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The prophylactic efficacy of various simulators against intoxication with the organophosphate soman: structure-activity studies*

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The effects were investigated of structural variations of the soman simulator pinacolyl dimethylphosphinate on its efficacy to prevent secondary failure of oxime-induced recovery of neuromuscular transmission and death after soman intoxication. The simulators were administered prophylactically to atropinized, HI-6 treated rats, dosed with 6 or 8 × LD50 soman. In these new simulators the pinacolyl moiety was varied, the phosphonyl oxygen atom was replaced by sulphur, or the phosphorous-bound methyl groups were replaced by ethyl or methoxy groups. All these variations appeared to be less active than pinacolyl dimethylphosphinate. Intravenously, the latter compound was very effective at a dose of 12 μmol kg^{-1}; its i.v. LD50 appeared to be higher than 1 mmol kg^{-1}.

When soman enters the body, part is stored and remains intact in a depot from where it is gradually released. So, by re-intoxication it may cause death in the 5–6 h following an initially successful oxime-therapy (Wolthuis et al 1981a, b; Benschop et al 1981). Soman storage and re-intoxication can be prevented by prophylaxis with so called soman-simulators, i.e. compounds that resemble soman in chemical structure and lipophilicity but which are devoid of anticholinesterase activity (Wolthuis et al 1981a, b; van Helden et al 1984).

With in-vitro diaphragm preparations, obtained from rats dosed with soman, it was demonstrated that at least part of the soman depot was localized in striated muscle. This storage could be prevented by prophylaxis with simulators (van Helden et al 1984). By substituting hydrogen, methyl or various alkoxy groups for the fluorine atom, and tests on the prevention of soman-induced reinhibition of neuromuscular transmission (NMT) and death, it was shown that the storage in muscle was a specific process. The more the simulator resembled the structure of soman the better it blocked its storage. So far, pinacolyl dimethylphosphinate, obtained by replacing the fluorine atom of soman by a methyl group, has been the most effective simulator (van Helden et al 1984).

However, the question remained whether variations in the pinacolyl moiety might further enhance the prophylactic efficacy of the soman simulator. This, in addition to tests with three other structural variations, is the subject of the present study.

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MATERIALS AND METHODS

Animals

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

Experiment 1: neuromuscular transmission (NMT)

Control rats were anaesthetized with hexobarbitone (175 mg kg^{-1}, i.p.) and given atropine sulphate (50 mg kg^{-1}, i.p.) 5 min before 8 × LD50 soman (3·6 μmol kg^{-1}, i.v.) was injected. HI-6 (150 μmol kg^{-1}, i.v.) was injected immediately after soman, which made artificial respiration unnecessary. Test rats were injected with one of the soman simulators (36, 12, 6 or 3 μmol kg^{-1}, i.v.; see compounds in Table 1), 10 min before soman and were otherwise treated identically; control rats received saline instead of simulator.

All animals were killed 25 min after the administration of soman, diaphragm strips were dissected, mounted in-vitro in Krebs-Ringer buffer and tested for their ability to sustain tetanic contractions on indirect stimulation at four standard frequencies as described earlier (Wolthuis et al 1981c; van Helden et al 1983a). This test for NMT was repeated five times at 10 min intervals (see Fig. 1). The first test at t = 0 started about 10 min after the animal had been killed, the last test was at t = 50 min, i.e. after 50 min in-vitro incubation. NMT was expressed as a per-

centage of the NMT determined separately in 20 untreated control preparations. Changes in NMT during the 50 min in-vitro incubation were expressed as $\%NMT_{t=0}-\%NMT_{t=50}$ (Fig. 2).

Experiment 2: survival

Groups of animals were treated identically as in experiment 1, except that 6 × LD50 soman was given instead of 8 × LD50 and the animals were not killed. 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 3: acute toxicity of simulator V

In an attempt to determine the i.v. LD50 of compound V in male rats, injections with this simulator, dissolved in 5% propylene glycol in distilled water, were given into the dorsal penis vein under light hexobarbitone sodium anaesthesia. Groups of 4 rats, weighing approximately 200 g, were injected with 0·14, 0·25, 0·42, 0·56, 0·84 or 1·1 mmol kg^{-1} and were monitored during 24 h following the injection by keeping them one to a cage and recording breathing movements with an ultrasonic detection device.

Chemicals

The new compounds X, XI, XIV and XVI were prepared from the appropriate alcohol (obtained from Ega Chemie, Brussels, Belgium) and dimethylphosphinochloridate by the same procedure as described earlier for compound V (van Helden et al 1984). Compound XIII was made similarly from pinacolyl alcohol and diethylphosphinochloridate. Compound IX was prepared from dimethylphosphinochloridothionate (Maier 1961) and pinacolyl alcohol in the presence of amine. Reaction of the sodium salt of pinacolyl alcohol with dimethyl phosphorochloridate gave compound XVII. Soman (1,2,2-trimethylpropyl methylphosphonofluoridate) was obtained according to standard procedures. All products were distilled until >98% purity (GLC, SE-30) and had satisfactory elemental analyses, ir-, NMR- and mass spectra. HI-6 (2-hydroxyiminomethyl-pyridinium-1-methyl-4′-carbamoylpyridinium-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. All simulators were dissolved in 5% propylene glycol in glass-distilled water, except compound IX, which was dissolved in solution Petit (25 g ethanol, 35 g glycerol, 40 g distilled water). The remaining 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 values for the degree of failure of NMT ($\%NMT_{t=0}-\%NMT_{t=50}$) between control and experimental groups and among experimental groups. The Fischer test (Finney 1948) was applied to compare survival between control and experimental groups and among experimental groups. In the text, significant indicates a real difference with a reliability of 95%, tested two-tailed.

RESULTS

Figs 1 and 2 show that in diaphragm preparations from control animals, the oxime-induced recovery of neuromuscular transmission (NMT), visible at the start of in-vitro testing, was followed by a gradual failure of NMT in the subsequent 50 min. In these control animals the value for the degree of failure of NMT ($\%NMT_{t=0}-\%NMT_{t=50}$, Fig. 2) is large. Upon pretreatment with all the soman-simulators, except for compound XI (Fig. 1A), this gradual secondary failure of NMT could be significantly prevented to varying degrees when compared with control values. Most effective in preventing failure of NMT was a pretreatment with simulator V (Fig. 1B). Deletion of one methyl group from the β-carbon of the pinacolyl moiety (compound X), or from the α-carbon (compound XIV), or the introduction of an extra methylene group between the α- and β-carbon of the pinacolyl moiety (compound XVI), decreased the efficacy of the simulator, albeit to a lesser extent than deletion of two methyl groups from the β-carbon atom (compound XI).

Replacement of the phosphoryl oxygen atom by sulphur (compound IX) and replacement of the phosphorus bound methyl groups of compound V by ethyl (compound XIII) or by methoxy (compound XVII) resulted in approximately the same negative effect on the protection of NMT as the permutations of one methyl group in the pinacolyl moiety mentioned above.

The same trends were found with regard to the effect of simulator prophylaxis on the survival of atropinized soman-dosed and HI-6 treated rats (see Table 1). In all groups pretreated with the high dose of simulators, i.e. 36 μmol kg^{-1}, survival was significantly better than in control animals, except

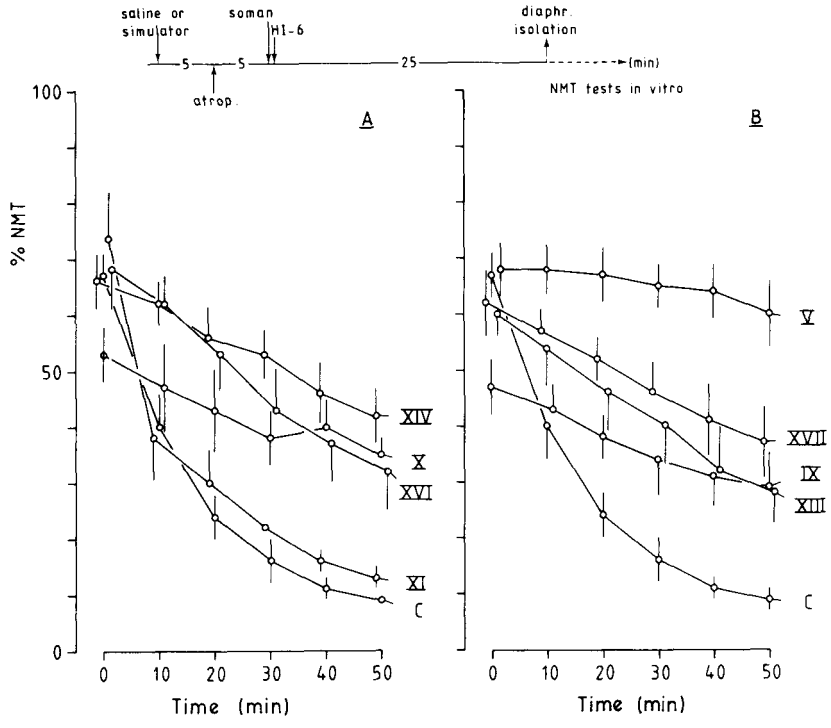


FIG. 1. Mean (± s.e.m.) neuromuscular transmission (NMT) in rat diaphragm preparations measured in-vitro and expressed as a percentage of the NMT determined separately in 20 untreated control preparations. Each experimental group consisted of 5 or 6 animals, the testing schedule is schematically shown at the top of the graphs. 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 structure of the simulators tested (compounds V, X, XIV, XI, XVI, IX, XIII and XVII) are shown in Table 1. In all cases the dose of the simulators was 36 μmol kg^{-1}, i.v.

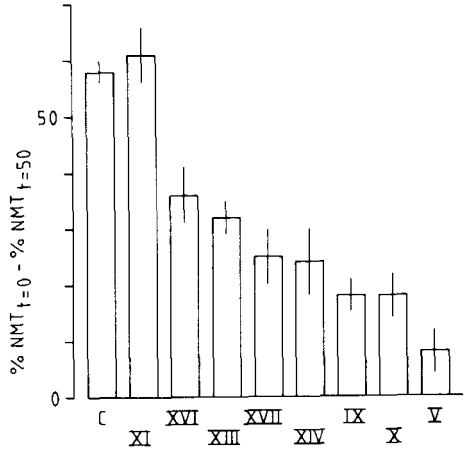


FIG. 2. The degree of failure of NMT during the in-vitro incubation of experiment 1. This is quantitatively expressed as the difference between the percentage of NMT at t = 0 (first test, Fig. 1) and the percentage of NMT at t = 50 min (last test) for each simulator.

for compound XI. At the lower dose (12 μmol kg^{-1}) survival in all groups was still significantly better than in the control group, except for the groups of animals pretreated with XIV, XI or XVI. Pretreatment with the lower dose of simulators resulted in a significant reduction of survival compared with the higher dose, except for V, X and XI. At still lower doses of compound V, 6 μmol kg^{-1} and 3 μmol kg^{-1}, the percentage survival was 92% and 17%, respectively.

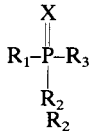
The mean survival time of the animals that died in spite of pretreatment with a simulator was longer than control animals, with the exception of those rats pretreated with compound XI or IX. In the group pretreated with compound IX the survival time was very short.

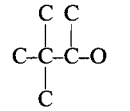
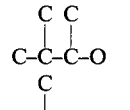
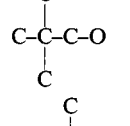
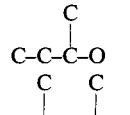
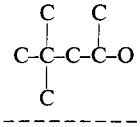
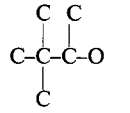
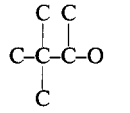
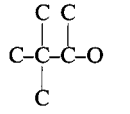
The i.v. LD50 of simulator V appeared to be higher than 1·1 mmol kg^{-1} (200 mg kg^{-1}), since all rats treated with this dose survived more than 24 h.

DISCUSSION

The synthesis of new simulators so far has been focussed on replacing the fluorine atom of soman by

Table 1. Effects of a prophylaxis with soman-simulators (compounds V–XVII) on 24 h survival and the time to death of non-survivors. All rats were anaesthetized, atropinized (50 mg kg^{-1}, i.p.), soman dosed (6 × LD50 = 2·7 μmol kg^{-1}, i.v.) and treated with HI-6 (150 μmol kg^{-1}, i.v.). The structure of soman is similar to that of compound V, except that R_3 the fluorine atom of soman is replaced by a methyl group. The experimental procedure of this experiment is similar to that shown at the top of Fig. 1, except for diaphragm isolation.



Prophylaxis	R_1	R_2	R_3	X	No. of survivors/group at 24 h (%) 36 μmol kg^{-1}	12 μmol kg^{-1}	Mean (±s.e.m.) time to death of non-survivors (h) 36 μmol kg^{-1}	12 μmol kg^{-1}
Control: Saline instead of simulators					0/36 (0)		7·6 ± 0·6	
Compound: V*		C	C	O	12/12 (100)	6/6 (100)	—	—
X		C	C	O	10/12 (83)	3/6 (50)	11·5 ± 3·2	10·4 ± 0·3
XIV		C	C	O	9/12 (75)	0/6 (0)	11·4 ± 5·4	9·1 ± 0·7
XI		C	C	O	3/12 (25)	0/6 (0)	7·9 ± 1·9	6·2 ± 0·9
XVI		C	C	O	11/12 (92)	2/6 (33)	(0·4)	13·0 ± 0·4
IX		C	C	S	12/12 (100)	3/6 (50)	—	1·7 ± 0·5
XIII		C–C	C–C	O	12/12 (100)	3/6 (50)	—	15·5 ± 2·5
XVII		O–C	O–C	O	12/12 (100)	3/6 (50)	—	19·9 ± 0·6

*At 6 μmol kg^{-1}, no of survivors/group = 11/12 (92%); mean time to death of non-survivors = 12 h; at 3 μmol kg^{-1}, no of survivors/group = 1/6 (17%); mean time to death of non-survivors = 7·8 ± 2·9 h.

various groups. In this series of compounds, it appeared that replacement of fluorine by methyl gave optimal results (compound V, see van Helden et al 1984). In the present experiments we investigated whether and how structural alterations in the pinacolyl group of simulator V would affect the efficacy of a simulator to prevent accumulation of soman in a tissue depot. In addition, three other analogues have been tested, in which the pinacolyl group was left intact (compounds IX, XIII, XVII).

A decrease of the neuromuscular transmission (NMT) in-vitro could be prevented almost completely by pretreating the animals with simulator V. It is apparent from Fig. 1 that this compound is more effective in preventing the storage of soman in striated muscles than the new simulators. Hence, the intact pinacolyl structure appears to be imperative for high efficacy. Removal of one methyl group from the β- (compound X) or α-position (compound XIV) of the pinacolyl moiety, or insertion of a methylene

group in between these positions (XVI) leads to a decrease in the protective efficacy. Removal of two methyl groups from the β-position (compound XI) abolishes the protective effect almost completely. From these and earlier findings (van Helden et al 1984) it is concluded that the efficacy of a simulator is very specific. Optimal results are obtained with a compound that bears the closest possible resemblance to soman, both with respect to the pinacolyl moiety and, to a lesser extent, to the size of the group replacing fluorine. However the generality of the latter conclusion should be regarded with some caution. Although we found previously (van Helden et al 1984) that replacement of the fluorine atom by the relatively small methoxy or ethoxy groups resulted in more effective simulators than replacement of fluorine by larger propoxy groups, we found afterwards that substitution of fluorine by an even bulkier n-butoxy group leads to an effective simulator (not published).

In addition to changes at the pinacolyl group three other variations were tested. The thiono analogue IX of compound V was tested for two reasons. First of all, compound IX may be regarded as a pro-drug of V, since the latter simulator might be formed upon in-vivo oxidation of the thiophosphyl group of IX, by analogy with the conversion of parathion into paraoxon. This might lead to a gradual formation of compound X, resulting in a prolonged protective time span. Secondly, it might be that compound IX is an effective simulator in its own right. In view of the reduced number of survivors upon lowering of the dose the latter assumption seems unlikely. Whether or not IX is metabolized into V remains questionable, the ill-understood short survival time of the non-survivors certainly does not point in this direction. The other two analogues XIII and XVII were more active than IX but all these three compounds were less effective than V. The striking, rather long survival times of rats pretreated with simulators XIII and XVII, are still inexplicable.

In attempts to determine the i.v. LD50 of compound V in rats, it appeared that this simulator did not cause toxic signs at a dose level of 0·14 mmol kg^{-1} i.v., whereas it was quite effective at doses below 0·05 mmol kg^{-1} i.v. At this stage of the investigation, therefore, a cutoff value of 1·1 mmol kg^{-1} i.v. was considered, at which dose no animals died.

In conclusion, all the synthesized and tested variations appeared to be less active than compound V, pinacolyl dimethylphosphinate.

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