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Influence of nimodipine on elimination of soman in rabbit blood and distribution of [^{3}H]soman in mice

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KEY WORDS soman; nimodipine; gas chromatography; drug metabolic detoxication; area under curve

ABSTRACT

AIM: To investigate the effect of nimodipine on the elimination of soman in rabbit blood and distribution of [^{3}H] soman in mice. **METHODS:** Chirasil capillary gas chromatographic analysis method with large volume injections was used to determine the concentration of C(±)P(-)soman in rabbit blood. [^{3}H]soman trace method was used to study the effect of nimodipine on soman distribution in mice. **RESULTS:** Nimodipine (10 mg/kg, ip, 1 h pre-treated) could significantly reduce the concentration of C(±)P(-)soman in rabbit blood from (54±13) to (19±12) μg/L blood at 15 s after soman injection (43.2 μg/kg, iv). Nimodipine could increase clearance rate [$CL_{(S)}$] from (20.8±1.5) to (31±11) $mL \cdot kg^{-1} \cdot s^{-1}$ and reduce AUC of C(±)P(-)soman from (2.08±0.15) to (1.6±0.4) $mg \cdot s \cdot L^{-1}$. Nimodipine (10 mg/kg , ip, 1 h pre-treated) treatment could significantly reduce the distribution amount of bound [^{3}H]soman in plasma, brain, lung, and liver, moreover increased the distribution amount of bound [^{3}H]soman in small intestine during 0-120 min after mice received [^{3}H]soman (0.544 GBq·119 μg/kg, sc) compared to soman control group. **CONCLUSION:** Nimodipine might alter the distribution of soman and reduce the initial concentration of soman in rabbit blood, then accelerated the metabolic detoxication of soman.

INTRODUCTION

Soman (*O*-1,2,2-trimethylpropyl methyl-phosphonofluoridate) is an extremely toxic organophosphorus agent and believed to interfere with the cholinergic nervous system resulting in hyperactivity such as salivation, bradycardia, hypotension, muscle tremors, convulsions, and respiratory depression. Due to the presence of a center of asymmetry at carbon in the pinacoloyl moiety and at the phosphorus atom, four steroisomers can be designated as C(+)P(+), C(+)P(-), C(-)P(+), C(-)P(-). These isomers vary widely in their toxicological and toxicokinetic properties. After absorption through respiratory track or skin, soman could distribute to brain, diaphragm, and other important target tissue and exert its toxic effects through pulmonary circulation and system circulation. The defensive mechanisms of the organism occur at the same time as soman poisoning. The relatively nontoxic C(±)P(+)-episomers are rapidly eliminated through hydrolyzing by A-esterase *in vivo*. Otherwise, detoxication of C(±)P(-)-episomers is accomplished through several different reactions, including reactions with proteins, particularly with carboxylesterase, hydrolysis, and distribution to tissue depots, *etc*[1]. It had been postulated that only a little amount of soman was transported to target organs and inhibit functional acetylcholinesterase. So how to enhance the detoxication ability of the organism itself was

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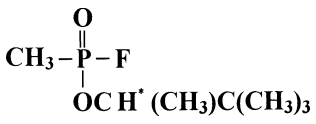
our concerned and studied task. The different organs contribute to detoxication process to an extent which is related to the blood flow through the organ. Soman could induce changes in organ blood flow *via* the autonomic innervation. Karlsson *et al* study showed that nimodipine (dissolved in dimethylsulphoxide), a calcium antagonist, could reduced the initial concentration of soman in the blood by reducing the peripheral vasoconstriction, thereby increasing the peripheral blood flow through the detoxifying organs such as liver and small intestine which contained abundant carboxylesteras (CaE), the detoxifying enzyme to soman[2,3]. In our study, chirasil capillary gas chromatographic analysis method with large volume injections was used to to study the influence of nimodipine (dissolved in plant oil) on the elimination of soman in rabbits blood. Furthermore, [^{3}H]soman trace method was used to study the effect of nimodipine on distribution soman in mice to infer the metabolic detoxication of nimodipine to soman.

METERIALS AND METHODS

Animals Nippon large-ear strain white rabbits (Grade I, Certificate No 01-5022) ♀, 2-2.5 kg and Kunming strain mice (Grade II, Certificate № 99001), ♀, 18-22 g, were obtain from the Laboratory Animal Center of Academy Military Medical Sciences, and housed under standard laboratory conditions with free access to water and food.

Chemicals and reagents Soman (*O*-1,2,2-trimethylpropylmethyl-phosphonofluoridate), [^{3}H]soman (more than 95 % pure by thin-layer chromatography, TLC, 839.9 GBq/mmol, labeled in pinacolyl) and diisopropyl phosphorofluoridate (DFP) were obtained from Institute of Pharmacology and Toxicology, Academy of Military Medical Sciences. Nimodipine was a gift of Dr FENG Hua-Song and its purity was more than 99 %. *N*-pentane and diethyl ether were of analysis grade. The structure of soman and labeled site of [^{3}H] was showed as below:



*** showed labeled site of [^{3}H]**

Animal experiments Nimodipine (10 g/L, dissolved in plant oil) or plant oil (control animal) was injected ip (10 mg/kg) 50 min before the rabbits were anaesthetized with penobarbitone (30 mg/kg, iv). A catheter was introduced into the carotid artery and the animals were tracheostomized to keep the upper airway free. One hour after the pre-treatment (nimodipine or plant oil), C(±)P(-)soman 43.2 μg/kg was injected into the marginal ear vein. Blood samples were withdrawn from the carotid artery 15, 30, 60, 90, 120, 180, 240, and 300 s after soman administration to determine soman concentration.

Work-up procedure and gas chromatography Blood samples for gas chromatographic analysis of soman isomers were stabilized and worked up as described before[4]. Gas chromatography was performed on a Hewlett-Packard 6890 gas chromatography equipped with large volume injector (the maximum injection volume was 200 μL), Alltech chirasil-val analytical column (length 50 m; inner diameter 0.32 mm; film thickness 0.2 μm), and an NP detector. The four stereoisomers of soman were well separated by using chirasil capillary gas chromatographic analysis method and DFP was used as internal standard. The calibration curves of C(±)P(-)-episomers were described[4].

Determination of tissue concentration of bound [^{3}H]soman For the isotope trace experiment, nimodipine (1 g/L, dissolved in plant oil) or plant oil (control animal) was injected (10 mg/kg, ip) 1 h before administration of [^{3}H]soman (0.544 GBq·119 μg/kg dissolved in saline) sc in the neck. Ten minutes, 30 min, 1 h, and 2 h after treatment, mice were decapitated and blood samples from the cervical wound were collected into haparinized tubes and centrifuged at 1000×*g*. The tissue of 100-150 mg were removed, weighed, and homogenized with a high-speed homogenizer in 600 μL ice-cold saline for 2 min. Homogenate was centrifuged at 8000×*g* for 10 min in a refrigerated centrifuge (0-4 ℃) and supernatants were removed. The pellets were resuspended in 600 μL ice-cold saline and centrifuged again. These procedure were performed for three times. After the last centrifugation, the pellets were resuspended in 100 μL ice-cold saline and dropped the small round filter paper uniformly. The filter papers were dried and added to spectrofluor 0.5 % PPO (2,5-diphenyl-oxazole, Sigma) and 0.02 % POPOP (1,4-bis-[5-phenyl oxazoyl-2]-benzene, Sigma) in xylene for determination of bound [^{3}H]soman concentration by liquid scintillation spectrometry (L1409, Wallac Company).

Counting efficiency was determined by external standardization.

Data analysis and statistical evaluation The 3p87pharmacokinetic program (Chinese Pharmacological Society) was used to generate the toxicokinetic parameters for soman iv administration. The areas under the curve [$AUC_{(0\text{-}300\ s)}$] for C(±)P(-)soman in rabbit plasma and $AUC_{(0\text{-}120\ min)}$ for bound [^{3}H]soman in mice plasma and different tissue were calculated by the linear trapezoidal rule to the last blood or tissue concentration. The concentration of bound [^{3}H]soman in per 100 mg tissue was a relative value compared to each experiment's bound [^{3}H]soman counting in 100 μL plasma of soman control group, so $AUC_{(0\text{-}120\ min)}$ for bound [^{3}H]soman in mice plasma and different tissue had not unit. The unpaired Student's *t*-test was used for the comparisons of nimodipine-pretreated group and soman control group.

RESULTS

Effects of nimodipine on the elimination of C (±)P(-)soman in rabbit blood after intoxication of soman *via* vein After rabbits received soman *via* vein for 15-300 s, C(±)P(+)soman was not detected in blood. Otherwise, the toxicokinetics of C(±)P(-)soman in rabbit could be described by a two-compartment model, first a fast distribution phase, then a slow elimination phase.

Nimodipine (10 mg/kg, ip, 1 h before treatment) could significantly reduce the concentration of C(±)P(-)soman in rabbit blood from (54±13) to (19±12) μg/L blood compared to soman-treated animal at 15 s after soman injection. But during 30 s to 300 s after soman intoxification, nimodipine did not significantly influence the concentration of C(±)P(-)soman in rabbit blood compared to soman control (Fig 1). Toxicokinetic parameters showed that nimodipine pretreatment could increase clearance rate [$CL_{(S)}$] from (20.8±1.5) to (31±11) $mL\cdot kg^{-1}\cdot s^{-1}$ and reduce AUC of soman in rabbits plasma from (2.08±0.15) to (1.6±0.4) $mg\cdot s\cdot L^{-1}$ (Tab 1).

Influence of nimodipine on distribution of bound [^{3}H]soman in mouse different tissues Nimodipine treatment could significantly reduce the tissue distribution of $AUC_{(0\text{-}120\ min)}$ for bound [^{3}H]soman in mouse plasma, brain, lung, and liver, but increase the tissue distribution of $AUC_{(0\text{-}120\ min)}$ for bound [^{3}H]soman in mouse small intestine after injection of [^{3}H]soman

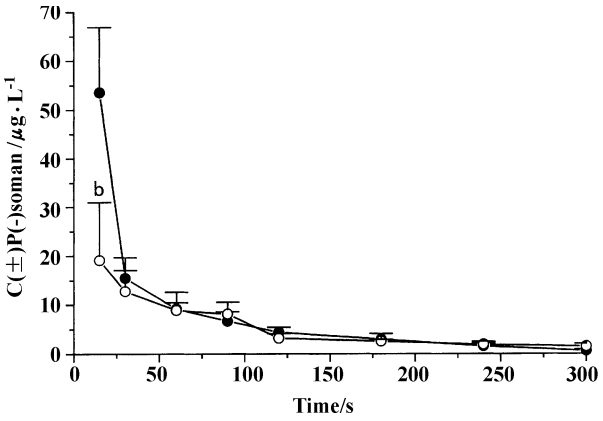


Fig 1. Influence of nimodipine(10 mg/kg, ip) on elimination of free C(±)P(-)soman in rabbit blood after injection of soman (43.2 μg/kg, iv). ● soman; ○ nimodipine pretreated+soman. *n*=4-6. Mean±SD. ^{b}*P*<0.05 *vs* soman control.

Tab 1. Influence of nimodipine (10 mg/kg, ip) on parameters of free C(±)P(-)soman in rabbit blood after injection of soman (43.2 μg/kg , iv). *n*=4-6. Mean±SD. ^{b}*P*<0.05 *vs* soman control.

Toxicokinetic parameters	Soman	Nimodipine+Soman
V_d/L·kg^{-1}	2.1±0.5	6±4
$t_{1/2\ \alpha}$ /s	4±3	3.7±2.9
$t_{1/2\ \beta}$ /s	72±21	26±15
$AUC_{(0\text{-}300\ s)}$/mg·s·L^{-1}	2.05±0.15	1.6±0.4^b
$CL_{(S)}$/mL·kg^{-1}·s^{-1}	20.8±1.5	32±11^b

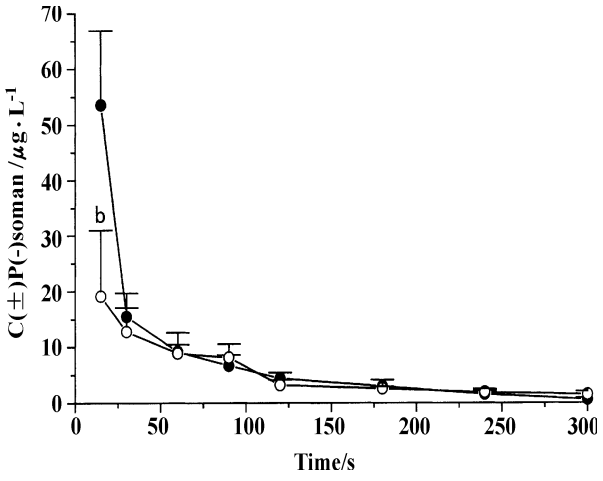


Fig 2. Influence of nimodipine on AUC (0-120 min) of bound [^{3}H]soman in mice plasma and different tissue after injection of [^{3}H]soman (0.544 GBq·119 μg/kg, sc). ■ soman control; □ nimodipine pre-treated+soman. *n*=5. Mean±SD. ^{b}*P*<0.05, ^{c}*P*<0.01 *vs* soman control.

(0.544 GBq·119 μg/kg, sc) as compared to soman control group (Fig 2).

DISCUSSION

Following the intravenous injection of soman, only the C(±)P(-)soman was detected during the first few minutes after administration in our study. So we used a pair of C(±)P(-)soman on making the calibration curve in rabbit blood and the linearity of the calibration curves was well (r>0.999) which the concentration range was 1−100 μg/L blood. The lower limit of detection was 0.1 μg/L blood. The precision was less than 10 % and averaged percentage extraction recovery was about 55 %[4]. The chirasil capillary gas chromatographic analysis method we established was stable and suitable to determine the C(±)P(-)soman in the rabbit blood.

Soman was rapidly eliminated *in vivo* through different processes involving in metabolism detoxication of protein and enzyme located in blood and other detoxication tissues[5]. Simultaneously, soman could cause a disturbance of cardiovascular system which affect the detoxifying processes. As described previously, the acetylcholine accumulation of soman intoxication could induce an increase in total peripheral vascular resistance which can affect the blood flow in different organs[6]. However, soman intoxication could also increase the blood pressue and heart rate in rats[7]. It was conceivable that the effects of decreased blood flow in different tissues especially detoxication tissues induced by soman intoxication maybe induce a lower ability to degrade the soman. If the effect on the cardiovascular system could be diminished and the circulation normalized, the more soman distributed in peripheral detoxication tissue, the more soman could be detoxified. Nimodipine dissolved in Me_2SO could reduce the initial concentration of soman in the blood[2,3,8]. Considering that the calcium block effect of Me_2SO and many cardiovascular effects which maybe interfere the effect of nimodipine[8], nimodipine was dissolved in plant oil to inject which could decrease the solvent's interference in our study. Fifteen seconds after soman injection intravenously was just in the fast distribution phase, so it was presumed that nimodipine could alter the distribution of soman by reducing the peripheral vasoconstriction and thereby increase the peripheral blood flow through the detoxifying organs such as small intestine which contained abundant CaE, the very important detoxifying enzyme to soman to accelerate the soman's elimination. Moreover, serum A-esterase is a calcium-dependant enzyme and calcium is first required for its catalyticac activity[9]. Nimdipine could inhibit calcium influx in red blood cell and calcium aggregation of platelet[10]. These effects might increase calcium concentration in serum and decrease blood viscosity resulting in accelerating hydrolysis of A-esterase to soman and improving blood flow being propitious to detoxication of soman. But these suppositions await further research to confirm.

Although there was no difference between the activity of acetylcholinesterase in different peripheral organs of control and nimodipine-treated animals in Karlsson's study[3], it was remarkable that the esteratic sites especially CaE available for detoxication of soman by irreversible binding in intestine were more than half of total esteratic sites in whole body[7]. So increasing of soman in intestine was a very significant detoxication process to soman. In present study, we determined the bound [^{3}H]soman in different organs, the distribution of bound [^{3}H]soman in detoxication tissues such as small intestine mainly indicated the detoxication degree of CaE in these tissues. On the other hand, the distribution of bound [^{3}H]soman in toxic target tissues such as brain mainly indicated the intoxication degree of AChE in these tissues. Our results suggested that nimodipine might enhance the contact of soman to detoxication enzyme especially to CaE in small intestine and reduced the concentration of soman in other tissues, and at a certain extend explained the reduction of nimodipine on the initial concentration of C(±)P(-)soman in the fast distribution phase following soman injection iv.

As a calcium antagonist, nimodipine could produce dilatation of pial arteries[11]. This effect maybe increase soman entering into brain to exert its toxic action, but it maybe counteract by its effect on enhancing soman flowing into small intestine. More details and exact mechanism of nimodipine's effects on metabolic detoxication of soman need deeply investigation.

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尼莫地平对兔血中梭曼的消除及[^{3}H]梭曼在小鼠组织分布的影响

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关键词 梭曼；尼莫地平；气相色谱法；药物代谢解毒；曲线下面积

目的：考察尼莫地平对兔血中梭曼的消除及结合[^{3}H]梭曼在小鼠组织分布的影响，以探索尼莫地平对梭曼代谢解毒的作用. **方法：**以二异丙基氟磷酸酯(DFP)为内标，用大进样量手性毛细管柱气相色谱法测定兔梭曼静脉染毒后血中游离C(±)P(−)梭曼浓度. 同位素示踪法测定结合[^{3}H]梭曼在小鼠组织中的分布. **结果：**尼莫地平(10 mg/kg, ip，预给药1小时)使兔梭曼染毒(43.2 μg/kg, iv) 15 s后，血中游离C(±)P(−)梭曼浓度从(54±13) μg/L下降到(19±12) μg/L，使血中C(±)P(−)梭曼的清除率从(20.8±1.5) mL·kg^{-1}·s^{-1}增加到(31±11) mL·kg^{-1}·s^{-1}，从而使AUC从(2.08±0.15) mg·s·L^{-1}降低到(1.6±0.4) mg·s·L^{-1}. 尼莫地平(10 mg/kg, ip, 预给药1小时)能显著降低在[^{3}H]梭曼皮下染毒(0.544 GBq·119 μg/kg) 0−120 min后小鼠血浆、脑、肺及肝脏中结合[^{3}H]梭曼的分布，而小肠中结合[^{3}H]梭曼的分布却显著升高. **结论：**尼莫地平可能通过改变梭曼的分布，降低了血中梭曼的初始浓度而起到促进梭曼代谢解毒的作用.

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