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Prophylactic and therapeutic efficacy of the soman-simulator, pinacolyl dimethylphosphinate

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Atropinized rats, intoxicated with 6 or 8 × LD50 soman and subsequently treated with the oxime HI-6 died several hours after intoxication. Oral or intravenous administration of the soman-simulator, pinacolyl dimethylphosphinate (PDP), given at progressively increasing time intervals before soman, appeared to be very active in preventing death and secondary failure of neuromuscular transmission that followed HI-6-induced recovery. Therapeutically, PDP was only effective when given immediately after soman intoxication and oxime treatment. In animals that received no treatment otherwise, intravenous administration of PDP 10 min before intoxication with 1 × LD50 soman did not enhance lethality.

It was found earlier that rats intoxicated with 6 × LD50 soman and treated with HI-6 and atropine, showed an initial recovery that was followed by a progressive decrease of their condition. Death occurred 5–6 h after the intoxication (Wolthuis et al 1981a, b; Benschop et al 1981). These results suggested that soman was temporarily stored and protected against degradation somewhere in the body, from which it was subsequently gradually released. This hypothesis was verified in experiments showing that purified electric eel acetylcholinesterase (AChE) injected intravenously at time intervals between 75 min and 3 h after soman intoxication, was progressively inhibited (Wolthuis et al 1981b; Van Helden et al 1984a). Soman storage and, consequently, re-intoxication and death could be prevented by a prophylaxis with soman simulators, compounds that resemble soman in chemical structure but that do not inhibit AChE (Wolthuis et al 1981a; Benschop et al 1981; Van Helden et al 1984a, b, 1986). With in-vitro diaphragm preparations, obtained from soman-treated rats given atropine and HI-6, it was demonstrated that at least part of the soman storage took place in striated muscle and that this storage could be prevented by administration of an effective simulator (Van Helden & Wolthuis 1983). During the testing of a large number of simulators, it appeared that the storage phenomenon in muscles was a rather specific process (Van Helden et al 1984b). The soman simulator, pinacolyl dimethylphosphinate (PDP), obtained by replacing the fluoro atom of soman by a methyl group, appeared to be most effective in preventing soman storage in muscles and death (Van Helden et al 1986). The present paper is a more detailed analysis of the effects of this active simulator on survival and on neuromuscular transmission in soman-intoxicated rats. The simulator was administered either prophylactically or therapeutically in conjunction with HI-6 and atropine.

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MATERIALS AND METHODS

Animals

Small male Wistar (WAG/Rij) rats, 180–200 g, were bred in the Medical Biological Laboratory TNO under SPF conditions.

Survival

Experiment 1. Control rats were anaesthetized with hexobarbitone (175 mg kg^{-1} i.p.) and received atropine sulphate (50 mg kg^{-1} i.p.) 5 min before 6 × LD50 soman (2·7 μmol kg^{-1} i.v.) was injected. As indicated at the top of Fig. 1, HI-6 (150 μmol kg^{-1} i.v.) was injected immediately after soman, which made artificial respiration unnecessary during the first few hours after intoxication. Similarly, treated experimental rats received an injection of PDP (3 to 1126 μmol kg^{-1} i.v.), at time intervals between 10 min and 18 h before soman and were otherwise treated identically; the above-mentioned control rats received saline instead of PDP. PDP was dissolved in 5% propylene glycol in distilled water; atropine sulphate, soman and HI-6 were dissolved in distilled water. The survival time of the animals was measured by keeping them one to a cage and recording breathing movements with an ultrasonic detection device.

Experiment 2. Groups of rats were treated as described for the first experiment except that in this case PDP was administered orally at various dose levels 1 to 4 h before soman intoxication (see schedule at the top of Fig. 3).

Experiment 3. In this experiment saline or PDP was administered therapeutically i.e. immediately after or at several time intervals after soman (6 × LD50 i.v.) intoxication and subsequent HI-6 (150 μmol kg^{-1} i.v.) treatment (see Table 3).

Experiment 4. To investigate whether PDP will increase the toxicity of 1 × LD50 i.v. soman without further therapy, groups of anaesthetized rats received saline (control groups) or PDP (36 μmol kg^{-1} or 144 μmol kg^{-1} i.v.) 10 min before 1 × LD50 soman. Survival times of the animals were measured (see Table 4).

Neuromuscular transmission (NMT)

Experiment 5. The efficacy of PDP in preventing secondary failure of oxime-induced recovery of NMT was investigated. Control rats were anaesthetized and atropinized (as described) 5 min before 8 × LD50 soman (3·6 μmol kg^{-1} i.v.) was injected (Fig. 4). HI-6 (150 μmol kg^{-1} iv.) was injected immediately after soman. Experimental rats received PDP (12 or 3 μmol kg^{-1} i.v.) 10 min before soman and were otherwise treated identically; control rats received saline instead of PDP. All rats were killed 25 min after soman and HI-6 administration, and diaphragm strips were dissected, mounted in-vitro in Krebs-Ringer solution and tested for their ability to sustain tetanic contractions on indirect stimulation at 4 standard frequencies as described earlier (Wolthuis et al 1981c; Van Helden & Wolthuis 1983). This test for NMT was performed six times at 10 min intervals (Fig. 4). The first test at t = 0 started about 10 min after the animal had been killed. NMT was expressed as a percentage of the NMT determined separately in untreated control preparations.

Experiment 6. Groups of rats were treated as described in experiment 5, except that in this experiment PDP (36 μmol kg^{-1} i.v.) was injected 2 or 4 h before soman intoxication (8 × LD50 i.v.) (see top of Fig. 5). Both groups consisted of 5 rats.

Experiment 7. To investigate whether PDP will be effective in preventing secondary failure of HI-6-induced recovery of NMT when administered after soman poisoning and HI-6 treatment, two groups consisting of 5 rats each were treated according to the scheme at the top of Fig. 6. One group received PDP immediately after HI-6 administration (1 min after soman), the other group at 5 min after soman. Anaesthesia, atropinization, soman poisoning, oxime treatment and handling of the diaphragm strips were performed identically as described in experiment 5. The control group is the same as used in experiment 5.

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-pyridinium-1′-methylether dichloride monohydrate) was graciously made available by Dr. P. A. Lockwood, Defence Research Etablishment, Suffield, Canada. Atropine sulphate and hexobarbitone sodium were purchased from Brocades Stheeman, Haarlem, The Netherlands, and from Bayer, Leverkusen, Germany, respectively.

Statistics

Variance analysis was performed 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%, tested two-tailed.

RESULTS

Experiment 1. From the dose-response data in Fig. 1 it appears that the optimal dose of PDP, when injected 10 min before soman intoxication, is between 12 and 36 μmol kg^{-1} i.v. When this time interval was extended to 4 h the optimal dose was between 72 and 144 μmol kg^{-1}. Whereas a dose of 144 μmol kg^{-1}, given 7 h before soman was ineffective, doubling the dose caused 75% survival. Doubling the dose again at a prophylactic time interval of 18 h was hardly effective in raising survival (from 42 to 55%).

Control animals as well as non-survivors of PDP-treated groups died between approximately 5 and 10 h after soman intoxication except in 2 groups (Table 1). From Fig. 1, median effective doses (ED50: doses at which 50% of the animals survive) have been roughly estimated by interpolation (see legend to Fig. 2). There seems to be a non-linear relationship between log ED50 and prophylactic time interval; at progressive time intervals between

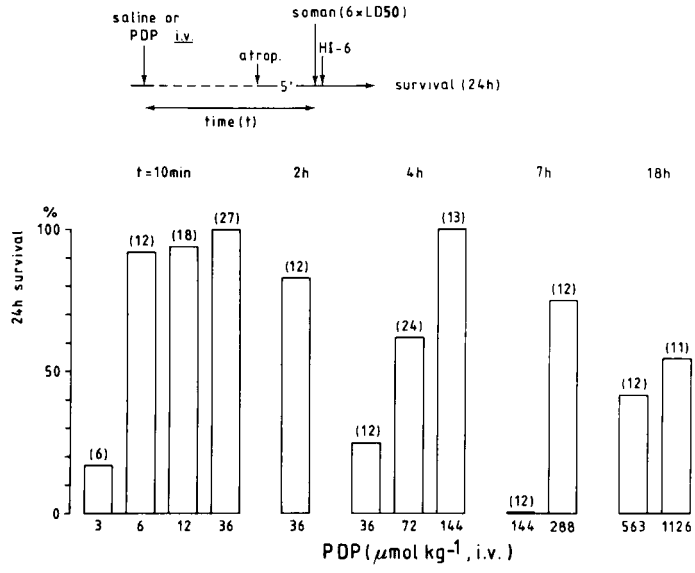

FIG. 1. Effects of a prophylaxis with PDP (i.v.) on 24 h survival of soman-intoxicated rats. All animals were anaesthetized, atropinized, soman-intoxicated and treated with HI-6 as described for experiment 1 (see schedule at the top). The time interval between PDP and soman administration varies from 10 min to 18 h along with the dose of PDP (3 to 1126 μmol kg^{-1} i.v.). The number of animals per group is shown in brackets at the top of each bar.

Table 1. Number and mean time to death of non-survivors after *intravenous* administration of PDP or saline from experiment 1.

Prophylactic time interval	PDP (μmol kg^{-1} i.v.)	Mean (±s.e.m.) time (h) to death of non-survivors PDP-treated (n/group)	Saline-treated (n/group)
10 min	3	7·8 ± 2·9 (5/6)	8·7 ± 1·4 (4/4)
	6	12·0 (1/12)	7·8 ± 1·4 (4/4)
	12	2·0 (1/18)	4·8 ± 1·6 (4/4)
	36	— —	5·6 ± 0·2 (8/8)
2 h	36	13·3 ± 4·0 (2/12)	6·8 ± 2·1 (3/3)
4 h	36	5·0 ± 1·7 (9/12)	2·0 ± 1·7 (4/4)
	72	1·5 ± 0·8 (9/24)	3·5 ± 1·3 (4/4)
	144	— —	10·0 ± 2·0 (4/4)
7 h	144	5·0 ± 0·5 (12/12)	5·6 ± 0·4 (4/4)
	288	6·4 ± 5·7 (3/12)	5·2 ± 1·0 (4/4)
18 h	563	10·6 ± 1·8 (7/12)	7·2 ± 0·4 (3/3)
	1126	7·0 ± 1·2 (5/12)	5·4 ± 1·1 (4/4)

10 min and about 4 h the ED50 increases more slowly than at more extended intervals.

Experiment 2. Survival after oral prophylaxis with 112 μmol kg^{-1} and 224 μmol kg^{-1} PDP 2 and 1 h before soman, respectively, was approximately the same (Fig. 3); at 3 h there was no difference in survival at 28 and 56 μmol kg^{-1}; at 112 μmol kg^{-1} it was poorer. Survival did not differ at a 4 h time interval at 25 and 112 μmol kg^{-1} PDP. Whereas the mean survival times in control groups (Table 2) were

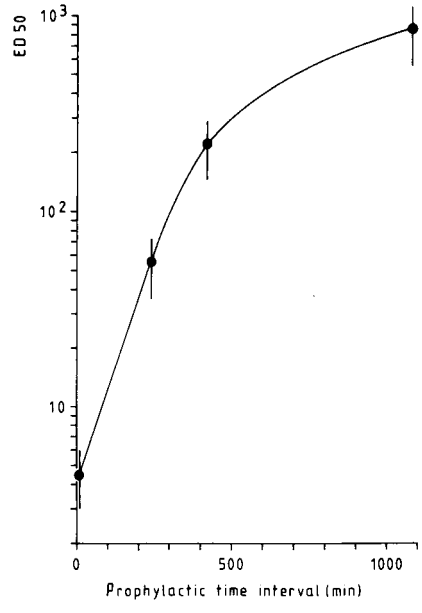

FIG. 2. The relation between prophylactic time intervals and median effect doses (ED50) of PDP roughly estimated by interpolation of the dose-response data of Fig. 1.

Prophylactic time int.	ED50 (μmol kg^{-1} i.v.) Between	Estimated
10 min	3– 6	4·3
4 h	36– 72	60
7 h	144– 288	240
18 h	563–1 126	920

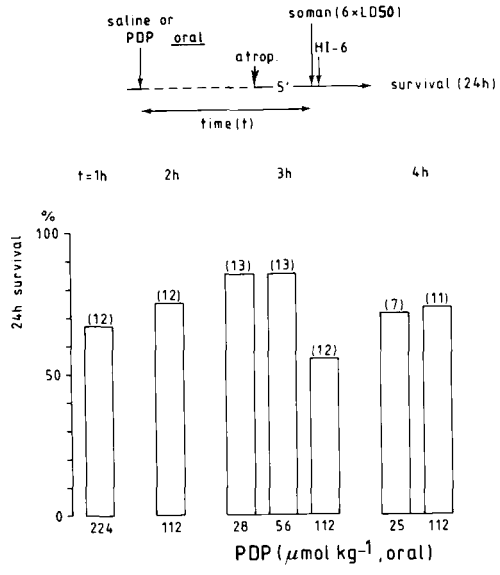

FIG. 3. Effects of an oral prophylaxis with PDP on survival time of soman-intoxicated rats. The experimental procedure is shown at the top of the Fig. The number of animals per group is represented at the top of each bar. Compare with Fig. 1.

Table 2. Number and mean time to death of non-survivors after *oral* administration of PDP or saline from experiment 2.

Prophylactic time interval (h)	PDP (μmol kg^{-1} oral)	Mean (±s.e.m.) time (h) to death of *non-survivors* PDP-treated (n/group)	Saline-treated (n/group)
1	224	2·1 ± 1·5 (4/12)	9·5 ± 1·6 (12/12)
2	112	6·1 ± 5·6 (3/12)	6·4 ± 3·7 (4/4)
3	28	5·3 ± 1·7 (2/13)	6·2 ± 0·8 (8/8)
3	56	0·6 ± 0·1 (2/13)	6·6 ± 1·1 (7/7)
3	112	0·5 ± 0·1 (5/12)	8·6 ± 1·6 (4/4)
4	25	10·2 ± 3·7 (2/7)	7·3 ± 2·3 (4/4)
4	112	2·5 ± 2·3 (3/11)	6·2 ± 0·6 (4/4)

Table 3. Effects of therapeutically administered PDP or saline (control) on survival time of soman-intoxicated rats treated with atropine and HI-6.

Time interval (min) between soman and PDP	PDP (μmol kg^{-1} i.v.)	No. of survivors/group at 24 h (%)	Mean (±s.e.m.) time (h) to death of non-survivors
1	36	15/20 (75)	7·4 ± 3·5
Control		1/25 (4)	6·4 ± 0·6
5	36	1/12 (8)	7·4 ± 1·1
Control		0/4 (0)	6·5 ± 0·7
5	144	0/23 (0)	5·0 ± 1·0
Control		0/4 (0)	6·5 ± 0·7
5	18	0/6 (0)	7·6 ± 2·0
Control		0/4 (0)	4 2 ± 0·5
15	36	0/9 (0)	8·9 ± 0·4
Control		0/3 (0)	11·2 ± 1·5
60	36	0/12 (0)	5·5 ± 0·2
Control		0/3 (0)	5·4 ± 0·5
60	144	0/12 (0)	4·9 ± 0·8
Control		0/3 (0)	2·3 ± 0·4

between 6 and 10 h, these were much shorter in the non-survivors of some PDP-treated groups.

Experiment 3. Upon therapeutical intravenous administration of PDP, i.e. 1 min after soman intoxication and HI-6 treatment, 75% of the animals survived whereas only 4% of control animals survived (Table 3). Later injection of PDP led to a sharp decrease in survival. A dose of 36 μmol kg^{-1} given 5 min after the intoxication caused 8% survival, whereas a higher dose (144 μmol) or a lower dose (18 μmol) was ineffective. In general the mean time to death of non-survivors did not differ much between PDP-treated and saline-treated groups (4–11 h).

Experiment 4. From Table 4 it appears that PDP did not increase the toxicity of 1 × LD50 soman; 54% of the rats treated with 36 μmol kg^{-1} PDP and 58% of the saline-treated animals survived. In the group treated with 144 μmol kg^{-1}, survival was the same as in the parallel running control group (67%).

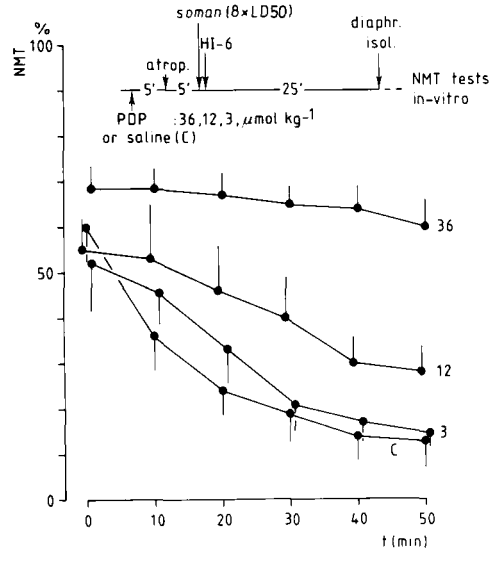

FIG. 4. Mean (±s.e.m.) neuromuscular transmission (NMT) in diaphragm preparations obtained from soman-intoxicated rats treated with atropine and HI-6 which were pretreated with PDP or saline (C). The NMT was measured in-vitro and expressed as a percentage of NMT determined separately in untreated control preparations. Each experimental group consisted of five animals, the control group of seven. The test schedule is shown at the top of the Fig. From each animal 2 diaphragm strips were tested and the results were averaged per animal. The dose of PDP was 36, 12 or 3 μmol kg^{-1} i.v.

Table 4. Per cent of survival and survival times of rats either treated with PDP or saline 10 min before 1 × LD50 soman intoxication. The animals received no atropine or HI-6 therapy.

PDP (μmol kg^{-1})	No. of survivors/group at 24 h (%)	Mean (± s.e.m.) time to death of non-survivors
36	13/24 (54)	n = 4: 2·7 ± 0·9 h n = 7: < 30 min
Saline	14/24 (58)	n = 3: 2·6 ± 1·1 h n = 7: < 30 min
144	14/21 (67)	n = 3: 4·3 ± 2·6 n = 4: <30 min
Saline	14/21 (67)	n = 2: 1·1 ± 0·3 n = 5: <30 min

Experiment 5. Fig. 4 shows that in diaphragm preparations from control animals, the HI-6 induced recovery of NMT, visible at the start of in-vitro testing, was followed by a gradual failure of NMT in the subsequent 50 min. Upon pretreatment with PDP (36 and 12 μmol kg^{-1}), this gradual failure of NMT could be significantly prevented. A dose of 3 μmol kg^{-1} was ineffective.

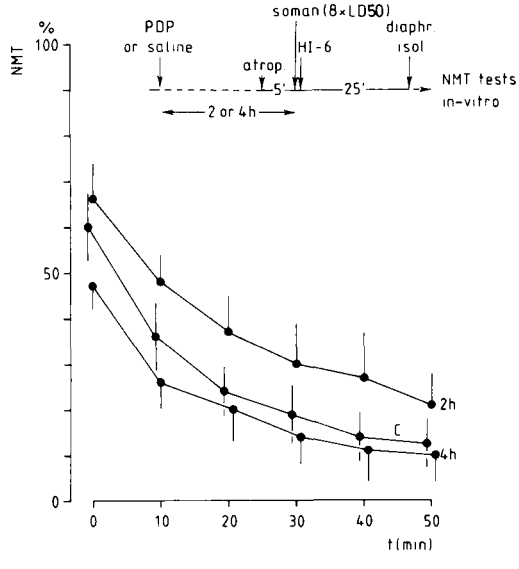

FIG. 5. Mean (±s.e.m.) neuromuscular transmission (NMT) in diaphragm preparations obtained from soman-intoxicated rats pretreated with PDP (36 μmol kg^{-1} i.v.) or saline (C) and treated with atropine and HI-6. The time interval between PDP injection and soman intoxication was 2 and 4 h as shown at the top of the Fig. The NMT was measured in-vitro and expressed as a percentage of the NMT determined separately in untreated control preparations. The control group (C) is the same as that in Fig. 4. Each experimental group consisted of 5 rats. From each animal 2 diaphragm strips were tested at 10 min intervals.

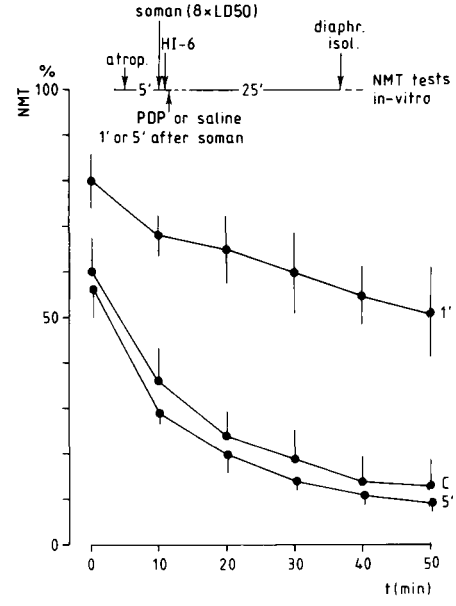

FIG. 6. Mean (±s.e.m.) neuromuscular transmission (NMT) in diaphragm preparations obtained from soman-intoxicated rats treated with atropine and HI-6 which received PDP (36 μmol kg^{-1} i.v.) or saline (C) one or five min after the intoxication (see schedule at the top). The NMT was measured in-vitro and expressed as a percentage of the NMT determined in untreated control preparations. The control group (C) is the same as that in Fig. 4. The PDP-treated group consisted of 5 rats.

Experiment 6. Upon increasing the time interval between PDP injection and soman intoxication from 10 min to 2 and 4 h (Fig. 5), the gradual failure of NMT could not be significantly prevented when compared with control values.

Experiment 7. From Fig. 6 it appears that PDP was only effective in preventing failure of oxime-induced recovery of NMT when administered immediately after soman intoxication and subsequent HI-6 treatment.

DISCUSSION

From fixed-schedule experiments it appeared that PDP was very effective in preventing secondary failure of NMT and death in atropinized oxime-treated rats intoxicated with high doses of soman (Van Helden et al 1986). In the present paper the activity of this simulator has been investigated at various prophylactic time intervals, dosages, and two routes of administration. When at the same dose of PDP the prophylactic time interval was progressively extended from 10 min to 4 h, first NMT and secondly

survival, deteriorated. For example, at a prophylactic time interval of 2 h and a dose of 36 μmol kg^{-1}, survival (Fig. 1) was high (83%), whereas NMT in the diaphragm was much inhibited (Fig. 5). At 4 h and the same dose, survival decreased to 25% (Fig. 1) and NMT dropped to control levels (Fig. 5). From the finding that a considerable failure of NMT was accompanied by a relatively high survival, it can be concluded that only a low degree of NMT in the diaphragm is necessary to survive. The failure of NMT at a prophylactic time interval of 2 h points to elimination of a large part of PDP before the soman intoxication takes place. At a prophylactic time interval of 10 min and a step by step enhancement of the dose of PDP from 3 to 36 μmol kg^{-1} (Fig. 1), the percentage of survival increased and the degree of failure of NMT decreased (Fig. 4). Again it became clear that, to obtain a high survival rate, a smaller amount of PDP is required compared with a dose needed to maintain a high level of NMT; at a dose of 12 μmol kg^{-1} (Fig. 1) the survival was 94% whereas the NMT (Fig. 4) was still much inhibited. A dose of 36 μmol kg^{-1} PDP given 10 min before the soman intoxication prevented soman storage in muscle tissue most effectively; there was hardly any secondary failure of NMT.

After a low intravenous dose (36 μmol kg^{-1}, Fig. 1) of PDP at a prophylactic time interval of 2 h, survival was approximately the same as after a high oral dose (112 or 224 μmol kg^{-1}, Fig. 3). At a longer interval (4 h) and at comparable doses of the simulator (36 μmol kg^{-1} i.v. and 25 μmol kg^{-1} oral), survival following oral administration of PDP was higher than after intravenous injection. This might be explained by a slow absorption of PDP after oral administration and consequently to a low blood level of the agent at short prophylactic time intervals. Therefore, at short prophylactic time intervals, higher oral doses of PDP may be required to obtain adequately protecting blood levels. Supposing a constant rate of elimination, the slow absorption of PDP also may explain that, following oral administration of the agent at a prophylactic time interval of 1 h and a high dose (224 μmol kg^{-1}), survival was approximately the same (70%) as after an interval of 4 h and a low dose (25 μmol kg^{-1}) (Fig. 3).

In general, the mean survival times of the PDP-treated groups fluctuated more than in control groups in which the mean survival time was roughly between 5 and 10 h (Tables 1 and 2). Deviations of survival times in PDP-treated groups did not correspond to deviations in control groups. On the one hand it might be expected that a beneficial effect of PDP on survival also would lead to better survival times of non-survivors. On the other hand, the hypothesis that PDP occupies binding sites of soman in the body, would result in larger amounts of free soman and make a dose of this agent more toxic. These two conflicting mechanisms might account for the unpredictable variability in survival times of the PDP-treated non-survivors.

Considering the beneficial effect of a PDP prophylaxis on survival, the conclusion is inevitable that the above supposed overload of free soman was broken down efficiently and, in so far as re-inhibition of AChE occurred, was counteracted by HI-6 administered immediately after the soman intoxication.

When animals intoxicated with 1 × LD50 soman were additionally pretreated with PDP, i.e. without HI-6 and atropine, this did not result in higher mortality (Table 4). At such a low dose of soman, storage probably does not occur.

Administering PDP therapeutically instead of prophylactically appeared to be effective only when given immediately after the soman intoxication and HI-6 treatment (Table 3). The NMT in the diaphragm of these animals was clearly less inhibited than in control animals (Fig. 6). It might be that the distribution of soman was not completed at that time so that there was still competition for binding sites between inhibitor and PDP. In addition, there is some evidence that PDP may have a higher affinity for these binding sites causing a release of soman into the blood stream (Van Helden et al 1984a). If such a release occurs at a moment when the HI-6 levels are still high, soman-inhibited cholinesterase is instantly reactivated and death may be prevented.

In the present experiments, rather high dosages of PDP were administered either prophylactically or therapeutically without paying attention to possible toxic signs. Attempts to determine the i.v. LD50 of PDP showed no toxic signs at a dose level of 140 μmol kg^{-1} i.v., whereas it was quite effective at doses below 50 μmol kg^{-1} i.v. Higher doses than 1100 μmol kg^{-1} i.v. (200 mg kg^{-1}) have not been tested; at this dose no animals died (Van Helden et al 1986).

It may be concluded that PDP was very active in preventing death at progressive prophylactic time intervals both at intravenous and oral administration. Therapeutically, it was only effective when given immediately after soman intoxication and oxime treatment. Secondary failure of HI-6 induced recovery of NMT, could be effectively prevented when PDP was given prophylactically as well as

therapeutically. The compound did not increase the toxicity of 1 × LD50 soman if given 10 min prophylactically.

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