marmoset was punctured using an Autoclix lancet (Boehringer, Mannheim, Germany). Blood samples (5 µl) were taken and immediately mixed with 50 µl 1% saponin (BDH, Poole, UK), frozen in liquid nitrogen, and stored at −70°C. After appropriate dilutions, AChE activity was assessed using a radiometric method (Johnson and Russell, 1975). The ACh end concentration used was 12 mM, [3 H]ACh iodide (NEN, Dreieich, Germany) was diluted to a specific activity of 602 MBq mmol⁻¹. Electric eel AChE was used as reference.

**Statistics**

An analysis of variance (ANOVA) followed by a Newman-Keuls post hoc test was used to assess statistical significance in all the behavioral test systems used and a paired T-test in the neurophysiologic test systems. For the symptomatology after soman intoxication a Fisher exact probability test was used. In all tests, p values < 0.05 were considered significant.

**RESULTS**

In this study, three aspects of subchronic PHY pretreatment were studied: a) the side effects during subchronic administration, b) the protection against lethality induced by 2 × LD50 soman, and c) the protection against postintoxication incapacitation after intoxication by 2 × LD50 soman.

**Side Effects during Subchronic Administration of PHY**

**Body weight and temperature after PHY.** Body weight was not affected by the subchronic administration of PHY. During the pretreatment period of subchronic PHY, a small, not significant decrease was observed in rectal temperature from 39.3 to 38.7°C. These rectal temperature changes are within the normal daytime range for such monkeys, which varies between 38.5°C and 40.0°C.

**Blood AChE inhibition after PHY.** The mean blood AChE inhibition of the subchronic PHY-treated animals (n = 6), measured as a percentage of their control value before osmotic pump implantation, at days 3, 5, 7, 10, and 12 after osmotic pump implantation, were: 35.4 ± 5.3%, 36.4 ± 4.5%, 26.8 ± 4.1%, 20.9 ± 4.8%, and 30.7 ± 7.5%, respectively.

**Side effects on behavioral parameters.** In Figure 1, the side effects of 10 days subchronic PHY administration on the behavioral parameters are shown. In the hand-eye coordination performance, a small but not significant decrease was found in the total number of hits during the pretreatment period. (An ANOVA analysis showed p > 0.05 in all the sessions.) This decrease was caused mainly by monkeys DP and CJ. DP had a high control performance of 40 hits in 42 trials. After pump implantation, DP scored 31, 29, and 18 hits, respectively.

The exploration/activity of the animals, measured by the number of compartment changes in the bungalow test, was also not affected by subchronic PHY administration. This was due mainly to the variation of exploration between the animals; some animals showed a decrease and some an increase in their activity.

In the startle reflex test, neither the amplitude of the response (Fig. 1) nor the latencies were affected by subchronic PHY administration compared with the control values. (At all test points, an ANOVA analysis showed p > 0.05 for all the parameters).

**Side effects on neurophysiologic parameters.** The total band power (V²) in the different frequency classes of the subchronic...
PHY-treated animals showed no difference compared with their control value (not shown). The VER consists of three positive and three negative peaks. The amplitude and the latency of each peak were measured. The data were averaged and compared with the control value (Fig. 2). The latencies were not affected in the animals receiving PHY compared with their control value. (At all registration points, a paired T-test analysis showed \( p > 0.05 \) for all the different parameters in the EEG and the latencies in the VER.) Only the amplitude of peak P1 at day 3 after osmotic pump implantation significantly decreased (\( p = 0.03 \)). At days 6 and 10, this effect was not found to be significant (baseline value: 108.3 \( \pm \) 38.2; day 3: –45.3 \( \pm \) 40.7; day 6: 27.8 \( \pm \) 63.7; day 10: 25.0 \( \pm \) 88.6).

Protection against Lethality Induced by \( 2 \times LD_{50} \) Soman

Body weight and temperature after PHY. After osmotic pump removal and 1 h after soman intoxication and atropine therapy (day 12), rectal temperature did not further decrease (38.6°C \( \pm \) 0.21; \( n = 6 \)), whereas in an animal that received only atropine therapy after soman intoxication, rectal temperature decreased further (32.6°C; \( n = 1 \)). Body weight was not affected by soman intoxication measured 24 h after the intoxication.

Blood AChE inhibition after PHY. The mean blood AChE inhibition of the PHY-treated animals, measured at day 12 after osmotic pump removal and just before soman intoxication, was 28.3 \( \pm \) 3.1 % (\( n = 6 \)), and 1 h after soman intoxication was 90.5 \( \pm \) 1.8 % (\( n = 6 \)) of the control value. This was in contrast to the unpretreated control animals, whose AChE levels were inhibited to 97.9 \( \pm \) 2.1 % (\( n = 3 \)). Shortly thereafter, these animals died.

Protective efficacy of PHY pretreatment against \( 2 \times LD_{50} \) dose of soman. All animals pretreated with subchronic PHY and treated with atropine against \( 2 \times LD_{50} \) soman (\( n = 6 \)) survived the intoxication, whereas all the animals treated only with atropine died after intoxication. The monkeys (FM, DJ, and FH) died at 2, 1.5, and 1 h after soman intoxication. The difference in survival was significant (Fisher exact probability test, \( p < 0.05 \), two-tailed).

Protection against Postintoxication Incapacitation after Intoxication by \( 2 \times LD_{50} \) Soman

Post intoxication incapacitation symptoms after \( 2 \times LD_{50} \) soman. The animals pretreated with subchronic PHY and treated with atropine against \( 2 \times LD_{50} \) soman (\( n = 6 \)) appeared to be in good condition. They showed only mild tremors and some ataxia, lasting from 10 min after intoxication until about 30 min after intoxication. During this time, the animals stayed alert and very active. Monkey GU (male) even showed sexual activity towards GV (female) that started 45 min after soman poisoning. This interest was mutual. All animals largely recovered about 1 h after intoxication and were judged to be able to perform behavioral tests. Two hours after soman they were completely free of apparent symptoms and started eating.

The animals of the control group (\( n = 3 \)), treated only with atropine, were in much worse condition compared with those pretreated with subchronic PHY. All animals showed severe tremors and convulsions lasting for periods of 10 to about 20 min., followed by a period of muscle fasciculations. The first symptoms started within 10 min after intoxication. During the convulsive activity, the animals started to suffer from dyspnea that lasted until they died. Shortly after the convulsions, all the animals became comatose. The clinical symptoms of the animals in this group were significantly worse compared with those pretreated with subchronic PHY (Fisher exact probability test, \( p < 0.05 \), two-tailed).

Postintoxication incapacitation effects on behavioral measurements. The postintoxication incapacitation effects on behavioral parameters after soman intoxication are shown in Figure 3. One and a half hours after soman intoxication, the animals performed differently in the hand-eye coordination task. Two monkeys, both male, showed very good performance (GT: 31 and GU: 38. Their control values were 36 and 38, respectively). In contrast, the female monkey performed very poorly in this task (DP: 13 and GV: 0). In the test cage of DP, three marshmallow rewards the animal had obviously spit out were found after the test. It could be that the motivation was decreased by queasiness, but after the tests all the animals started to eat their normal food. One day later, monkey GV was recovered completely in this task (performance score of 40), whereas monkey DP was still performing poorly (score of 7). The other animals maintained their performance well. Five days after soman intoxication (day 17), monkey DP was back to normal performance (score of 37).

One hour after soman, the animals were tested in the bungalo test; no effect on activity was observed. The mean number of compartment changes was 48.8 \( \pm \) 13.3 (\( n = 4 \)).
Twenty four hours after soman (day 13), the mean number of compartment changes was not significantly decreased compared with 1 h after soman intoxication (35 ± 3.7; n = 4; GT, GU, GV, DP) and also not when compared with the baseline value (ANOVA analysis p > 0.05).

In the startle reflex test, a not significant increase of the startle response amplitude was observed 1 h after soman intoxication. Twenty-four hours later (day 13), this tendency had disappeared (2.5 ± 0.3; n = 6).

Postintoxication incapacitation effects on neurophysiological measurements. Two hours after soman intoxication, the EEG and VER were registered. Total power of the different frequency classes from the EEG is summarized in Table 1. In all animals, a shift of the power towards the higher frequencies after soman intoxication was noted. This remained during the study and was significant only in the first frequency band (0.8–2.0 Hz) at 24 h (day 13) and 1 week (day 19) after soman intoxication.

The amplitude and the latency of each peak were measured, averaged, and compared with the control values. For all test points, no effect was found on the latency of the VER peaks after 2 × LD50 soman intoxication in PHY-pretreated animals compared with their control (at all registration points p > 0.05). The amplitudes of the VER peaks were also unaffected; only the amplitude of peak P3 at 24 h after soman intoxication was significantly decreased compared with the control value (p = 0.04) (Table 2). Thereafter, this effect disappeared.

DISCUSSION

In this study, the side effects, protective efficacy against soman 2 × LD50-induced lethality, and the postsoman intoxication incapacitation after subchronic pretreatment with PHY were investigated in marmosets.

After a subchronic pretreatment with PHY (0.0125 mg/kg/hr) for 12 days at a therapeutic dose, resulting in a blood AChE inhibition of about 30%, no behavioral or neurophysiologic side effects were observed in the tests performed. In an earlier study in guinea pigs, following almost the same protocol as in the present study, we demonstrated that subchronic PHY caused no behavioral and neurophysiologic side effects (Philippens et al., 1998). In contrast, in a previous investigation it was shown that a single dose of PHY (0.6 mg/kg), leading to a comparable AChE inhibition, caused unacceptable side effects in this animal species (Philippens et al., 1996). This was also confirmed in the marmoset (Wolthuis et al., 1995). A single dose of PHY (0.02 mg/kg im) that caused an inhibition of the blood AChE activity > 20% resulted in a significantly high performance decrement in the hand-eye coordination test. Obviously, the plasma concentration of the PHY after a bolus injection will be higher when compared with a subchronic infusion and may be responsible for the observed side effects. Another explanation for the lack of side effects after subchronic PHY administration could be the well-known phenomenon of development of tolerance for AChE-inhibitors (Costa et al., 1982; Lim et al., 1987; Van Dongen and Wolthuis, 1989; Wolthuis et al., 1990). Taken together, this study and a previous one in guinea pigs indicate that subchronic administration of PHY is the route of choice to prevent the development of unwanted side effects.

The results presented herein also demonstrate that a subchronic pretreatment with PHY efficiently protects marmoset monkeys from soman 2 × LD50 induced lethality and limits...
severe postintoxication incapacitation symptoms. The efficacy against soman-induced lethality in this study is similar to that found in a previous study in guinea pigs (Philippines et al., 1998). This finding is in accordance with other studies; guinea pigs continuously treated with PHY via osmotic minipumps were protected against soman-induced toxicity (Lim et al., 1988). In contrast, these investigators did not observe this protection after a single-dose PHY pretreatment. The lack of convulsions and the relative good clinical condition of the marmosets after soman intoxication in this study suggest that protection after subchronic PHY pretreatment in the marmoset is much more effective than in the guinea pig. This discrepancy can be attributed to subtle species differences in the AChE molecule. This may result in different reactivation kinetics of AChE after reversible binding of PHY to AChE. The fact that pretreatment with a carbamate combined with postintoxication atropine therapy effectively protects against soman poisoning has already been reported for different animal species. However, there is a marked species variation in the efficacy of this treatment regime (Berry and Davies, 1970; Gordon et al., 1978). Obviously nonhuman primates are very well protected in this way. This could indicate that man would also benefit greatly from such a treatment regime.

Based on the results presented herein, one is inclined to conclude that subchronic pretreatment with PHY for 12 days (together with postintoxication atropine therapy) offers even better protection against 2\(\times\)LD50 soman intoxication than therapy with an oxime (combined with atropine). This observation is based on previous findings; marmoset monkeys intoxicated with 2\(\times\)LD50 soman followed 1 min later by a therapy of HI-6 and atropine sulphate (0.5 mg/kg im) showed much worse postintoxication symptoms than reported here (Busker et al., 1996). In that study, the animals remained unresponsive to their environment for at least 1 day and were not able to move, eat, or drink by themselves for 3 to 4 days. In one animal it took 18 days before she started to eat by

**TABLE 1**

Effects on EEG of 2\(\times\)LD50 Soman Intoxication Followed by Atropine Therapy in PHY Pretreated Animals

<table>
<thead>
<tr>
<th>Test day</th>
<th>Power (V(^2) ± SE) of the different frequency classes (Hz)</th>
<th>0.8–2</th>
<th>2–3.5</th>
<th>3.5–5.5</th>
<th>5.5–7.5</th>
<th>7.5–10</th>
<th>10–12.5</th>
<th>12.5–18</th>
<th>18–25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>307.7 (54.0)</td>
<td>242.8 (58.0)</td>
<td>225.2 (52.7)</td>
<td>195.2 (71.8)</td>
<td>197.0 (88.8)</td>
<td>158.7 (40.0)</td>
<td>247.5 (88.2)</td>
<td>242.0 (75.7)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>159.7 (35.8)</td>
<td>202.5 (66.3)</td>
<td>159.7 (24.4)</td>
<td>110.8 (17.5)</td>
<td>121.5 (16.2)</td>
<td>152.2 (17.8)</td>
<td>361.8 (38.3)</td>
<td>298.0 (19.4)</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>*143.3 (32.3)</td>
<td>130.8 (15.5)</td>
<td>124.7 (11.2)</td>
<td>101.3 (17.3)</td>
<td>104.5 (19.1)</td>
<td>178.8 (22.9)</td>
<td>354.8 (16.2)</td>
<td>370.2 (29.8)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>150.0 (51.7)</td>
<td>147.5 (21.7)</td>
<td>165.7 (23.2)</td>
<td>107.0 (19.1)</td>
<td>124.3 (15.6)</td>
<td>182.3 (25.8)</td>
<td>380.0 (12.7)</td>
<td>337.7 (42.7)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>*98.2 (16.3)</td>
<td>123.3 (23.1)</td>
<td>124.0 (18.5)</td>
<td>93.8 (11.4)</td>
<td>127.8 (25.4)</td>
<td>138.8 (21.1)</td>
<td>399.8 (0.3)</td>
<td>351.3 (34.3)</td>
<td></td>
</tr>
</tbody>
</table>

Note. At day 12 the EEG was registered 2 hours after soman intoxication. Effects expressed as the mean (± SEM) (\(n = 4\)) band power (V\(^2\)) of the different frequency classes measured before the start of the study (Day 0) and on Day 12, 13, 17, and 19.

* Significantly different from control value (\(p < 0.05\)).

**TABLE 2**

Effects on VER of 2\(\times\)LD50 Soman Intoxication Followed by Atropine Therapy in PHY Pretreated Animals

<table>
<thead>
<tr>
<th>Day</th>
<th>P1 (mV ± SE)</th>
<th>N1 (mV ± SE)</th>
<th>P2 (mV ± SE)</th>
<th>N2 (mV ± SE)</th>
<th>P3 (mV ± SE)</th>
<th>N3 (mV ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>108 (38)</td>
<td>−413 (58)</td>
<td>380 (22)</td>
<td>241 (85)</td>
<td>415 (66)</td>
<td>−169 (120)</td>
</tr>
<tr>
<td>12</td>
<td>20 (113)</td>
<td>−405 (96)</td>
<td>344 (169)</td>
<td>97 (170)</td>
<td>358 (144)</td>
<td>−248 (114)</td>
</tr>
<tr>
<td>13</td>
<td>63 (45)</td>
<td>−442 (130)</td>
<td>245 (110)</td>
<td>69 (150)</td>
<td>*100 (97)</td>
<td>−427 (75)</td>
</tr>
<tr>
<td>17</td>
<td>173 (113)</td>
<td>−415 (135)</td>
<td>288 (130)</td>
<td>101 (84)</td>
<td>238 (136)</td>
<td>−296 (71)</td>
</tr>
<tr>
<td>19</td>
<td>108 (119)</td>
<td>−387 (174)</td>
<td>407 (230)</td>
<td>151 (125)</td>
<td>262 (125)</td>
<td>−213 (55)</td>
</tr>
</tbody>
</table>

Note. At day 12 the VER was registered 2 hours after soman intoxication. Values are mean (±SEM) amplitudes (\(n = 4\)) of the positive (P1, P2, P3) and negative (N1, N2, N3) peaks of the VER measured before the start of the study (Day 0) and after 2\(\times\)LD50 soman intoxication followed by a atropine therapy in PHY pre-treated animals (Days 12, 13, 17, 19).

* Significantly different from control value (\(p < 0.05\)).
herself. A possible explanation for the lower efficacy of oxime therapy against soman poisoning in marmosets could be the aging phenomenon of the soman-AChE complex. Soman-inhibited AChE of marmosets and man “ages” with a $t_{1/2}$ of 1–1.5 min (Talbot et al., 1988). This means only a small amount of soman-inhibited AChE can be reactivated after an acute intoxication, as AChE is already “aged”. Therefore, therapy with an oxime may be less effective than good pretreatment with PHY.

Busker et al. (1996) also reported a difference in response between male and female marmoset monkeys. The females needed more time to recover from soman intoxication completely. In this study we also noticed a sex difference. After soman intoxication, both female monkeys showed a very strong performance decrement in the hand-eye coordination task, whereas the performance in the male monkeys was not affected. Therefore, it seems that female marmosets are more sensitive to soman intoxication than males (Sket, 1993).

Thus, subchronic PHY pretreatment in combination with atropine therapy seems to be a very effective protection against 2 × LD50 soman intoxication. Whether this is also valid for other OPs has to be further elucidated. However, based on literature data (Harris et al., 1991) one should expect this to be the case.

With the combination of subchronic PHY and atropine sulphate therapy, not only was survival improved, but postintoxication incapacitation was hardly present. One hour after soman intoxication, the animals were already able to perform the behavioral tests. The male monkeys even showed a normal hand-eye coordination. Furthermore, all the monkeys were very alert and showed a normal pattern of activity. This was also expressed in the bungalow task. Performance in motor activity was normal, with a tendency toward increase of the motor activity. This increase may be the result of stimulation of ACh receptors by increased amounts of ACh in the synaptic cleft. At test time, the AChE inhibition was 90.5% and a nonsignificant increase of the amplitude of the startle response was observed. This effect cannot be due to AChE inhibition and ACh accumulation. It has been reported that most anti-ChE drugs failed to increase the startle reflex (Davis et al., 1982). Presumably, direct effects on ACh receptors are involved in this effect (Philippens et al., 1997). In the EEG power spectrum, a shift from lower toward higher frequencies was found and is also explained by the stimulation of ACh receptors. Delta activity may be associated with behavioral impairment (Vanderwolf, 1971). A decrease in this slow wave activity suggests an increase of the animal’s activity. This is in accordance with our findings. In a previous study with rats, an increase in delta activity was found 1 h after 0.5 × LD50 soman intoxication (Wolthuis et al., 1991). Furthermore, 24 h after soman, a high degree of EEG synchronization was observed around 8 Hz. In this study, no effect was found at this frequency. This can also be explained by the activity of the animals at that time. Frequencies around 8 Hz represent “walking” behavior in rats. In the study of Wolthuis et al. (1991), the rats were forced to walk on a wheel while the EEG was recorded.

In this study, the animals were not forced to any active behavior. The absence of the increase of the delta activity suggests that PHY pretreatment counteract the effects of soman on the EEG. The increasing tendency of the high frequencies is hard to explain. One week after soman intoxication, this effect was still present. This suggests that a permanent change had occurred in the brain. The impact of this change in EEG activity on long-term neurologic effects needs further investigation. On the other hand, no effects of physiologic importance on the VER were found. This finding is in accordance with other studies (DeBruyn et al., 1991). DeBruyn and colleagues reported that PHY pretreatment combined with a treatment of mecamylamine (a nicotine receptor antagonist) and atropine reduced the effect of soman on the VER in the cat.

In conclusion, subchronic treatment with PHY seems to be a good alternative for current PYR pretreatment. In particular, this pretreatment shows no side effects in the tests used, protects more efficiently against 2 × LD50 soman intoxication, and thereby leads to less postintoxication incapacitation effects in marmoset monkeys. Although this study strongly favors the use of subchronic PHY pretreatment as an effective protection against soman intoxication, several points need consideration. When this pretreatment is applied to man, a continuous stable plasma level of PHY should be realized. For practical everyday use, a route of administration other than chronic infusion via osmotic minipumps should be developed. This can be realized only by equipment similar to the osmotic pump. In this respect, transdermal application has to be considered. Irregularities in the plasma level may result in less effective protection. Furthermore, during the first stage of subchronic PHY pretreatment when a certain plasma level must be reached, protection may be less than adequate. Effective plasma levels can be reached by an initiation dose of PHY at the start of the pretreatment period. In that case, combination with an anticholinergic drug such as scopolamine is recommended. This allows a quicker achievement of a symptom-free protective plasma level of PHY. In our opinion, one should try to reach a steady-state plasma level within 48 h. In that case, one should further investigate how well one is protected during that first 48 h of PHY application. This will be the purpose of future investigations.

REFERENCES


