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Comparative antidotal effects of diphenhydramine and atropine against dichlorvos-induced acute toxicosis in rats

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ABSTRACT

The antidotal effect of diphenhydramine against acute toxicosis induced by the organophosphorus insecticide dichlorvos in male rats was evaluated and compared with that of the standard antidote atropine. Dichlorvos at 45 mg/kg orally induced cholinergic toxicity in the rats with cholinesterase inhibition in the plasma (62%), erythrocytes (59%) and whole brain (37%) in comparison with the control group. Intraperitoneal administration of diphenhydramine or atropine at 20 mg/kg 5 minutes after the dichlorvos dosing significantly decreased the incidences of toxic manifestations and gradually decreased the severity of toxicosis during a 60-minute observation period. The antidotal effect of diphenhydramine appeared to be comparable with that of the atropine. However, addition of diazepam did not improve the antidotal efficacy of diphenhydramine. The reductions in blood and brain cholinesterase activities occurred in all the dichlorvos-treated rats regardless of antidotal therapy. The data indicate the antidotal efficacy of diphenhydramine against dichlorvos-induced poisoning in rats in a manner comparable with that of atropine.

Key words: organophosphate, cholinesterase, anticholinesterase, antihistamine

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Introduction

Diphenhydramine is a histamine-H1 receptor antagonist with considerable antimuscarinic effects (GARRISON, 1990). It is effective in preventing physostigmine and neostigmine-induced toxicosis in mice (MOHAMMAD et al., 1987) as well as organophosphate (OP) poisoning in mice (MOHAMMAD et al., 1989; FARIS and MOHAMMAD, 1996a; 1997) and dogs (CLEMMONS et al., 1984; FIKES, 1990). The antidotal efficacy of diphenhydramine has been also demonstrated when given after OP (dichlorvos) in mice (FARIS and MOHAMMAD, 1997) and after carbamate (methomyl) in rats (AL-BAGGOU' and MOHAMMAD, 1999). The protective and therapeutic effects of diphenhydramine against cholinesterase (ChE) inhibitors-induced poisoning were attributed to the antimuscarinic (FIKES, 1990; FARIS and MOHAMMAD, 1997) and possibly antinicotinic (CLEMMONS et al., 1984) action of this antihistamine. Further, the interaction of diphenhydramine and inhibitors at the level of ChE suggests that diphenhydramine does not potentiate inhibition of true ChE caused by OP (FARIS and MOHAMMAD, 1996b) or by carbamates (AL-BAGGOU' and MOHAMMAD, 1999).

A recent study indicates that the antidotal effect of diphenhydramine against methomyl-induced toxicosis in rats is comparable with that of the standard antidote atropine (AL-BAGGOU' and MOHAMMAD, 1999). Such a comparison is lacking in case of OP poisoning. Therefore, the present study was undertaken to examine and compare the antidotal effect of diphenhydramine alone or combined with diazepam with that of atropine in rats acutely intoxicated with dichlorvos. Diazepam is the preferred anticonvulsant agent to alleviate convulsion induced by OP (ANONYMOUS, 1986; DOMINO, 1987).

Materials and methods

Male albino Wistar rats with masses of 150-200g were used. They were housed at a temperature of 23-25 °C with a 14-h light and 10-h dark cycle. The rats had free access to food and water.

Rats were dosed orally with dichlorvos (Aforfos, 50% EC, KN Efthymiadis, Athens, Greece) at 45 mg active ingredient/kg body mass.

This dose was predetermined to induce cholinergic toxic manifestations in rats, and it represents approximately 60% of the oral LD_{50} of dichlorvos in this species (GAINES, 1960). Five minutes after dichlorvos dosing, the rats (5/treatment group) were treated intraperitoneally (i.p.) with either physiological saline solution (positive control), diphenhydramine HCl (SDI, Iraq) at 20 mg/kg, atropine sulfate (1%, Bimeda, Ireland) at 20 mg/kg or with diphenhydramine at 20 mg/kg followed immediately by diazepam (1%, La Roche, Switzerland) at 10 mg/kg. The doses of the antidotes were obtained from literature (DOMINO, 1987; FARIS and MOHAMMAD, 1997; AL-BAGGOU' and MOHAMMAD, 1999). Dichlorvos was diluted with distilled water to a concentration of 4.5%. Diphenhydramine was prepared in physiological saline solution at a concentration of 2%. The volume of administration of dichlorvos and diphenhydramine was at 1 ml/kg body mass. The injectable solutions of atropine and diazepam were used without further dilution. A saline-treated control group of rats was also included in the study.

After dichlorvos dosing, each rat was individually observed for the occurrence of signs of cholinergic toxicity (BUCCAFUSCO et al., 1988; FARIS and MOHAMMAD, 1997; AL-BAGGOU' and MOHAMMAD, 1999). These signs included straub tail, muscle fasciculation, piloerection, lacrimation, defecation, urination, salivation, tremors, gasping and convulsion, and they were recorded at time 0 (5 minutes after the dichlorvos dosing), and then at 15, 30, 45 and 60 minutes after antidote administration. The latency to onset of death within one hour was recorded.

The severity of toxicosis within each group was scored by summing the grades assigned to the percentage of occurrence of signs of toxicosis as described earlier (AL-BAGGOU' and MOHAMMAD, 1999):

1. 1-25%
2. 26-50%
3. 51-75%
4. 76-100%

One hour after antidote administration the rats were anesthetized with ether and blood was obtained by a heparinized syringe from the heart. After cervical dislocation the whole brain was dissected out. The blood

samples were centrifuged at 3000 rpm for 15 minutes to obtain the plasma and erythrocytes. The brain was homogenized in a pH 8.1 phosphate-barbital buffer at 3 ml/100 mg brain mass, and the homogenate was used for the assay of ChE activity (MOHAMMAD et al., 1997; 1999). Aliquots of 0.2 ml of plasma, packed erythrocytes or brain homogenate were used for the determination of ChE activity by an electrometric method previously described (MOHAMMAD et al., 1997; AL-BAGGOU' and MOHAMMAD, 1999). The enzyme activity was expressed as delta pH/30 minutes (pH_1 before incubation – pH_2 after 30 minutes incubation).

Frequency data were analyzed by Fisher's exact probability test (RUNYON, 1977). Data of the ChE activity were analyzed by one-way analysis of variance followed by the least significant difference test (BRUNING and KINTZ, 1977). The level of significance was $P<0.05$.

Results

Dichlorvos dosing in rats produced signs of cholinergic poisoning which included straub tail, muscle fasciculation, piloerection, lacrimation, defecation, excessive salivation, whole body tremor, gasping and convulsion. The occurrence of these signs in the dichlorvos-intoxicated rats ranged between 20-100% (Table 1). Intraperitoneal administration of diphenhydramine or atropine at 20 mg/kg 5 minutes after the dichlorvos dosing gradually and significantly decreased the occurrence of toxic manifestations in the rats over the 60-minute observation period in comparison with the control (dichlorvos + saline) group (Table 1). Correspondingly, diphenhydramine and atropine gradually reduced the severity of dichlorvos-induced toxicosis (Table 2).

The antidotal effect of diphenhydramine against dichlorvos toxicosis was comparable with that of the standard antidote atropine (Tables 1 and 2). However, addition of diazepam (10 mg/kg, i.p.) to the antidotal therapy with diphenhydramine was not advantageous over those of diphenhydramine or atropine given alone. This is because one rat in this group succumbed to death 35 minutes after dichlorvos dosing, and the reduction in the severity of toxicosis was not more than those produced by the other two drugs (Table 2). Further, diazepam-treated rats suffered from muscle relaxation.

Table 1. Antidotal effects of diphenhydramine (20 mg/kg, i.p.), atropine (20 mg/kg, i.p.) and diphenhydramine-diazepam combination (20 + 10 mg/kg, i.p.) in dichlorvos (45 mg/kg, orally) - intoxicated rats

Time (min) after antidote	Percent occurrence									
	Straub -tail	Muscle fasciculation	Piloerec-tion	Lacrima -tion	Defeca-tion	Urina-tion	Saliva-tion	Tremors	Gasping	Convul-sion
Dichlorvosandsaline (control)										
0	80	60	100	80	80	20	20	20	0	20
15	100	100	100	100	60	20	60	80	20	20
30	80	80	80	80	20	20	100	80	0	0
45	80	40	40	60	20	40	60	80	0	0
60	60	20	40	40	0	0	40	20	0	0
Dichlorvos and diphenhydramine										
0	100	0	100	80	60	60	20	0	0	0
15	60	0*	0* [c]	40	20	20	0	0*	0	0
30	0* [c]	0*	0* [c]	20	40	20	0*	0*	0	0
45	0* [c]	0	0 [c]	0 [c]	0	20	0	0*	0	0
60	0c	0	0 [c]	0 [c]	0	20	0	0	0	0
Dichlorvos and atropine										
0	100	40	100	60	60	0	40	40	20	20
15	40	20*	0* [c]	20	0	0	20	0*	0	0
30	20 [c]	0*	0* [c]	20	0	0	0*	0*	0	0
45	20 [c]	0	0 [c]	20	0	0	0	0*	0	0
60	20 [c]	0	0 [c]	20	0	0	0	0	0	0
Dichlorvos and diphenhydramine with diazepam										
0	60	60	60	60	60	20	60	60	0	0
15	40	40	20*	40	0	0	20	80 [ab]	20	0
30	20	20	0*	0	0	0	0*	20	0	0
45	0*	25	0	0	0	0	0	0* [d]	0	0
60	0*	25	0	0	0	0	0	0 [d]	0	0

Antidotes were injected 5 minutes after the dichlorvos dosing. n = 5/group.
Time 0 (5 minutes after the dichlorvos dosing) represents the time before the administration of the antidotes.
Significant differences ($P<0.05$)
* from control (dichlorvos + saline);
[a] from the dichlorvos + diphenhydramine group;
[b] from the dichlorvos + atropine group;
[c] and [d] from the respective values at times of 0 and 15 min., respectively.

Rats dosed with dichlorvos and then treated with saline had significantly lower ChE activity in the plasma (62%), erythrocytes (59%) and brain (37%) in comparison with the non-treated control group (Table 3). The reductions in ChE activities occurred in all the rats dosed with dichlorvos

Table 2. Severity of toxicosis in rats treated by dichlorvos (45 mg/kg, orally) alone or in combination with different antidotes

Time (min)	Toxicity scores			
	Control	Diphenhydramine	Atropine	Diphenhydramine+Diazepam
5	23	19	22	22
15	29	7	5	13
30	26	4	2	3
45	21	1	2	1
60	11	1	2	1

The antidotes were injected 5 minutes after dichlorvos dosing. See materials and methods for the calculation of scores of severity of toxicosis from Table 1.

Table 3. Cholinesterase activities in rats treated by dichlorvos (45 mg/kg, orally) alone or in combination with different antidotes

Treatment	Cholinesterase activity (delta pH/30 minutes)		
	Plasma	Erythrocyte	Brain
Saline	0.29 ± 0.01	0.29 ± 0.02	0.19 ± 0.03
Dichlorvos+ Saline	0.11 ± 0.02*	0.12 ± 0.01*	0.12 ± 0.02*
Dichlorvos+ Diphenhydramine	0.08 ± 0.01*	0.09 ± 0.01	0.13 ± 0.03*
Dichlorvos+ Atropine	0.18 ± 0.01*[ab]	0.14 ± 0.03*	0.12 ± 0.02*
Diphenhydramine+ Diazepam	0.13 ± 0.02*[bc]	0.15 ± 0.03*[b]	0.12 ± 0.02*

Significant differences ($P<0.05$)
* from control
[a] from dichlorvos + saline
[b] from dichlorvos + diphenhydramine
[c] from dichlorvos + atropine
The antidotes were injected 5 minutes after dichlorvos dosing. The enzyme activity was measured one hour after the antidote administration.
Values are mean ± SE of 5 rats/group.

regardless of the concurrent antidotal therapy (Table 3). The antidotes did not protect rats against the ChE inhibition caused by the dichlorvos dosing.

Discussion

As expected, dichlorvos at 45 mg/kg, orally induced signs of poisoning in rats characteristic of ChE inhibition. This dose of dichlorvos represents approximately 60% of its oral LD_{50} value in rats (GAINES, 1960). Accordingly, the dose of dichlorvos used was not lethal in rats, but it did cause significant ChE inhibition in the tissues examined, and the rats manifested cholinergic signs of toxicosis. These findings are in agreement with the known anticholinesterase toxicity of dichlorvos in animals (GAINES, 1960; COHEN and EHRICH, 1976; OSWEILER et al., 1985).

Diphenhydramine treatment effectively alleviated the severity of dichlorvos-induced toxicosis in the rats. This result is in accordance with previous reports regarding the protective and antidotal efficacy of diphenhydramine in mice intoxicated with OP insecticides (MOHAMMAD et al., 1989; FARIS and MOHAMMAD, 1996a; 1997) as well as in rats intoxicated with carbamate insecticide methomyl (AL-BAGGOU' and MOHAMMAD, 1999). The present study further extends these findings and indicates the successful antidotal therapy in rats already showing signs of parasympathetic overstimulation produced by dichlorvos (Table 1). The antidotal effect of diphenhydramine was also comparable with that of the standard antidote atropine. A similar conclusion was reported earlier in rats poisoned with methomyl (AL-BAGGOU' and MOHAMMAD, 1999).

Addition of diazepam to the antidotal therapy of anticholinesterase poisoning is recommended, especially where there is central nervous system excitation and convulsion (ANONYMOUS, 1986; DOMINO, 1987; FIKES, 1990). In the present study, the concurrent administration of diphenhydramine and diazepam did not prove to have an added advantage over that of diphenhydramine when given alone. Instead, one rat died as a result of the combined diazepam-diphenhydramine treatments. It is possible that the absence of significant occurrence of central excitation and convulsion in rats dosed with dichlorvos precluded the appearance of the beneficial anticonvulsant effect of diazepam. However, diphenhydramine was found

to reduce convulsion episodes in rats intoxicated with methomyl (AL-BAGGOU' and MOHAMMAD, 1999).

Dichlorvos is known to variably inhibit blood and tissue ChE activities (COHEN and EHRICH, 1976; ANONYMOUS, 1986; KHAN et al., 1990). In the present study, dichlorvos inhibited plasma, erythrocyte and brain ChE activities regardless of the concurrent antidotal therapy. It appears that modulation of ChE inhibition is not a detrimental factor in the antidotal therapy with diphenhydramine in case of poisoning with OP insecticides. Also, diphenhydramine did not affect the inhibitory action of dichlorvos on erythrocyte and brain ChE activities in mice (FARIS and MOHAMMAD' 1996b). Our data further show that none of the antidotal treatments altered the extent of brain ChE inhibition. Inhibition of true ChE in the nervous tissues is the most important factor of OP poisoning (ANONYMOUS, 1986; FIKES, 1990; OSWEILER, 1996).

In conclusion, diphenhydramine was found to reduce dichlorvos-induced toxicosis in rats in a manner similar to that of the standard antidote atropine.

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SAŽETAK

Protuotrovni učinak difenhidramina protiv trovanja uzrokovanog diklorvosom u štakora uspoređen je sa standardnim djelovanjem atropina. Diklorvos u dozi 45 mg/kg primijenjen oralno uzrokovao je kolinergično trovanje u štakora inhibicijom kolinesteraze plazme (62%), funkcije eritrocita (59%) te inhibicijom funkcije mozga (37%) u odnosu na kontrolnu skupinu. Intraperitonejska primjena difenhidramina ili atropina u dozi 20 mg/kg pet minuta nakon unošenja otrova znatno je smanjila toksični učinak i u tijeku 60 minuta postupno smanjivala opasnost od trovanja. Antidotni učinak difenhidramina u potpunosti je usporediv s djelovanjem atropina. Dodatna primjena diazepama nije sinergistički djelovala na učinak difenhidramina. Smanjenje aktivnosti kolinesteraze u krvi i mozgu uočeno je u svih životinja bez obzira na primjenu antidota.

Ključne riječi: organofosfati, kolinesteraza, antikolinesteraza, antihistamin