95. THE PROTECTIVE EFFICACY OF CALCIUM CHANNEL BLOCKERS IN SOMAN POISONED RATS

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INTRODUCTION

Organophosphorus anticholinesterase inhibitors are important insecticides, which account for a number of deaths every year and furthermore, stock piling these agents as potential weapons represents an ongoing societal concern. The mechanisms of their toxic action appear to involve at least two processes: a) the irreversible phosphorylation of cholinesterase leading to acetylcholine accumulation at central and peripheral cholinergic neuroeffector junctions (1) and b) the induction of presynaptic repetitive discharges leading to enhanced acetylcholine release and consequently augmented postjunctional responses (2). Calcium channel blockers (CCBs) have been shown to suppress such discharges and the accompanying fasciculation, and accordingly, may influence the organophosphate toxicity (3).

CCBs represent not only chemically, but functionally heterogenous group with various binding sites on calcium channels both in peripheral tissues and in neuronal structures (4). The present experiments were designed to determine whether structural differences among CCBs as well as the time of their application in relation to that of soman, a highly toxic organophosphorus chemical warfare nerve agent, may influence the acute soman toxicity and efficacy of its standard antidotes in rats.

MATERIAL AND METHODS

Adult male Wistar rats weighing 180 to 220 g were used in all experiments. Tested drugs were freshly prepared as follows: atropine sulphate ("Sigma"), oxime HI-6 ("Bosnalijek", Sarajevo), verapamil ("Lek", Ljubljana) and diltiazem ("Alkaloid", Skopje, injection vial of 25 mg liophilizate) were dissolved in saline. Nicardipine ("Lek", Ljubljana) was dissolved in 10% (v/v) ethanol. Diazepam was used from commercially available parenteral solution ("Apaurin" ampoules, "Lek", Ljubljana). All solutions were prepared in concentrations so that 0.1 ml contained the amount of substance to be given per 100 g body weight. The doses of drugs used were: atropine 10 mg/kg, oxime HI-6 10 mg/kg, diazepam 2.5 mg/kg, verapamil 10 mg/kg, nicardipine 10 mg/kg and diltiazem 25 mg/kg. CCBs (verapamil, diltiazem and nicardipine) and soman were administered by *sc* injection, and standard antidotes (atropine, oxime HI-6, diazepam) via *im* route. CCBs were given either 30 minutes before or immediately after application of soman. Standard antidotes were always injected immediately after soman administration. The median lethal doses (LD-50) of soman in absence or presence of CCBs and standard antidotes were calculated on the basis of 24-hour survival of poisoned animals and served for evaluation of protective efficacy of drugs given (5).

RESULTS AND DISCUSSION

Efficacy of CCBs tested, given alone or in combination with standard antidotes, in protection of rats acutely poisoned with soman, was presented in Table 1 and 2, respectively.

Among CCBs tested only verapamil (phenylalkylamine derivative), given either before or immediately after soman, produced significant protective effect (Table 1), but without enhancing the protective effect of standard antidotes (Table 2). Nicardipine (1,4-dihydropyridine derivative) failed to offer the protection in poisoned animals (Table 1) and, moreover, lowered the protective efficacy of standard antidotes (Table 2). Diltiazem (benzothiazepine derivative) alone produced significant protective effect only when given as pretreatment (Table 1), but failed to increase the protective efficacy of standard antidotes, even reduced it when given immediately after soman (Table 2).

Similarly to our work Dretchen et al. (6) were used various CCBs (verapamil, diltiazem and three different dihydropyridine derivatives, nifedipine, nitrendipine and nimodipine) in protection against DFP toxicity in the mouse. Given 30 minutes before the organophosphate only verapamil and dihydropyridine derivatives provided significant protection against the lethal effects of DFP. However, in combination with standard antidotes atropine and oxime 2-PAM, only verapamil and nifedipine produced a still higher level of protection (6). Karlsson and Sellstrˆm (7) also reported that nimodipine was effective when used as a pretreatment, alone or in combination with atropine and pyridostigmine, in mice acutely poisoned with soman. These results, except those related to verapamil, are in contrast to our ones. In the present study nicardipine, a member of dihydropyridine CCBs, failed to offer any protection regardless the time of administration in relation to that of soman, and, even, reduced the protective effect of standard antidotes when given in combination with them. This finding is similar to that of Milovanovic et al. (8) which used nicardipine and nimodipine, alone or in combination with standard antidotes (atropine, oxime HI-6, diazepam), in protection of mice intoxicated with soman. Both CCBs, administered immediately after the poison, enhanced soman toxicity and reduced protective efficacy of the antidotes given (8).

It is very difficult to interpret these results at the moment. In above mentioned studies various CCBs have been used in protection against various organophosphates, in different species, and in different both dose and treatment regimens. Besides, different CCBs have different affinity to the binding sites on calcium channels in different tissues. The site of action of verapamil and diltiazem is the intracellular domain of the calcium channel, whereas dihydropyridine CCBs produce their action via the extracellular domain of the channel (4). Furthermore, verapamil and diltiazem have stronger affinity to the cardiac tissue, and dihydropyridine CCBs to the vascular smooth muscle. Corbier and Robineau (9) reported that inhibition of Na^+/K^+ATPase by an organophosphate compound, methylphosphonothiolate, could lead to ventricular arrhythmia that is proposed to be caused by intracellular calcium overload. It has been demonstrated that soman may contribute partially to the overload by reducing intracellular calcium uptake into microsomes and mitochondria (10). Having in mind these facts it could be concluded that the protection against soman obtained in our study with verapamil and, partially, with diltiazem might be a consequence of cardioprotective action of these CCBs. Our earlier results demonstrated the excellent cardioprotective effect of verapamil in soman-intoxicated rats (11).

CONCLUSION

Our results suggest that both the protective efficacy of CCBs in soman-poisoned rats and their influence on protection afforded by standard antidotes might be, at least in part, dependent on their chemical structure as well as the time of their administration in relation to that of soman.

SUMMARY

This study was designed to determine whether structural differences among calcium channel blockers (CCBs) as well as the time of their application in relation to that of soman may influence the acute soman toxicity and efficacy of its standard antidotes in rats.

The experiments were performed on adult, male Wistar rats. CCBs, phenylalkylamine derivative verapamil (10 mg/kg), 1,4-dihydropyridine derivative nicardipine (10 mg/kg) and benzothiazepine derivative diltiazem (10 mg/kg), were injected *sc* 30 minutes before or immediately after soman (*sc*), alone or aloÓg with atropine (10 mg/kg *im*), oxime HI-6 (10 mg/kg *im*) and diazepam (2.5 mg/kg *im*), always administered immediately after the poison. The median lethal doses of soman with or without CCBs and standard antidotes were calculated on the basis of 24-hour survival of poisoned animals and served for evaluation of protective efficacy of drugs given.

Among CCBs tested, only verapamil, given either before or immediately after soman, produced significant protective effect, but without enhancing the protective effect of standard antidotes. Nicardipine failed to offer the protection in poisoned animals and, moreover, lowered the protective efficacy of standard antidotes. Diltiazem alone produced significant protective effect only when given as pretreatment, but failed to increase the protective efficacy of standard antidotes, even reduced it when given immediately after soman.

These results suggest that protective efficacy of CCBs in soman-poisoned rats and their influence on protection afforded by standard antidotes might be dependent on their chemical structure as well as the time of their administration in relation to that of soman.

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KEYWORDS

nerve warfare agents, soman, calcium channel blockers, verapamil, nicardipine, diltiazem

FIGURES AND TABLES

Table 1. Protective efficacy of various calcium channel blockers against soman toxicity in rats

Pretreatment time (min)	LD-50 of soman	(95% Conf. Limits)	Protective index (PI)
-	0	(90.56-108.19)	1
30	0	(104.92-215.78)	1.52*
0	0	(104.92-215.78)	1.52*
30	0	(184.13-326.63)	1.67*
0	0	(45.53-213.80)	0
30	0	(45.53-213.80)	0
0	0	(45.53-213.80)	0

$$PI = \frac{\text{LD-50 of soman in treated animals}}{\text{LD-50 of soman in the control}}$$; *$p<0.05$ *vs* the control

Table 2. Protective efficacy of various channel blockers given in combination with standard antidotes (atropine + oxime HI-6 + diazepam) against soman toxicity in rats

Treatment	Pretreatment time (min)	LD-50 of soman	(95% Conf. Limits)	Protective index (PI)
Control	-	0	(90.56-108.19)	1
Standard antidotes	0	0	(117.79-292.98)	1.88*
Verapamil (10 mg/kg sc) +Standard antidotes	30	0	(113.04-255.37)	1.56*
	0	0	(140.55-307.83)	2.10*
Diltiazem (25 mg/kg sc) +Standard antidotes	30	120.75	(63.35-230.62)	0
	0	0	(129.66-314.76)	2.04*
Nicardipin (10 mg/kg sc) +Standard antidotes	30	121.75	(91.06-163.32)	0
	0	0	(113.82-171.24)	1.41*

Standard antidotes: atropine (10 mg/kg *im*) + oxime HI-6 (10 mg/kg *im*) + diazepam (2.5 mg/kg *im*) were always given immediately after soman (*sc*)

$$PI = \frac{\text{LD-50 of soman in treated animals}}{\text{LD-50 of soman in the control}}$$; *$p<0.05$ *vs* the control