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The Effect of the Calcium Antagonist Nimodipine on the Detoxification of Soman in Anaesthetized Rabbits

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Abstract

The effect of nimodipine, a vasoactive calcium antagonist, on the disappearance of soman from blood was studied in anaesthetized rabbits intoxicated with soman (10·8 $\mu g\ kg^{-1}$ i.v.). Blood samples from the left heart ventricle and femoral artery were used to investigate soman detoxification. The concentrations of the soman isomers C + P − and C − P − in blood samples were determined by gas chromatography coupled with high-resolution mass spectrometry.

During the sampling, 15–300 s after soman injection, the soman concentration in control animals decreased from 50 to 0·029 ng mL^{-1}; in animals pre-treated with nimodipine (10 mg kg^{-1}) it decreased from 15 to 0·033 ng mL^{-1}. In animals pre-treated with nimodipine the soman concentration was significantly reduced during the first minute of sampling. No differences were detected between soman concentrations in samples from the heart and femoral artery. Acetylcholinesterase inhibition was also used as an indicator of soman activity; there was no difference between the activity of this enzyme in different peripheral organs of control and nimodipine-treated animals.

Nimodipine reduces the initial concentration of soman in the blood, which might be of significance in the treatment of soman intoxication.

Soman (*O*-1,2,2-trimethylpropyl methylphosphonofluoridate), a highly toxic organophosphate, interferes with the cholinergic nervous system, resulting in hyperactivity. After soman intoxication in rats, the circulatory system responds with changes in blood pressure and heart rate (Bataillard et al 1990). The magnitude of these changes is related to the extent of the inhibition of the acetylcholinesterase in the brain, so-called central inhibition (Brezenoff et al 1984). In the periphery the accumulation of acetylcholine as a result of the inhibition of acetylcholinesterase, and the consequent over-activity, can induce changes in organ blood flow via the autonomic innervation (Vetterlein & Haase 1979). Soman is subject to different detoxifying processes, as discussed by Benschop et al (1987). The different organs contribute to this detoxification process to an extent which is related to the blood flow through the organ in question (Maxwell et al 1987). Thus, a compound with circulatory effects such as nimodipine (a 1,4-dihydropyridine derivative) might alter the detoxifying process. Nimodipine is a Ca^{2+}-channel blocker which prevents or reduces intracellular loading of Ca^{2+} and has dilatory effects on cerebral vessels and on blood vessels in peripheral organs (Cain & Nicholson 1989).

In a preliminary study we found that nimodipine given with atropine and pyridostigmine protected mice against soman intoxication (Karlsson & Sellström 1986). In an attempt to explain the mechanism underlying this protective effect, we studied the influence of nimodipine pre-treatment on the clearance of administered soman from the blood. We found that this pre-treatment considerably increased the rate of disappearance of soman (Karlsson et al 1994), presumably by reducing the peripheral vasoconstriction and thereby increasing the peripheral blood flow through the detoxifying organs.

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The disappearance of soman was so rapid in the Karlsson & Sellström (1986) study that the soman must have been hydrolysed or bound to the tissue during the first passage through the body.

Because of the extremely rapid disappearance of soman in rabbits pre-treated with nimodipine (Karlsson et al 1994) it is of interest to address the following questions:

Is soman detectable in the left heart ventricle after its first passage through the lung tissue and subsequent passages through the body in animals pre-treated with nimodipine? If soman is not detectable after its passage through the lungs the detoxifying process must have been increased tremendously either in the lungs or in the blood.

Is there a major difference between the soman concentration in the blood collected from the left heart ventricle compared with that sampled a distance from the heart? If there is, the detoxifying process occurs in the blood itself.

Materials and Methods

Animals

Albino rabbits of the Swedish country strain, 2·5–3·5 kg, were housed in individual cages (18–20°C, relative humidity 50 ± 5%). They had restricted access to Ewos rabbit pellets K1; tap water was freely available. The animals were maintained on a 12-h alternating light/dark cycle, with artificial light between 1800 and 0600 h. The animal experiments were approved by the Regional Research Ethical Committee in accordance with national laws (SFS 1988:539, LSFS 1989:41).

Materials

Soman, neopentyl sarin (*O*-2,2-dimethylpropyl methylphosphonofluoridate) and the internal standard, deuterated soman (CD_3P-soman), were prepared at this establishment according

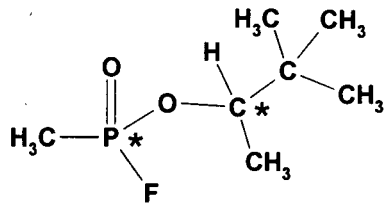

FIG. 1. The structure of *O*-1,2,2-trimethylpropyl methylphosphonofluoridate (soman). *Asymmetric centres.

to standard procedures. The soman standard solutions for quantification were prepared from the racemic mixture assuming a 55:45 diastereoisomeric ratio (C+P−, C−P+)/(C+P+, C−P−) and equal amounts of the enantiomers in each pair (Fig. 1). Stock standard solutions (1 mg mL^{-1}) were prepared in hexane and stored at −20°C. Soman stock solutions for intravenous injection were prepared from the undiluted agent in deionized water immediately before each experiment.

Solvents used were of analytical reagent grade or better. Solid-phase extraction was performed with BondElut C_{18} cartridges (Analytichem, CA) connected to a vacuum manifold (Supelco).

A 2-propanol solution containing the internal standard and neopentyl sarin was added to the buffer. Calibration standards were freshly prepared in ethyl acetate for each experiment.

Nimodipine was a generous gift from Bayer, Wuppertal, Germany, and was dissolved in dimethylsulphoxide (DMSO) (Fluka AB p.a.).

Injection of drugs and antidotes

DMSO (control animals) or nimodipine (10 mg kg^{-1}, dissolved in DMSO), was injected intraperitoneally 45 min before induction of anaesthesia. The animal was anaesthetized by injection of pentobarbitone (30 mg kg^{-1}; Apoteksbolaget, Sweden) via a marginal ear vein. The left ventricle of the heart was cannulated via the brachial artery. A catheter was introduced into the femoral artery. The animals were tracheostomized in order to secure a free upper airway. Arterial blood gases were analysed (ABL 500, Radiometer, Copenhagen, Denmark) before and after the injection of soman.

One hour after the pre-treatment (DMSO or nimodipine), 10·8 μg kg^{-1} soman (0·2 mL) was injected into the marginal ear vein. This corresponds to the dose causing death in 50% of all animals treated (Harris et al 1981).

Before soman administration, blood samples were withdrawn from the femoral artery for blank and recovery tests. Blood samples were withdrawn from the central catheter and from the femoral artery 15, 30, 60, 90, 120, 180, 300 s after soman injection for determination of soman concentration.

Sample preparation

Blood samples were immediately mixed with ice-cold acetate buffer (pH 3·5; 0·2 M, 6 mL) containing aluminium nitrate (1·65 mM), neopentyl sarin (0·1 μg mL^{-1}) and CD_3P-soman internal standard (2 ng mL^{-1}). The samples were centrifuged at 1500 *g* for 10 min using a Beckman TJ-6 refrigerated centrifuge.

The supernatant was transferred to a C_{18} cartridge preconditioned with methanol and deionized water (4 mL of each). Vacuum was applied and the flow-rate adjusted to approximately 4 mL min^{-1}.

Ethyl acetate (500 μL) was slowly passed through the C_{18} cartridge to extract the soman. Residual water was removed from the extracts by freezing, before transfer to the autosampler glass vial. Extracts expected to contain a low concentration of soman were concentrated to approximately 50 μL, immediately before the analysis, on a SpeedVac sample concentrator (Savant Instruments, NY).

In all experiments the accuracy of the procedures was checked by analysing blank blood and blood samples to which soman (10 ng) had been added. The soman solution used for intravenous injection was checked by adding a volume containing 10 ng soman to 6 mL of the buffer and determining the concentration using the normal sample preparation procedure.

Gas chromatography–mass spectrometry

Gas chromatography–high resolution mass spectrometry (GC-HRMS) was performed with a VG 70-SQ high-resolution hybrid tandem mass spectrometer interfaced with a Hewlett-Packard 5890 gas chromatograph; the chromatograph was equipped with a 30 m × 0·25 mm i.d. fused-silica capillary column coated with a 0·25-μm film of DB-5MS (J & W Scientific, CA).

Samples were introduced by splitless injection at a column temperature of 50°C. The column temperature was then programmed at 10°C min^{-1} to 180°C. The injector and interface temperatures were 150 and 160°C. The helium carrier gas pressure was 50 kPa, giving an average carrier gas velocity of approximately 30 cm s^{-1}.

An electron impact (EI)-only source was used at 200°C with an electron energy of 40 eV, a trap current of 500 mA and a source pressure of approximately 1×10^{-6} mbar. The mass spectrometer was tuned to a minimum resolution of 5000 and was operated in the selected ion recording mode. Mass calibration was performed daily or whenever the tuning was changed. The ions selected for the measurement of soman and the internal standard were m/z 126·0246 and 129·0434, respectively. The perfluorokerosene ion at m/z 118·9920 was used as a lock-mass. The measuring time at the soman and internal standard channels was 80 ms, that at the lock-mass channel was 50 ms; the delay time between the channels was 10 ms. This resulted in a cycle time of 240 ms and approximately 10 sampling points measured over each GC peak.

Tissue preparation and analysis of cholinesterase

After the withdrawal of the last blood sample the rabbit was killed by intravenous injection of anaesthetic and saturated KCl. Organs were removed by autopsy and frozen until cholinesterase analysis, when they were thawed, cut into small pieces and homogenized in phosphate buffer (0·1 M, pH 7·4) with a Potter-Elvehjelm homogenizer.

Cholinesterase activity was assayed by a modification of the method of Augustinsson et al (1978). 4,4′-Dithiodipyridine (PDS; 0·28 mM, 75 μL) in phosphate buffer (0·1 M, pH 8·0) was added to each well of a flat-bottomed microwell plate (Nunc, Roskilde, Denmark). Samples (25 μL), in each well, of homogenates diluted 50–500 times and buffer (blank) were added in triplicate. After 10–15 min incubation on a shaker at room temperature the reaction was started by adding acetylthiocholine iodide (2 mM; 100 μL) as substrate to each well.

The rate of increase in absorbance at $\lambda = 340$ nm was measured for 10 min and the results were processed by use of a microtitre plate reader (Labsystem iEMS reader MF, Genesis software). This modified method gave results for activity measurement approximately in accordance with those obtained with the method of Augustinsson et al (1978) with a factor of 2·6. Enzyme activity was expressed as mol s^{-1} (mg protein)$^{-1}$.

Measurements of protein

The protein content of the tissue was measured as described by Bradford (1976), using Coomassie brilliant blue G-250 dye (Bio-Rad). The absorbance was measured by means of a Perkin-Elmer 557 spectrophotometer at $\lambda = 595$ nm.

Statistical evaluation

The unpaired Student's *t*-test was used for the comparisons of cholinesterase activity. Analysis of variance was used to test the significance of differences in soman concentrations between controls and nimodipine pre-treated animals. When appropriate, the Bonferroni correction was used as a post-hoc test. Results are reported as means ± s.e.m.

Results

Clear signs of intoxication were observed soon after administration of soman. These included salivation, fasciculation, miosis and occasionally convulsions in both groups. Table 1 shows the pH and arterial blood gases in the animals.

There were no significant differences between cholinesterase activity in the peripheral organs of controls and the nimodipine pre-treated rabbits 5 min after the injection of soman (Table 2). There was a marked difference between the cholinesterase activity in the organs. In the control animals the highest activity was in the kidney and the lowest in the skin. After nimodipine pre-treatment the relative cholinesterase activities were slightly changed (Table 2).

Although there was no difference between the concentrations of soman isomers (C + P −, C − P −) in rabbit blood collected from the heart ventricle and from the femoral artery, during the first minute of intoxication the soman concentration in samples from both the heart ventricle and the femoral artery was significantly lower in nimodipine-treated rabbits than in the control animals (Fig. 2).

Discussion

Soman is a highly toxic organophosphate which inhibits the hydrolysing enzyme acetylcholinesterase irreversibly (Hopff et al 1984). Synaptically released acetylcholine cannot be degraded, which results in its accumulation in different parts of the body and increased nervous activity. Visible signs of overstimulation of the cholinergic part of the nervous system include miosis, salivation, fasciculation, tremor and convulsions (Lotti 1992). Indeed the animals in this study showed miosis, salivation, fasciculation and sometimes convulsions, with appearance of the first sign soon after the soman injection.

Different detoxifying processes degrade the soman with varying degrees of efficiency as discussed by Benschop et al

Table 1. pH and blood gases in rabbits before (control) and after (soman) injection of soman (10·8 μg kg^{-1}, i.v.).

Group		n	pH	pCO_2 (kPa)	pO_2 (kPa)
Dimethylsulphoxide	Control	6	7·48 ± 0·02	4·48 ± 0·21	9·10 ± 1·12
	Soman	6	7·45 ± 0·07	4·54 ± 0·64	9·52 ± 1·26
Nimodipine	Control	9	7·41 ± 0·02	4·21 ± 0·20	9·96 ± 0·29
	Soman	9	7·42 ± 0·03	4·23 ± 0·36	9·50 ± 0·63

The rabbits were treated with nimodipine (10 mg kg^{-1}) or dimethylsulphoxide (vehicle; 1 mL kg^{-1}) 1 h before soman exposure. Values represent means ± s.e.m.

Table 2. Cholinesterase activity (nmol s^{-1} mg^{-1}) in different organs 5 min after injection of soman (10·8 μg kg^{-1} i.v.) preceded 1 h earlier by treatment with nimodipine 10 mg kg^{-1} or dimethylsulphoxide (vehicle; 1 mL kg^{-1}).

Organ	Dimethylsulphoxide (vehicle) pre-treatment Activity	Nimodipine pre-treatment Activity
Kidney	4·970 ± 0·538	4·599 ± 0·931
Brain	0·859 ± 0·286	0·632 ± 0·159
Intestine	0·637 ± 0·049	0·582 ± 0·065
Liver	0·623 ± 0·091	0·607 ± 0·088
Diaphragm	0·374 ± 0·042	0·353 ± 0·034
Muscle	0·335 ± 0·049	0·213 ± 0·036
Lung	0·266 ± 0·073	0·272 ± 0·081
Heart	0·234 ± 0·016	0·156 ± 0·026
Skin	0·051 ± 0·026	0·214 ± 0·055

Values represent means ± s.e.m. (n = 6).

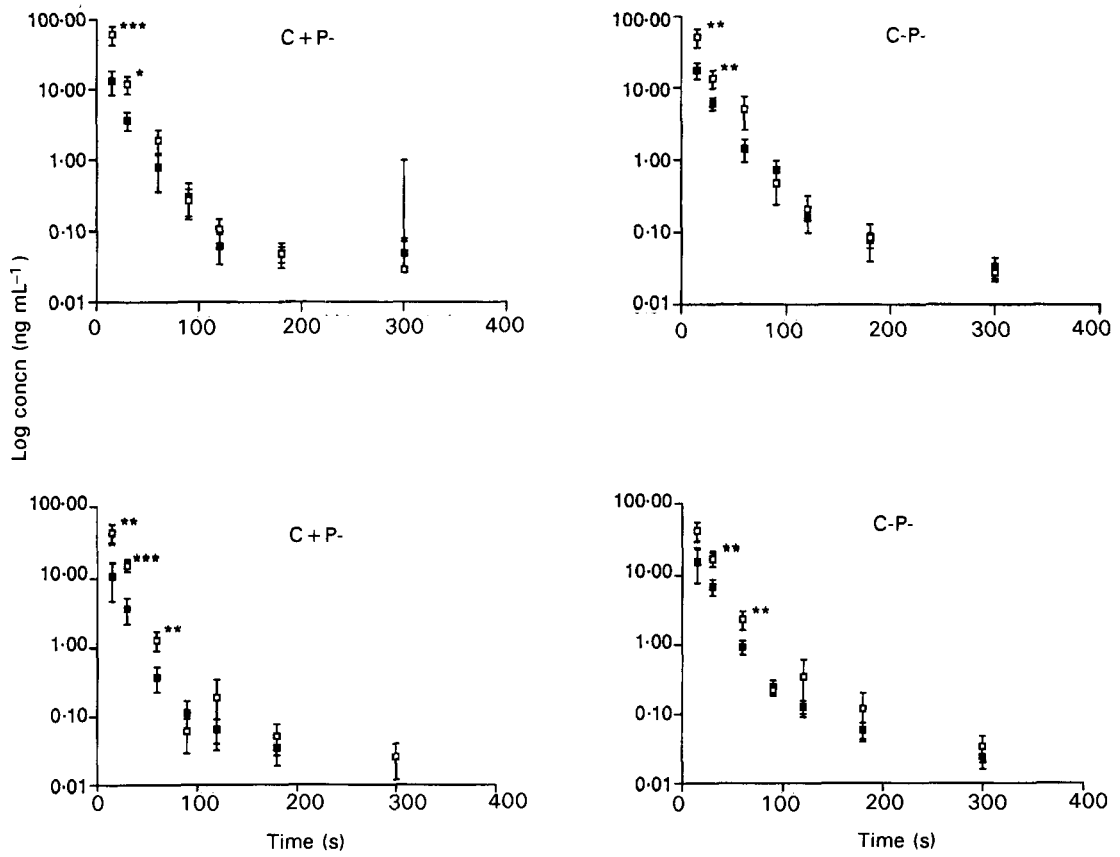

FIG. 2. Concentrations of soman, ng mL^{-1}, in blood withdrawn from the femoral artery (upper graphs) and from the left ventricle of the heart (lower graphs) 15–300 s after injection of soman (10·8 $\mu g\ kg^{-1}$, i.v.), in animals pre-treated intraperitoneally one hour earlier with nimodipine (■) 10 mg kg^{-1} (1 mL kg^{-1}; n = 10) or dimethylsulphoxide (□) (vehicle, control; 1 mL kg^{-1}; n = 5). Values represent mean ± s.e.m. Statistical evaluation was performed by analysis of variance and the Bonferroni correction was used as a post-hoc test. $^{*}P < 0.01$, $^{**}P < 0.05$, $^{***}P < 0.005$.

(1987). Exposure to soman causes a disturbance of the cardiovascular system which might affect the detoxifying processes, and this can be reflected as changes in the soman concentration in the blood.

In an attempt to determine whether the pulmonary system is crucial for the previously reported extremely rapid detoxification of soman in nimodipine pre-treated animals (Karlsson et al 1994), we measured the soman concentration in blood collected from the left heart ventricle. As soman was injected via a marginal ear vein, the only major detoxifying organ it passes before entering the left heart ventricle is the lungs. Soman was clearly detected but in a lower concentration in the nimodipine pre-treated rabbits as compared with the controls. This was observed even in blood withdrawn 15 s after the injection of soman. These results indicate that nimodipine pretreatment significantly reduces the concentration of soman in the blood of the rabbit during the first minute after soman injection. One can assume that the pulmonary system contributes significantly to this process. Indeed, increased venous return in animals pre-treated with nimodipine could result in a reduced concentration of soman because of a dilution effect. Nimodipine has been shown to increase cardiac output in the rabbit (van der Griessen et al 1990).

The accumulation of acetylcholine is reported to induce vasodilation which can affect the blood flow in different organs (Vetterlein & Haase 1979). Soman intoxication, however, also immediately induces an increase in blood pressure and decrease in heart rate both in conscious unrestrained rats (Bataillard et al 1990) and in pentobarbital-anaesthetized rats (Maxwell et al 1987). The vasodilation initially induced by an increase in acetylcholine as a result of exposure to soman might be counteracted by the activity in the sympathetic nervous system (Vetterlein & Haase 1979). An increase in total peripheral vascular resistance after soman intoxication might have a pressor effect (Kentera et al 1982). It is conceivable that a decreased blood flow in different organs will result in a lower ability to degrade the soman. In soman-intoxicated animals the degree of cholinesterase inhibition in peripheral organs correlates with the blood flow through that organ (Maxwell et al 1987). Thus, the detoxifying capacity will be reduced if the blood flow is reduced in a particular organ. In this study cholinesterase activity was not significantly altered after nimodipine treatment before soman intoxication.

Accumulation of acetylcholine in the heart might alter cardiac output (Stewart & McKay 1961). A reduction in the heart rate after soman poisoning might result in a transient reduction in cardiac output (Kentera et al 1982). Cardiac output is also reduced by a negative inotropic effect of soman exposure, an effect that is species-dependent, with the guinea-pig as the most sensitive (82·5% reduction) compared with rats and rabbits, with reductions of 50·8 and 41·5%, respectively (Maxwell et al 1991). If the effect on the cardiovascular system could be diminished and the circulation normalized, more soman could be detoxified.

Nimodipine is a Ca^{2+}-channel antagonist. Haws et al (1983) found that it increased the blood flow in the brain, heart and skeletal muscle in unanaesthetized rabbits. They also observed an increase in heart rate, whereas arterial pressure decreased. In conscious pigs, nimodipine caused dose-dependent increases in the blood flow of the stomach, myocardium and skeletal muscle of 95, 97 and 267%, respectively (van der Griessen et al 1990) and the cardiac output and heart rate were also increased, by 54 and 42%, respectively. Nimodipine could, therefore, be beneficial in soman poisoning, because it counteracts the deleterious cardiovascular effects of soman intoxication. In a previous study we found that nimodipine together with atropine and pyridostigmine increased the protection ratio in mice exposed to soman (Karlsson & Sellström 1986). Nimodipine also enhanced the disappearance of soman from the blood in pentobarbital-anaesthetized rabbits, as previously reported (Karlsson et al 1994). This effect might be produced by prevention of the soman-induced vasoconstriction in peripheral organs (Karlsson et al 1994). In this study we have confirmed that nimodipine reduces the concentration of soman in the blood during the first minute after soman intoxication. The magnitude of the effect on soman disappearance was not as great in this study as we previously reported (Karlsson et al 1994). After the first minute the difference between nimodipine pre-treated and control animals was no longer significant.

As there was no difference between the soman concentration in the central and peripheral arterial blood it seems unlikely that the detoxifying process takes place in the blood itself.

Thus, nimodipine seems to affect the concentration of soman in the blood. The exact mechanisms are not clear but the pulmonary system could contribute to this effect. Interestingly, however, no difference in the inhibition of cholinesterase was observed between the controls and the nimodipine pre-treated animals. Further investigation is required to determine whether the effect of the calcium blocker is exclusively related to its vascular actions or involves other mechanisms.

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