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Retention of Soman in Rats, Guinea-pigs and Marmosets: Species-dependent Effects of the Soman Simulator, Pinacolyl Dimethylphosphinate (PDP)

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Abstract—Whether the temporary retention of intact soman in the rat and its subsequent delivery from tissues into the circulation of the blood is also demonstrable in guinea-pigs and marmosets has been investigated as was whether the soman simulator PDP (pinacolyl dimethylphosphinate) prevented this retention. Electric eel AChE, intravenously injected 1·5 h after an intravenous soman intoxication into anaesthetized, atropinized and artificially ventilated guinea-pigs (150 μg kg^{-1} soman), marmoset monkeys (100 μg kg^{-1} soman) and rats (330 and 172·5 μg kg^{-1} soman) lost its activity faster than enzyme injected in non-intoxicated animals. Electric eel AChE incubated in the presence of pectoralis or diaphragm muscle isolated from soman-intoxicated rats, guinea-pigs and marmosets 0·5 or 1·5 h after the intoxication, was progressively inhibited, indicating that those muscles still delivered soman into the incubation medium. In rats, PDP (6·4 mg kg^{-1} i.v.) pretreatment was effective in preventing inhibition of intravenously injected electric eel AChE 1·5 h after intoxication with a high dose of soman (330 μg kg^{-1}). But after intoxication with a low dose (172·5 μg kg^{-1}), PDP pretreatment was ineffective in this action, however, it did lead to less soman delivery from muscle tissue isolated 30 min following the 172·5 μg kg^{-1} soman intoxication, suggesting that there was less soman in the tissue. In PDP (6·4 mg kg^{-1} i.v.)-pretreated marmosets (100 μg kg^{-1} soman) and guinea-pigs (150 μg kg^{-1} soman), to the contrary, the trend was for the injected AChE to be more inhibited, whereas only slightly less soman was delivered from isolated muscle tissue. It is suggested that after PDP treatment to rats the elimination of soman from the blood circulation is faster than in marmosets and guinea-pigs.

In contrast to previous assumptions, it has appeared that after intoxication with soman, this agent is not rapidly eliminated or rendered harmless. Evidence is accumulating that a portion of the soman temporarily resides and remains protected from degradation in tissues. From these tissues the agent is gradually released into the blood circulation thereby causing the reappearance of signs of intoxication and death. Wolthuis et al (1981a, b) reported that upon intoxication of rats with 6 × LD50 soman and subsequent treatment with atropine and HI-6, this therapy was only initially successful. The condition of the animals deteriorated gradually in the following hours after HI-6 treatment and many of them died with symptoms of organophosphate poisoning. The possibility that soman temporarily resides in the body tissues and can slowly be released, has been discussed by several investigators (Sterri et al 1980; Benschop et al 1981; Clement 1982; Van Helden et al 1984a; Nordgren et al 1984; Reynolds et al 1985). Retention of soman in rats and subsequent re-intoxication could partly be prevented by prophylaxis with a non-toxic so-called soman simulator in which the fluorine atom of soman was replaced by an ethoxy group (Wolthuis et al 1981a, b; Benschop et al 1981). With diaphragm preparations from soman-intoxicated rats treated with atropine and HI-6, the retention of soman could be demonstrated in striated muscle (Van Helden & Wolthuis 1983; Van Dongen et al 1986) and lung tissue (Van Dongen & De Lange 1987) and this storage could be manipulated by simulator activity. From structure-activity studies testing a number of soman simulators it appeared that retention of soman in muscle tissue could best be prevented by those structures resembling soman (Van Helden et al 1984b); i.e. the more the structure resembled soman the higher its efficacy. PDP (pinacolyl dimethylphosphinate) appeared to be most active in this respect, as well as in preventing death (Van Helden et al 1986a). In recent work it has been shown that PDP is active even if given several hours before or just after the soman intoxication (Van Helden et al 1986b) and may completely relieve post-intoxication disturbances of motor coordination (Wolthuis et al 1986).

In the present study, we investigated whether the temporary retention and release of soman could be shown in the guinea-pig and the marmoset, and if so whether PDP prevented this in these species.

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Methods and Results

Animals

Male guinea-pigs (300–500 g) used were of a Cpb-GpHi65 strain obtained from the Central Animal Breeding Centre TNO at Zeist. Male and female marmosets (250–400 g) were obtained from the Primate Centre TNO at Rijswijk. Male rats of the strain Small WAG/Rij (180–200 g) were bred in the Medical Biological Laboratory TNO under SPF conditions.

Chemicals

The soman simulator, pinacolyl dimethylphosphinate (PDP) was prepared by Dr H. P. Benschop from the Prins Maurits Laboratory TNO as described earlier (Van Helden

et al 1984b). Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) was obtained according to standard procedures. Soman and PDP were distilled until >98% purity. HI-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-4′-carbamoyl-pyridimium-1′-methylether dichloride monohydrate) was kindly made available by Dr P. A. Lockwood, Defence Research Establishment, Suffield, Canada. Atropine sulphate and hexobarbitone sodium were purchased from Brocades Stheeman, Haarlem, The Netherlands, and from Bayer, Leverkusen, Germany, respectively.

Effect of prophylactic and therapeutic PDP administration on electric eel AChE activity in soman-intoxicated rats

Three separate in-vivo experiments (A, B, C) were performed at intervals of several weeks. Anaesthetized (hexobarbitone, 175 mg kg^{-1} i.p.), atropinized (50 mg kg^{-1} i.p.) and cannulated (carotid artery and trachea) rats were used. The experiments varied only in the time that PDP was administered. Experiment A consisted of 3 treatment groups (each of n = 5) i.e. (1) only saline, (2) soman 4 × LD50 i.v. (330 μg kg^{-1}) and (3) PDP (6·4 mg kg^{-1} i.v.) followed 10 min later by 4 × LD50 soman. Experiment B consisted of 2 groups (each of n = 5) (1) soman; (2) PDP administered immediately after soman. Experiment C consisted of 2 groups (n = 5 each) (1) soman; (2) PDP given 91 min after soman. In all 3 experiments the animals were i.v. injected with 300 u electric eel AChE (Sigma) 90 min after soman intoxication. This enzyme was dissolved in 0·2 mL 0·05 M phosphate-buffered saline pH 7·4. The inhibition of this enzyme in the circulation of the blood was studied by taking 1 μL blood samples just before and at various times after AChE administration. AChE activity was determined radiometrically using [^{3}H]ACh (500 mCi mmol^{-1}, Amersham) as a substrate, essentially according to Potter (1967). Intravenous injections were given into the dorsal penis vein. PDP was dissolved in 5% propylene glycol in distilled water; atropine sulphate and soman in distilled water. Electric eel AChE injected 90 min following a 4 × LD50 soman intoxication, was almost completely inhibited within 15 min (Fig. 1, left graph). Pretreatment with PDP substantially prevented this inhibition; the values came much closer to those for the spontaneous decrease in enzyme activity in the circulation of untreated animals. PDP administration just after soman intoxication (middle graph, Fig. 1) also partially prevented the enzyme inhibition. From the right graph it appears that PDP injected one min after the AChE-injection, i.e. 91 min after the intoxication, led to an increased rate of inhibition. The enzyme activities measured at t = 1 min did not differ significantly in both groups. These results agree with previous work and show that if a high dose of soman is administered in rats, PDP may clearly affect the amount of soman in the blood stream.

To investigate whether PDP was still effective at lower levels of soman intoxication, experiments A and C were repeated using 172·5 μg kg^{-1} i.v. soman. Three groups of 4 rats each were included and treated, respectively, with: (1) saline (i.p.), followed after 5 min by soman; (2) PDP (6.4 mg kg^{-1} i.v.) instead of saline; (3) saline only. At 90 min post-intoxication, each animal was injected i.v. with electric eel AChE (300 u). Non-intoxicated rats showed an enzyme activity of 29·5 u mL^{-1} at t = 1 min (Fig. 2, right graph). That would be an activity of 30 u mL^{-1} at t = 0 (after extrapolation), giving the total blood volume of the animal as 10 mL. Corrected for spontaneous decline, the enzyme inhibition due to soman was 9·5 u mL^{-1} (32%) at t = 1 min and 4·5 u mL^{-1} (15%) at t = 60 min. The results indicate that at this low level of soman intoxication, PDP pretreatment did not result in a decreased amount of soman in the circulation of the blood.

Effect of PDP pretreatment in rats on the delivery of soman from isolated muscle tissue

Two groups of anaesthetized, atropinized and artificially ventilated rats were intoxicated with soman (172·5 μg kg^{-1}

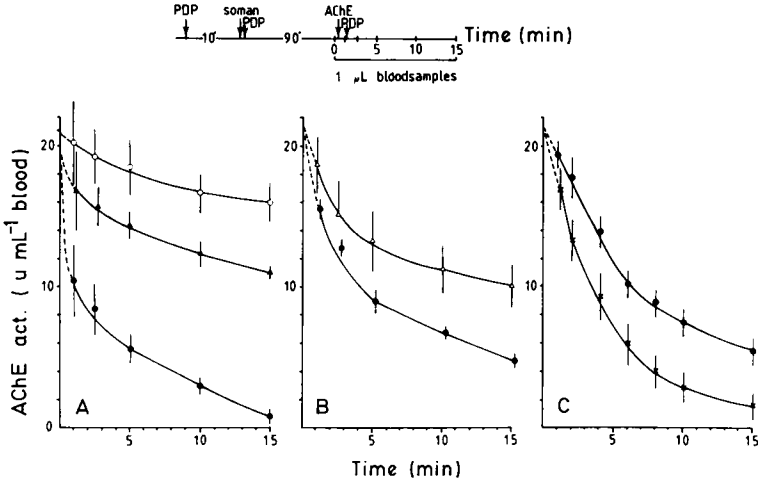

FIG. 1. Effect of prophylactic and therapeutic PDP (6·4 mg kg^{-1} i.v.) administration on electric eel AChE activity in soman-intoxicated (4 × LD50 i.v.) rats. A: saline (○); soman (●), PDP (▲) followed 10 min later by soman (n = 5). B: soman (●); PDP (△) administered immediately after soman (n = 5). C: soman (●); PDP(X) was given 91 min after soman (n = 5) (see schedule at the top of Fig.). Rats were i.v. injected with 300 u electric eel AChE 90 min after some intoxication. Bars indicate s.e.m.

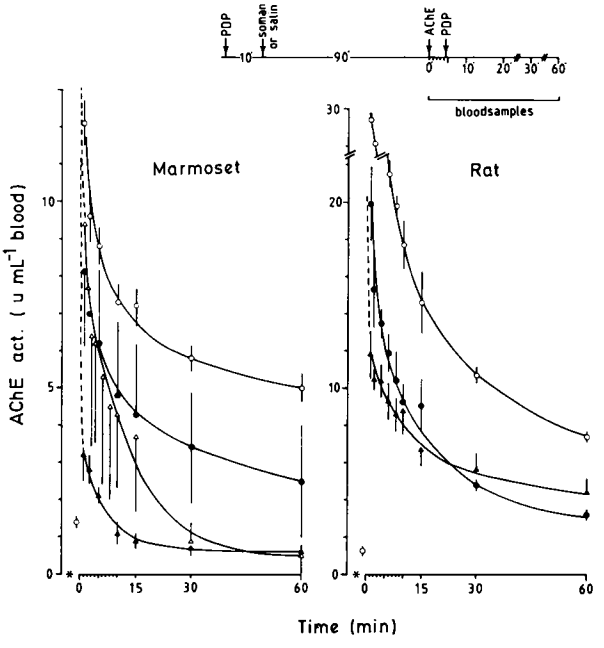

FIG. 2. AChE activity in blood samples taken from marmosets and rats. Anaesthetized, atropinized animals were i.v. injected with either saline (○, n = 4) or soman (100 μg kg^{-1} i.v. and 172·5 μg kg^{-1}, respectively). The first soman group received no further treatment (●), the second was treated with PDP (36 μmol kg^{-1} i.v.) 10 min before soman (▲), the third group (marmosets) was treated with a similar dose of PDP 94 min after soman (△). (See schedule at the top of the Fig.). Time 0 of the graph started 90 min after the administration of soman (or saline). * denotes endogenous blood ChE activity in soman intoxicated animals just before injection with enzyme. Bars indicate s.e.m. some of which have been omitted for clarity.

i.v.). One group was pretreated with PDP (6·4 mg kg^{-1} i.v.) 10 min before soman whereas the other group received saline (control group). At 30 and at 90 min post-intoxication, 4 PDP-treated and 4 control rats were killed, each diaphragm and 1 g of each pectoralis muscle were dissected and incubated separately in 1 mL of 0·05 M phosphate-buffered saline of pH 7·4 at 37 °C for 30 min. Following addition of electric eel AChE (30 mu) to the medium, 50 μL samples were collected at various times for determination of enzyme activity.

During a 30 min incubation in the presence of 1 g pectoralis muscle (not illustrated) isolated from soman-intoxicated rats 30 min following the intoxication, the AChE activity present in the medium, decreased by 80% (Fig. 3, left graph). If pretreated with PDP, the decline was only 35%. Muscle tissue derived from soman-intoxicated animals 90 min post-intoxication, caused only a 30% enzyme inhibition which was not influenced by PDP pre-treatment. PDP pretreatment clearly decreased the amount of soman leaking from muscle tissue isolated 30 min following the acute intoxication. Spontaneous decrease of enzyme activity in the presence of 1 g pectoralis muscle tissue from untreated rats amounted only to 15% during a 30 min incubation.

Effect of prophylactic and therapeutic PDP administration on electric eel AChE activity in soman-intoxicated guinea-pigs

After guinea-pigs were anaesthetized with ketamine (40 mg kg^{-1} i.m.), administered in combination with 2 mg per animal Vetranquil, carotid and tracheal cannulae were inserted. Five min before 6 × LD50 soman (150 μg kg^{-1} i.v.) or saline injection, they received 7 mg kg^{-1} i.v. atropine sulphate. The soman group was divided into groups of 5 animals each. The first received no further treatment, the second was prophylactically treated with PDP (6·4 mg kg^{-1} i.v.) 10 min before soman injection, the third was treated with a similar dose of PDP 94 min after soman. Ninety min following the soman or saline injection, all animals were i.v. injected with 600 u of electric eel AChE dissolved in 0·4 mL 0·05 M phosphate-buffered saline pH 7·4. 1 μL blood samples were then collected from the carotid cannula just before and 1, 2·5, 5, 10 and 15 min following the enzyme injection. Intravenous injections were given into the external jugular vein. Non-intoxicated guinea-pigs, receiving 600 u AChE intravenously, showed

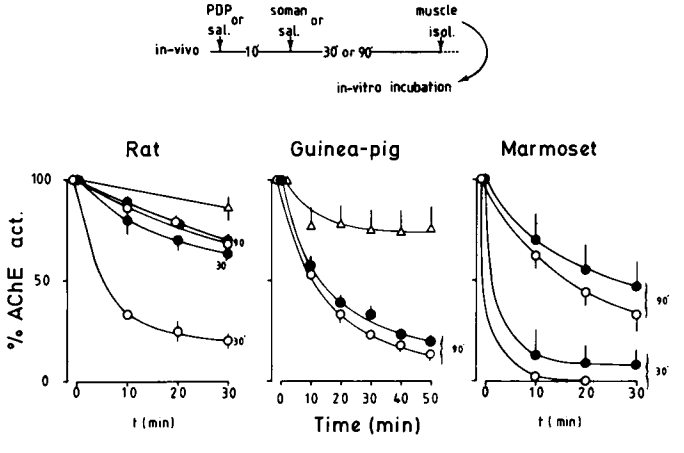

FIG. 3. Effect of PDP pretreatment on the delivery of soman from pectoralis muscle isolated from soman-intoxicated rats and marmosets and from diaphragm muscle derived from soman-intoxicated guinea-pigs. Rats, guinea-pigs and marmosets pretreated with PDP (6·4 mg kg^{-1} i.v., ●) or saline (○), were i.v. intoxicated with soman (1 × LD50 + 90 μg kg^{-1}, 1 × LD50 + 125 μg kg^{-1} and 1 × LD50 + 92 μg kg^{-1}, respectively). Spontaneous decline of enzyme activity (△) was measured in the presence of 1 g muscle tissue from untreated rats and guinea-pigs. The activity at t = 0 is taken as 100% and represents the activity measured just after adding the enzyme to the medium and just before adding the muscle tissue. Bars indicate s.e.m.

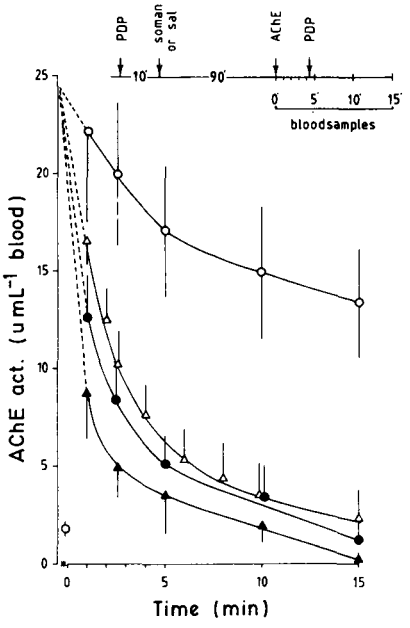

FIG. 4. AChE activity in blood samples taken from anaesthetized, atropinized guinea pigs and i.v. injected with either saline (○) or 6 × LD50 soman (150 μg kg^{-1}). The first (●) not further treated, (▲) treated with PDP (36 μmol kg^{-1} i.v.) 10 min before soman, (△) treated with a similar dose of PDP 94 min after soman. (See schedule at the top of Fig.) AChE (600 u) was given 90 min later. Time 0 of the graph started 90 min after the administration of soman (or saline). * denotes endogeneous blood ChE activity just before injection with AChE. Bars indicate s.e.m.

an enzyme activity of 22 u mL^{-1} in the first blood sample at t = 1 min (Fig. 4). Extrapolation (see interrupted line) led to an activity of about 25 u mL^{-1} at t = 0, indicating a total blood volume of about 24 mL. There was no difference in the course of enzyme inhibition between the three groups. Approximately a 50% decrease in AChE activity might be attributed to inhibition by soman, the rest was due to elimination of the enzyme by other processes. PDP, at the dosage used, is therefore not effective in influencing the amount of soman in the blood circulation.

Effect of PDP pretreatment in guinea-pigs on the delivery of soman from isolated diaphragms

Three groups of 3 guinea-pigs each were treated similarly as the first experiment 1 (i.e. only saline, only soman or PDP 10 min before soman), except that the animals were not injected with AChE, but were killed 90 min after the soman intoxication. Each diaphragm (≈1 g wet wt) was dissected and incubated immediately in 10 mL Krebs-Ringer bicarbonate buffer pH 7·4 at 37 °C was gassed with carbogen (95% O_2, 5% CO_2). Electric eel AChE (30 u) was added to the medium from which 10 μL samples were collected just before and after 10, 20, 30, 40 and 50 min. The activity of AChE in the presence of 1 g diaphragm muscle from saline-treated guinea-pigs declined approximately 25% during a 50 min incubation (Fig. 3, middle graph). In the presence of a diaphragm isolated from animals 90 min following a 6 × LD50 soman intoxication, the enzyme activity decreased about 85%. PDP pretreatment hardly decreased the amount of soman delivered from the muscle tisue.

The efficacy of PDP in preventing failure of neuromuscular transmission (NMT) in diaphragms isolated from soman-intoxicated guinea-pigs

Control guinea-pigs were anaesthetized with ketamine (40 mg kg^{-1} i.m.) and then atropinized (7 mg kg^{-1} i.v.) 5 min before 8 × LD50 soman (200 μg kg^{-1} i.v.). HI-6 (56·5 mg kg^{-1} i.v.) was injected immediately after soman (Fig. 5). Test animals received PDP (6·4 mg kg^{-1} i.v.) 10 min before soman and were otherwise treated identically. Control animals received saline. Groups of 3–6 animals were killed either 25 min, 2 h or 3·5 h following the intoxication. Diaphragm strips were dissected, mounted in-vitro in Krebs-Ringer solution and were tested for their ability to sustain tetanic contractions on indirect stimulation at 4 standard frequencies (Van Helden & Wolthuis 1983). This test was repeated at 10 min intervals; beginning at t = 0 about 10 min after an animal had been killed. NMT was expressed as a percentage of the NMT determined separately in untreated control preparations. Diaphragms isolated from soman-intoxicated and HI-6 treated guinea-pigs showed a NMT of 60–80% at t = 0 (at the start of in-vitro incubation) regardless whether the muscle was isolated at 25 min, 2 h or 3·5 h following the intoxication (Fig. 5). The inhibition of NMT in-vitro, was less at increasing time intervals between intoxication and muscle isolation. PDP-pretreatment hardly influenced the course of inhibition of NMT, except in muscles isolated at 3·5 h in which the NMT was even significantly more inhibited.

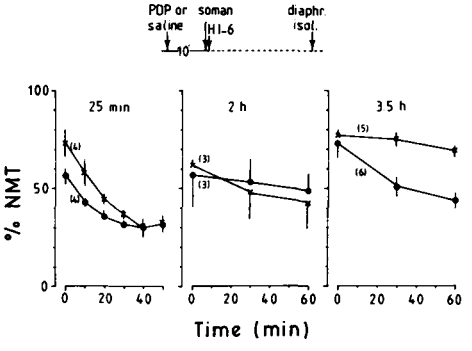

FIG. 5. Mean (±s.e.m.) neuromuscular transmission (NMT) in diaphragm preparations from soman intoxicated (200 μg kg^{-1} i.v.) guinea-pigs treated with atropine (20 mg kg^{-1} i.p.) and HI-6 (150 μmol kg^{-1} i.v.) pretreated with PDP (●) (36 μmol kg^{-1} i.v.) or saline (×). At 25 min 2 h or 3·5 h following the intoxication the diaphragms were isolated and incubated in vitro. The NMT was measured in vitro and expressed as a percentage of NMT determined separately in untreated control preparations. The schedule is shown at the top of the figure. The number of animals per group is shown in brackets. Bars indicate the s.e.m.

Efficacy of PDP in preventing delayed death in guinea-pigs following soman intoxication and HI-6 treatment

Control guinea-pigs were anaesthetized and atropinized as described, before receiving 8 or 10 × LD50 soman (i.v.). Similarly treated animals received PDP (6·4 mg kg^{-1} i.v.) 10 min before the soman. All animals were treated with HI-6 (56·5 mg kg^{-1} i.v.) immediately following soman, which made artificial respiration unnecessary. Survival

Table 1. Per cent of 24 h-survival and survival times of anaesthetized and atropinized (7 mg kg^{-1} i.v.) guinea-pigs pretreated with either PDP (6·4 mg kg^{-1}) or saline 10 min before intoxication with 8× or 10 × LD50 soman (i.v.). Immediately after soman, the animals were treated with HI-6 (56·5 mg kg^{-1} i.v.).

% 24 h-survival (n/group)				Survival times			
8 × LD50		10 × LD50		8 × LD50		10 × LD50	
PDP	sal.	PDP	sal.	PDP	sal.	PDP	sal.
75 (9/12)	64 (7/11)	58 (7/12)	50 (6/12)	n = 2 < 60′ n = 1 < 90′	n = 3 < 60′ n = 1 : 7 h	n = 2 : 6·5 h n = 3 < 30′	n = 1 : 10·6 h n = 5 < 30′

times were measured by keeping the animals one to a cage and recording breathing movements with an ultrasonic detection device. About 50% of guinea-pigs given 8 or 10 × LD50 soman and treated with HI-6 and atropine, survived (Table 1). In both cases, PDP pretreatment did not significantly increase the percentage survival. Survival times (when shorter than 24 h) did not differ in the groups.

Effect of prophylactic and therapeutic PDP administration on electric eel AChE activity in soman-intoxicated marmosets

Following anaesthesia with ketamine (100 mg kg^{-1} i.m.), injected in combination with Vetranquil (2 mg per animal), carotid and tracheal cannula were inserted. This experiment included 4 groups of 4 marmosets. The respective groups were treated with: (1) saline (0·9% NaCl solution, i.p.), followed after 5 min by atropine sulphate (10 mg kg^{-1} i.p.) and after another 5 min by 100 µg kg^{-1} i.v. soman; (2) PDP (6·4 mg kg^{-1} i.v.) instead of saline 10 min before soman and otherwise treated identically as the first group; (3) PDP (6·4 mg kg^{-1} i.v.) given 94 min after instead of before the soman intoxication (see schedule at the top of Fig. 2); (4) saline only. All animals were artificially ventilated. At 90 min post-soman (or after the second saline injection in group 4), each animal received an injection in the jugular vein containing 300 u electric eel AChE dissolved in 0·2 mL 0·05 M phosphate-buffered saline pH 7·4. For the determination of AChE activity in blood, 1 µL blood samples were collected from the carotid cannula just before and 1, 2·5, 5, 10, 15, 30 and 60 min after the enzyme injection. Non-intoxicated marmosets showed an enzyme activity of 12 u mL^{-1} at t = 1 min (Fig. 2, left graph). Those values would correspond (after extrapolation) to an activity of 13 u mL^{-1} at t = 0, indicating that the mean total blood volume of the animals was approximately 23 mL. The spontaneous decrease in enzyme activity was 1 u mL^{-1} (7·6%) at t = 1 min and 8 u mL^{-1} (62%) at t = 60 min. Corrected for this spontaneous decline, the enzyme activity in blood samples taken from soman animals at t = 1 min (i.e. 91 min following the intoxication) had decreased with 4 u mL^{-1} (31%), whereas in PDP-pretreated animals this decrease was 9 u mL^{-1} (69%). At t = 60 min these values were 2·5 u mL^{-1} (19%) and 4·5 u mL^{-1} (35%), respectively. In animals treated with PDP 94 min after soman, the decline in enzyme activity was also 35% at t = 60 min. The results demonstrate that, in marmosets, PDP, given either before or after the soman, tends to increase the blood level of soman.

Effect of PDP pretreatment in marmosets on the delivery of soman from isolated muscle tissue

Anaesthetized and atropinized marmosets received tracheal cannulae for artificial ventilation. Two groups of 7 animals each received soman (100 µg kg^{-1} i.v.). One group was pretreated with PDP (6·4 mg kg^{-1} i.v.) 10 min before soman, whereas the control group received saline. Thirty min post-intoxication, 3 PDP-treated and 3 control animals were killed. Ninety min post-intoxication, the remaining 4 PDP-treated and 4 control animals were killed. One gram of each pectoralis muscle and of each diaphragm were dissected and incubated separately in 1 mL of 0·05 M phosphate-buffered saline of pH 7·4 at 37 °C for 30 min. Electric eel AChE (30 mu) was added to the medium then 50 µL samples were collected just before and 10, 20 and 30 min after adding the muscle tissue. During the first 10 min of incubation in the presence of 1 g pectoralis or diaphragm (not shown) from marmosets 30 min after soman, the AChE activity added declined almost completely (Fig.3, right graph). In the presence of muscle tissue from PDP-pretreated animals, this enzyme was slightly less inhibited in 10 min. Incubation of tissue isolated 90 min after the soman without PDP pretreatment, caused a 70% inhibition of enzyme activity during a 30 min incubation, whereas in the case of PDP pretreatment, about 50% of the enzyme activity was inhibited. There was hardly any difference between pectoralis and diaphragm muscle in the amounts of soman leaking from the muscles. These results show that PDP pretreatment of the animals does not result in a decrease in the amount of soman leaking from muscle tissue isolated 30 or 90 min following the soman intoxication.

Statistics

Curves representing enzyme activity were submitted to a two-way repeated analysis of variance; the Newman-Keuls test was used for simultaneous comparison of the means, according to Winer (1971). The Welch test (Hald 1952) was used to compare the values for the degree of failure of NMT between control and experimental groups. The Fischer test (Finney 1948) was applied to compare survival between control and experimental groups. In the text the word significant indicates a difference with a reliability of 95%.

Discussion

The present study was undertaken to investigate whether (1) retention of soman could also be demonstrated in

species other than the rat and (2) whether the favourable effects of PDP, found earlier in the soman-intoxicated rat (Van Helden et al 1986a, b), are also present in the other species. If so this could lead to further evaluation of PDP for potential use in man.

With respect to the first question it is clear that retention of soman occurs also in the guinea-pig and the marmoset. For the three species the results show that even 90 min after the soman intoxication: (a) sufficient soman is circulating in the blood stream to cause a rapid inhibition of i.v. injected AChE, (b) sufficient soman may leak from the isolated muscle in-vitro to cause substantial inhibition of AChE present in the incubation medium.

The results on survival and delayed death (Table 1), as well as those on the decrease of NMT (Fig. 5) are supportive of the proposition that soman also persist in guinea-pigs (marmosets were not tested in a similar fashion), but to a lesser extent than in the present and previous experiments with rats.

Thus, it would appear that the persistence of soman is a general phenomenon. Also in man intoxicated with organophosphorus pesticides, relapses following initial successful treatment have been observed (Davies et al 1975; Ecobichon et al 1977; Dutoit et al 1981).

The small or absent effects of PDP in guinea-pigs and marmosets are harder to explain. The main difficulty is that in all experiments the dose of soman is expressed in terms of the LD50. However, the i.v. LD50 values in these species are widely divergent: for the marmoset this value is approximately 8 $\mu g\,kg^{-1}$*, for the guinea pig 25 $\mu g\,kg^{-1}$ and for the rat 82·5 $\mu g\,kg^{-1}$ (both determined in our laboratory). It was supposed that, at a 1 × LD50 dose of soman, most ChE and aliesterase activity would be inhibited. All overload above this dose could be stored in the body. In one of the present experiments with rats and marmosets we tried to give a similar overload of inhibitor; the rats received 172·5 $\mu g\,kg^{-1}$ i.e. 1 × LD50 + 90 $\mu g\,kg^{-1}$; the marmosets 100 $\mu g\,kg^{-1}$ i.e. 1 × LD50 + 92 $\mu g\,kg^{-1}$; in experiments with guinea-pigs, which were performed much earlier, a dose of 6 × LD50 was used, corresponding with a slightly higher overload: 1 × LD50 + 125 $\mu g\,kg^{-1}$. This still means that the marmoset was injected with at least 12·5 × LD50, the guinea-pig with 6 × LD50 and the rat with approximately 2 × LD50. As earlier preliminary results had suggested, PDP had practically no effect in rats intoxicated with 2 × LD50; the degree of inhibition of i.v. injected eel AChE did not differ when PDP was given (Fig. 2) and soman released from muscle tissue in PDP-treated animals was only decreased when tested 30 min after intoxication, but not after 90 min (Fig. 3). In contrast, clear effects of PDP on the amount of soman in the blood stream of rats could be seen (Fig. 1) when 4 × LD50 (330 $\mu g\,kg^{-1}$) instead of 2 × LD50 soman (172·5 $\mu g\,kg^{-1}$) was administered. These results suggest that, to be able to detect clear effects of PDP administration, the absolute dose of soman is more relevant than the dose in terms of LD50.

* To limit the number of marmosets, an LD50 value for soman was not determined. Dirnhuber et al (1979) reported a subcutaneous LD50 of 8 $\mu g\,kg^{-1}$. The i.v. dose given in the present experiment (100 $\mu g\,kg^{-1}$) certainly amounts to at least 12·5 × LD50 (see also Van Helden et al 1983).

An additional problem is that only one dose of PDP, i.e. 6·4 mg kg^{-1}, was used for all species. It seems unlikely that a higher dose would be more effective for the following reasons. In the first place a broad dose-range of PDP appears to be effective in the rat in preventing secondary recovery of NMT and death following HI-6 treatment. Secondly, the dose of PDP given to the rats in the present experiments represents 20 times the amount of soman on a molecular basis, in the guinea-pig 45 times and in the marmoset 72 times. Therefore increasing the dose of PDP to obtain effects seems an unlikely solution. At best it may be worthwhile to test two or three other soman simulators of a different chemical structure.

It was initially thought that the species differences could be explained by differences in the speed of elimination of soman once it had returned into the blood stream. If the disappearance of soman from rat blood was much faster than in the other two species, it was argued, this might explain the decrease of soman in rat blood after PDP in contrast to the increase in the other two species. However, this possibility is not likely since it does not explain why this does not occur after 2 × LD50 soman has been administered (see Fig. 2).

Summarizing, the results of the present experiments show that following an intoxication with soman this agent is retained for a period in the organism. This occurs not only in rats, but also in guinea-pigs and marmosets. The similarity of the results in these three species points to a phenomenon of a more general nature and it seems likely that such persistence may also occur in man, particularly since relapses following therapy in severe organophosphate-poisoned patients have been observed, e.g. parathion (Braeckman et al 1980; Eigenberg et al 1983).

The prospects for the potential use of PDP in humans are rather pessimistic. If the explanation for the species differences is that an effect of PDP can only be seen at very high levels of intoxication with soman, further research with this drug does not seem useful. If other explanations are found, it will depend on the type of explanation and the possible approaches whether these investigations will be continued.

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