J. Pharm. Pharmacol. 1984, 36: 305–308
Received October 10, 1983

New simulators in the prophylaxis against soman poisoning: structural specificity for the depot site(s)

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The oxime HI-6 is effective as an antidote in the soman poisoned (6-8LD50) rat, however, successfully treated animals subsequently show a gradual relapse of signs of poisoning and eventually die after several hours. The relapse is caused by the reappearance of soman at specific sites, after having been elsewhere in the body. Diaphragms isolated from poisoned rats successfully treated with HI-6 also showed a 'secondary' relapse of poisoning. Eight compounds chemically related to soman–soman-simulators—have been tested as prophylactic agents, for their potency in preventing the reappearance of poisoning. The idea was that such compounds may block the non-synaptic binding sites for soman. Three of the 8 compounds proved very effective, which gave some insight into the chemical structure needed for this type of prophylactic action.

With few exceptions, most experiments aimed at a prophylaxis of soman poisoning have been performed with reversible acetylcholinesterase (AChE) inhibitors of the carbamate type or with enzyme reactivating oximes. From earlier experiments in our laboratory it appeared that oxime-therapy after severe soman poisoning (6–8 × LD50) may lead to a rapid initial recovery of the animals, followed by a deterioration of the condition of the animals and subsequent death in the hours thereafter. This latter phenomenon appeared to be due to the endogenous re-intoxication by soman from a depot formed somewhere in the body (Wolthuis et al 1981a, b; Benschop et al 1981). At least part of this soman depot appeared to be localized in muscle tissue (Van Helden et al 1983).

In addition, these studies demonstrated that the accumulation of soman in the depot can be influenced by a soman simulator (pinacolyl ethyl methylphosphonate—also named 'som-sim'), a non-toxic compound with similar physicochemical characteristics to soman, but devoid of anticholinesterase activity. Given prophylactically, this compound can prevent death after a few hours.

In the present experiments a total of eight soman simulators, including 'som-sim', have been tested to obtain some insight into the chemical structure needed for this type of prophylactic action. Apart from som-sim (compound II), two more compounds were found to be effective, both closely related to II.

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MATERIALS AND METHODS

Animals

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

Experiment A; neuromuscular function

Control rats were anaesthetized with hexobarbitone (175 mg kg^{-1} i.p.) and received atropine sulphate (50 mg kg^{-1} i.p.) just before 8 × LD50 soman (3·6 μmol kg^{-1} i.v.) was injected. HI-6 (150 μmol kg^{-1}) was injected i.v. immediately after soman, which made artificial respiration unnecessary.

The experimental rats received an injection of one of the soman-simulators (36 μmol kg^{-1} i.v.; compounds I–VIII, Table 1) 10 min before soman and were otherwise treated identically.

All animals were killed 25 min after the administration of soman, diaphragm strips were dissected and mounted in-vitro in Krebs-Ringer buffer. The ability of the muscle strips to sustain tetanic contractions was tested with three 3 s periods of indirect supramaximal stimulation, at 30 s intervals and at frequencies of 25, 50 and 100 Hz respectively (see also Wolthuis 1981c). The percentage of neuromuscular transmission (% NMT) was calculated as the height in the middle of each tetanic response expressed as a percentage of the height of the response by direct stimulation of the muscle; the mean value of these 3 percentages is used as a measure for % NMT.

Experiment B: survival

In this experiment the animal groups were treated identically as in experiment A, except that 6 × LD50 soman was given instead of 8 × LD50. The survival times of the animals were measured by keeping them one to a cage and recording breathing movements with an ultrasonic detection device.

Chemicals

The simulators I–IV were prepared from 1,2,2-trimethylpropyl methylphosphonochloridate (Christol et al 1966) by reaction with the appropriate alcohol in the presence of an equimolar amount of triethylamine. Compound V was obtained in a similar way from dimethylphosphinochloridate and 1,2,2-trimethylpropanol. The products were washed with aqueous sodium carbonate before final distillation, in order to remove pyrophosphonates or pyrophosphinates. Compound VI was prepared according to Pelchowicz et al (1963), VII and VIII as well as 1,2,2-trimethylpropyl methylphosphonofluoridate (soman) were obtained according to standard procedures. All products were >98% pure (glc) and had satisfactory elementary analysis, nmr- and ir-spectra. HI-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-4′-carbamoyl-pyridinium-1-methyl ether dichloride monohydrate) was kindly made available by Dr P. A. Lockwood, Defence Research Establishment, Suffield, Canada. Atropine sulphate and hexobarbital-sodium were purchased from Brocades Stheeman, Haarlem, The Netherlands. All solutions were made in glass-distilled water.

Statistics

Variance analysis was performed according to Winer (1971). The Welch test (Hald 1952) was used to compare the mean % NMT-values for (A–F) between control and experimental groups. The Fisher test (Finney 1948) was applied to compare survival between control and experimental groups. In the text, significant indicates $P < 0{\cdot}05$, two-tailed test.

RESULTS

Fig. 1 shows the time schedule and the results of experiment A. Following the oxime-induced recovery of the neuromuscular transmission (NMT) of the rat isolated diaphragm at the start of in-vitro testing, a gradual 'secondary' failure of NMT was observed upon repeated testing of the diaphragm strips from control animals not pretreated with one of the simulators. Upon pretreatment with different simulators, i.e. I–VIII (see Table 1 for chemical structures), this gradual secondary failure of NMT could be prevented to various degrees. When R = *O*-alkyl (I–IV), Fig. 1a, the effect seemed optimal when the alkyl group is methyl (I) or ethyl (II) and decreases markedly upon further chain lengthening (III) or branching (IV). Substitution of the *O*-alkyl chain by a methyl group or by hydrogen, as in compounds V or VI respectively, Fig. 1b, resulted in a significantly better prevention of secondary failure compared with control preparations. Two other simulators structurally unrelated to soman i.e. VII and VIII, were ineffective.

Table 1. Effects of prophylaxis with soman-simulators (I–VIII, 36 μmol kg^{-1} i.v.) on survival (times) of anaesthetized atropinized (50 mg kg^{-1} i.p.) rats poisoned with 6 × LD50 soman (2·7 μmol kg^{-1} i.v.) and subsequently treated with HI-6 (150 μmol kg^{-1} i.v.). In soman R = fluorine. * = Significantly different from control animals.

Prophylaxis	No. survivors/ group at 24 h (%)	Mean (± s.e.m.) time to death of non-survivors (h)	Chemical structure
Saline (control)	0/12 (0)	4·3 ± 0·4	
Compound: I	9/12 (75)*	3·3 ± 2·6	R = O – CH_3
II	9/12 (75)*	2·6 ± 1·8	R = O – CH_2 – CH_3
III	6/12 (50)*	4·0 ± 1·7	R = O – CH_2 – CH_2 – CH_2
IV	4/12 (30)	0·7 ± 0·1*	R = O – $CH(CH_3)$ – CH_3
V	15/15 (100)*		R = CH_3
VI	7/12 (58)*	5·8 ± 2·6	R = H
VII	2/12 (16)	4·2 ± 1·4	$[(CH_3)_2CHO]_3P=O$
VIII	0/12 (0)	3·3 ± 0·3	$(CH_3CH_2CH_2O)_3P=O$

With regard to survival of the intact rat, the same trends were found (see Table 1). Survival in the groups pretreated with I or II was significantly better than in control animals and tended to be better than in animals pretreated with III or IV. Survival following pretreatment with IV did not differ significantly from that of control animals. As predicted from the in-vitro results in experiment A, the administration of V or VI resulted in a significantly improved survival rate compared with control animals, whereas prophylaxis with V prevented death in all animals. Although VII prevented death in 2 out of 12 rats, this compound as well as VIII can be considered as ineffective, which is in line with the results of experiment A.

All animals that died, in spite of pretreatment with a simulator, did so after roughly the same time span after soman administration as animals not pretreated with the simulator, with the exception of those rats pretreated with IV, which had a significantly shortened survival time, i.e. less than 1 h (Table 1).

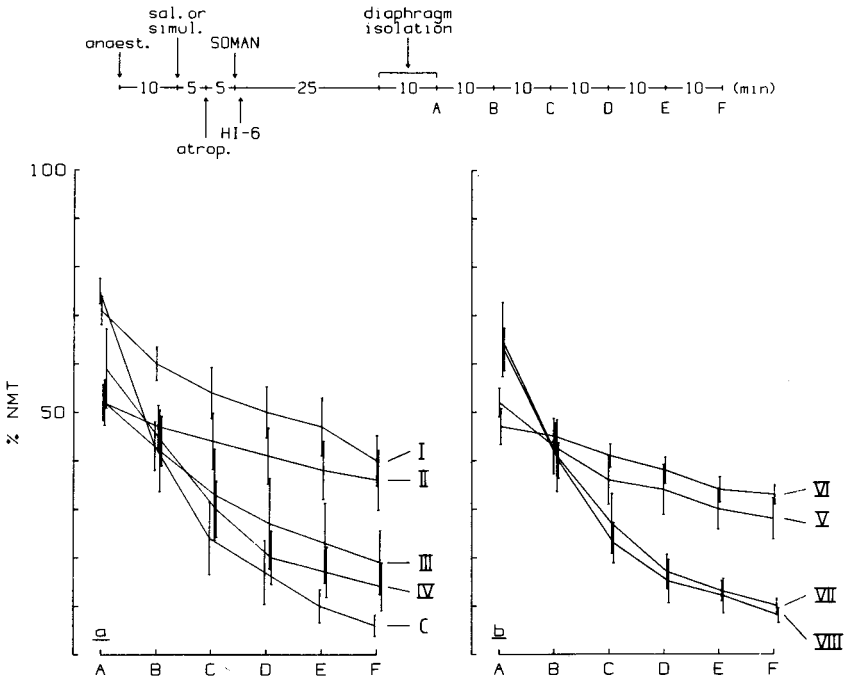

FIG. 1. Mean (± s.e.m) neuromuscular transmission (NMT) in rat diaphragm preparations expressed as a percentage of the NMT determined separately in 20 untreated control preparations. Each experimental group consisted of 5 animals and the control group (C) of 9 animals. From each animal 2 diaphragm strips were tested. The soman dose was 8 × LD50 (3·6 μmol kg^{-1} i.v.) whereas the HI-6 dose was 150 μmol kg^{-1} i.v. The structures of compounds I–VIII are shown in Table 1. In all cases the dose of these compounds was 36 μmol kg^{-1} i.v. The time schedule is schematically shown at the top of the Figure. The Figure shows that pretreatment with various simulators (I–VIII) prevented the gradual decrease of NMT to different degrees.

DISCUSSION

In conjunction with earlier findings (Wolthuis et al 1981a, b; Benschop et al 1981; Van Helden et al 1983) the present results (experiment A) indicate that after poisoning with large dosages of soman and subsequent treatment with the oxime HI-6, soman is stored in the muscle from which it is gradually released causing secondary failure of NMT.

The interpretation of the findings on the basis of the final results at the end of the testing period in experiment A (see Fig. 1) is somewhat confounded by the observed differences in the NMT at the start of the in-vitro tests. These differences might be explained by the differences in efficacy of the simulators in occupying the binding sites of the depot. When this efficacy is low, relatively large amounts of soman can—temporarily—be bound in the depot and will not be available for initial NMT inhibition, in that case NMT at the start will be relatively good. Upon its gradual release, subsequent failure of NMT will be pronounced at the end of the 50 min in-vitro testing period. This phenomenon can be observed in muscle preparations from control animals, in preparations from animals pretreated with the ineffective simulators VII or VIII and to a lesser degree in preparations from rats pretreated with IV. NMT in these preparations starts at high and falls to low values. Conversely, when the efficacy of a simulator is high, it can be expected that larger amounts of soman will remain in circulation due to blockage of its binding sites, causing a lower quality of NMT at the start of the 50 min testing period. Because of the smaller amounts of soman in the depot, the subsequent failure will then be less than in control preparations. Moreover, at the end of this testing period, as explained above, NMT will then be better than in control preparations. In short, the curve will then run a flatter course, as seen in the figure with muscle preparations from animals pretreated with II, V and VI. An exception which does not fit well into this picture is the effect of I: NMT starts high, falls off but is still high at the end of testing.

The results suggest that the efficacy of a simulator

is rather specific and that optimal results are obtained with compounds that most closely resemble soman.

As mentioned, a higher amount of soman may remain in circulation when the depot is rendered inaccessible by the prophylaxis with an effective simulator. This might result in a higher lethality. Although we did not find increased toxicity of an LD10 of soman in rats with pretreatment 10 min before soman with compound II (7·5 mg kg^{-1} i.v.), Talbot et al (1983) recently reported a 25% enhanced sensitivity of rats to soman by pretreatment with this simulator (15 mg kg^{-1} i.m.), 30 min before soman.

The unexpected reduction of the survival time after pretreatment with compound IV (see Table 1) remains unexplained. Since the toxicity of all the simulators—except that of compound II—still has to be determined, it may be that this effect of compound IV is due to an unknown side-effect.

In conclusion, it may be said that (i) the in-vivo–in-vitro model appears to be a useful technique for investigation of the soman-depot and the ways in which its formation can be manipulated by these soman-simulators, (ii) a further search for better simulators than the original som-sim seems warranted, (iii) the limited data available so far suggest reversible, but specific binding at the depot site(s) in that replacement of the fluorine atom of soman by small groups leads to efficient simulators, whereas replacement by larger groups renders them ineffective.

Acknowledgements

Many thanks are due to Mr G. R. van den Berg for the synthesis of the simulators, to Mrs H. J. van der Wiel for her very skilful technical assistance, to Mrs M. J. Wijnans for statistical evaluation of the results and to Dr E. Meeter for critical proof reading of the manuscript.

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