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Metoclopramide protection of diazinon-induced toxicosis in chickens

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The efficacy of metoclopramide for preventing organophosphate insecticide-induced (diazinon) toxicosis was evaluated in 7~14 days old chicks. Injection of metoclopramide at 25 mg/kg, s.c. 15 min before diazinon increased the oral 24 h median lethal dose of the insecticide in the chicks by 80%. Metoclopramide alone inhibited the *in vitro* and *in vivo* plasma and whole brain cholinesterase activities of the chicks. Metoclopramide pretreatment at 100 mg/kg, s.c. reduced the extent of cholinesterase inhibition that was caused by diazinon (10 mg/kg, p.o.) in the plasma and whole brain by 24% and 7%, respectively. Diazinon at 10 mg/kg, p.o. produced signs of cholinergic toxicosis in the chicks, and these signs included salivation, lacrimation, gasping and convulsions within 2 h, and the 2-h and 24-h lethalities were 88 and 100%, respectively. Metoclopramide at the dose rates of 12.5, 25, 50, 100 and 200 mg/kg, s.c. given 15 min before diazinon (10 mg/kg, p.o.) variably decreased the occurrence of toxic manifestations in the chicks. The highest dose of metoclopramide (200 mg/kg, s.c.) reduced the 2-h and 24-h lethality of diazinon to 75% each and it reduced the overall toxicity score of diazinon by 32%. The data suggest that metoclopramide pretreatment only partially protected chicks against the acute toxicity of diazinon.

Key words: cholinesterase, diazinon, metoclopramide, organophosphate, poisoning

Introduction

Organophosphates are widely used as insecticides in public health, veterinary practice and agriculture [8,15,28]. The single most important mechanism of the toxic action of these insecticides in man and animals is inhibition of acetylcholinesterase at the nerve terminals, and this causes acetylcholine accumulation that subsequently causes a series of muscarinic, nicotinic and central nervous system effects [4,16,26]. The cardinal treatment for organophosphate poisoning includes atropine to counteract the muscarinic signs and symptoms, and oximes to reactivate the inhibited cholinesterases [4,16,26,28]. However, the clinical results of administering antidotal therapy for organophosphate poisoning are far from perfect [4,6,16,26,28], and there are many ongoing experimental trials using other therapeutic and protective agents for controlling the signs and symptoms of poisoning, and especially those related to the nicotinic effects that are not controlled by atropine. Examples of such trials include antihistamine diphenhydramine [5,12], alpha-2-adrenoceptor agonists [7,29] and metoclopramide, which is a dopamine D2 receptor antagonist [14,22-24].

Metoclopramide is used clinically as a prokinetic and antiemetic agent [25]. Other than the antidopaminergic and possible serotonergic effects, metoclopramide has been found to possess anticholinesterase properties [13,14,22-24]. The drug weakly inhibits cholinesterase activity both *in vitro* [22,23] and *in vivo* [14,24], and so it supposedly prevents further enzyme inhibition by organophosphate compounds. This protective effect of metoclopramide on the cholinesterase is thought to be of practical usefulness for the treatment of organophosphate poisoning [22,23]. Further *in vivo* protection studies in rats that were poisoned by the organophosphate paraoxon demonstrated that metoclopramide was less effective as a protective agent than pralidoxime, without taking into consideration the possible effects of metoclopramide on the signs of poisoning [14,24].

Other than preventing additional cholinesterase inhibition, it is not known whether metoclopramide can modify the signs and symptoms of acute organophosphate poisoning in experimental animals. The aim of the present study was to evaluate the protective effect of metoclopramide in a chick model of acute diazinon-induced toxicosis. Diazinon is an organophosphate insecticide that's widely used in veterinary medicine [1].

Materials and Methods

Seven to fourteen days old, mixed breed broiler chicks of either sex (52-95 grams each) were used in the experiments. They were maintained in batches of 20-30 chicks in a room

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with constant lighting at a temperature of 30-34°C, which was controlled by electric heaters. The floor litter consisted of wood shavings. Water and feed were given *ad libitum*. Metoclopramide HCl (Yuhan, Korea) was dissolved in physiological saline solution for s.c. administration at a volume of 5 ml/kg body weight [3]. A commercial insecticidal concentrated solution of diazinon (60%; Novartis, Switzerland) was further diluted in distilled water to obtain the desired concentrations of the insecticide for oral dosing, at a volume of 5 ml/kg body weight, by using a gavage needle. The choices of the metoclopramide and diazinon doses were based on our preliminary experiments on chicks, as well as on a previous study [3].

The acute (24 h) median lethal doses (LD50) of diazinon with or without metoclopramide (25 mg/kg, s.c., 15 min before diazinon dosing) were determined in the chicks by the up-and-down method [10]. The chicks were individually observed for the appearance of signs of toxicosis for 1 h and then the 24 h lethality was recorded. The protective ratio for metoclopramide was calculated as follows:

Protective ratio = LD50 of diazinon with metoclopramide/ LD50 of diazinon with saline

***In vitro* cholinesterase inhibition**

Six chicks were killed by decapitation after performing ether anesthesia, and blood samples were collected using heparinized test tubes [9]. The plasma was separated from the erythrocytes by centrifugation at 3,000 rpm (Centurion, UK) for 15 min. The whole brains were also obtained from the chicks. All the samples were kept at –20ºC pending cholinesterase analysis that was done within one week. The whole brain was homogenized on an ice bath by using a glass homogenizer with phosphate barbital buffer (pH 8.1) at 3 ml/100 mg wet weight [17,20]. Samples of the plasma and brain homogenates were separately pooled. The inhibitor-cholinesterase incubation method was used to cause *in vitro* inhibition of cholinesterase activities by metoclopramide in aliquots of the pooled plasma and the brain homogenates as has been described before [18,19]. The desired concentrations of metoclopramide were prepared in distilled water and then individually added, in a volume of 0.1 ml, to the enzymatic reaction mixtures of the plasma or brain homogenate (5 samples/concentration). The final concentrations of metoclopramide in the reaction mixtures were 9.4, 18.8, 37.5 and 75 μM, respectively. The control reaction mixtures did not contain metoclopramide and they were used for measurement of the baseline cholinesterase activities in the plasma and brain samples. The reaction mixtures containing metoclopramide were initially incubated at 37°C for 10 min to facilitate cholinesterase inhibition [18,19]. Thereafter, the residual cholinesterase activity (ΔpH/30 min) in the mixtures was measured by an electrometric method that's been described earlier (17-19]. The % of cholinesterase inhibition was calculated as follows:

% Cholinesterase inhibition =

[Cholinesterase activity (without metoclopramide) – Cholinesterase activity (with metoclopramide)/Cholinesterase activity (without metoclopramide)] × 100

***In vivo* effect of metoclopramide on cholinesterase activity**

Eighteen chicks were randomly divided into 3 groups of 6 birds each. The chicks were treated with either the physiological saline solution at 5 ml/kg, s.c. (the control) or with metoclopramide at 100 or 200 mg/kg, s.c., respectively. Thirty minutes after the metoclopramide treatment, the chicks were euthanized to obtain the plasma and whole brain for determining the cholinesterase activity [17-19]. The choices of the metoclopramide doses and the time of obtaining the samples depended on a previous finding in which the pharmacological effects of metoclopramide appeared in the chicks within 30 min after s.c. injection [3].

In another experiment, chicks (6/group) were treated with the physiological saline solution at 5 ml/kg, s.c. (the control) or with metoclopramide at 100 mg/kg, s.c. 15 min before an oral dosing with diazinon at 10 mg/kg. The experiment also included a control group that was not subjected to diazinon or metoclopramide treatments. Thirty minutes after the administration of diazinon, the chicks were euthanized to obtain their plasma and whole brain for determining the cholinesterase activity, as was described earlier.

Effect of metoclopramide on diazinon-induced toxicosis

Chicks (8/group) were treated s.c. with the physiological saline solution at 5 ml/kg (the control) or with metoclopramide at dose rates of 12.5, 25, 50, 100 or 200 mg/kg of body weight, respectively. Fifteen minutes after injection, all the chicks were dosed orally with diazinon at 10 mg/kg of body weight. The choice of the metoclopramide doses depended on a previous study that reported the pharmacological effects of the drug on chicks [3]. The dose of diazinon (10 mg/kg) was determined in a preliminary experiment on chicks, and this dose was found to induce death in 80 to 100% of the dosed chicks within 24 h. After the diazinon dosing, the chicks were observed individually for the occurrence of the signs of organophosphate poisoning that are characteristic of cholinergic toxicity [11,12,17]. The signs included salivation, lacrimation, gasping and convulsion. The 2-h and 24-h lethalities were also recorded. The latencies to onset of any sign of poisoning and death were recorded within 2 h. The severity of toxicosis within each treatment group was scored as has been described before [2]. The following grades were assigned to the percentage of occurrence of the signs of poisoning (salivation, lacrimation, gasping and convulsions), including the 2-h and 24-h lethalities: Grade 1 (1~25%), Grade 2 (26~50%), Grade 3 (51~75%) and Grade 4 (76~100%).

For reference, the highest toxicity score within a group showing all the signs of poisoning (salivation, lacrimation,

gasping and convulsions) and the 2-h and 24-h lethalities would be 24 (4 × 6 signs or death with 100% occurrence).

The present study was approved by the Scientific Committee of the College of Veterinary Medicine at the University of Mosul (Iraq). All the experiments complied with regulations addressing animal use, and proper attention and care was given to the chicks used in this study.

Statistics

The data as multiple means were subjected to the analysis of variance followed by the least significant difference test [21]. The frequency data were subjected to the Fisher's exact probability test, and the scores of the severity of toxicosis were analyzed by the Wilcoxon signed rank test [21]. The level of statistical significance was $p < 0.05$.

Results

The acute (24 h) oral LD50 value of diazinon alone in the chicks was 9.2 mg/kg (Table 1). The signs of cholinergic toxicosis in the chicks appeared within 11-42 min. The signs were salivation, lacrimation, gasping, frequent defecation, convulsions and recumbency before death. Injection of metoclopramide at 25 mg/kg, s.c. increased the oral LD50 value of diazinon by 80% to 16.53 mg/kg, with a protective ratio of 1.8 (Table 1).

Metoclopramide, in a concentration-dependent manner (9.4~75 μM), inhibited the *in vitro* plasma and whole brain cholinesterase activities by 3-28% and 2-19%, respectively (Table 2). Subcutaneous injection of metoclopramide at 100 and 200 mg/kg dose-dependently reduced the plasma (11%

Table 1. Determination of the 24-h median lethal dose (LD50) of diazinon alone or in combination with metoclopramide in chicks by the up-and-down method*

Variable	Result
Diazinon alone (saline pretreatment)	
LD50	9.20 mg/kg, p.o.
Range of the doses used	15~10 = 5 mg/kg, p.o.
Initial dose	15 mg/kg, p.o.
Last dose	10 mg/kg, p.o.
Number of chicks used	6 (XXOXOO)
Increase or decrease in dose	5 mg/kg, p.o.
Range of latency to the onset of poisoning	42~11 = 31 min
Signs of poisoning	salivation, lacrimation, gasping, frequent defecation, convulsions and recumbency
Diazinon with metoclopramide pretreatment	
LD50	16.53 mg/kg, p.o.
Range of the doses used	20~10 = 10 mg/kg, p.o.
Initial dose	15 mg/kg, p.o.
Last dose	15 mg/kg, p.o.
Number of chicks used	5 (OXXOO)
Increase or decrease in dose	5 mg/kg, p.o.
Range of latency to the onset of poisoning	53~22 = 31 min
Signs of poisoning	salivation, lacrimation, gasping, frequent defecation, convulsions and recumbency

X = death; O = survival.
*Metoclopramide was injected 15 min before the diazinon dosing.

Table 2. *In vitro* inhibition of the plasma and whole brain cholinesterase activities in chicks by metoclopramide

Metoclopramide concentration (μM)	Plasma		Whole brain	
	ΔpH/30 min	% inhibition	ΔpH/30 min	% inhibition
0 (baseline)	0.32 ± 0.018		0.180 ± 0.010	
9.4	0.31 ± 0.030	3	0.177 ± 0.002	2
18.8	0.30 ± 0.020	6	0.173 ± 0.005	4
37.5	0.27 ± 0.010	16	0.160 ± 0.005	11
75	0.23 ± 0.010*	28	0.145 ± 0.005*	19

Cholinesterase activity values are mean ± SE, n = 5/each concentration.
*Significantly different from the respective baseline value ($p < 0.05$).

Table 3. *In vivo* inhibition of the plasma and whole brain cholinesterase activities in chicks by metoclopramide

Metoclopramide (mg/kg, s.c.)	Plasma		Whole brain	
	ΔpH/30 min	% inhibition	ΔpH/30 min	% inhibition
0 (saline-control)	0.45 ± 0.050		0.16 ± 0.01	
100	0.40 ± 0.050	11	0.13 ± 0.01	19
200	0.24 ± 0.020*,†	47	0.09 ± 0.01*,†	44

Cholinesterase activity values are means ± SE, n = 6 chicks/group. Cholinesterase activity was determined 30 min after the metoclopramide injection.
*Significantly different from the respective control value ($p < 0.05$).
†Significantly different from the respective value of the 100 mg/kg dose group of metoclopramide ($p < 0.05$).

Table 4. Effect of metoclopramide on the plasma and whole brain cholinesterase activities in the diazinon-treated chicks

Treatment	Plasma		Whole brain	
	ΔpH/30 min	% inhibition	ΔpH/30 min	% inhibition
Saline (control)	0.33 ± 0.05		0.15 ± 0.02	
Diazinon	0.07 ± 0.02*	79	0.12 ± 0.01	20
Metoclopramide + Diazinon	0.15 ± 0.02*,†	55	0.13 ± 0.01	13

Cholinesterase activity values are means ± SE, n = 6 chicks/group. Metoclopramide was injected 15 min (100 mg/kg, s.c.) before the diazinon dosing. Cholinesterase activity was determined 30 min after the diazinon dosing (10 mg/kg, p.o.).
*Significantly different from the respective control value ($p < 0.05$).
†Significantly different from the respective value of the diazinon-treated group ($p < 0.05$).

Table 5. Effect of metoclopramide treatment on diazinon-induced toxicosis in chicks*

Metoclopramide (mg/kg, s.c.)	Occurrence rate (%)					
	Salivation	Lacrimation	Gasping	Convulsions	2 h death	24 h death
0 (saline control)	88	63	63	88	88	100
12.5	75	13	63	88	88	88
25	88	13	25	100	100	100
50	75	25	88	100	100	100
100	63	25	63	88	88	88
200	75	25	50	75	75	75

*Metoclopramide was injected 15 min before the diazinon dosing (10 mg/kg, p.o.). n = 8 chicks/group.

and 47%) and whole brain (19% and 44%) cholinesterase activities of the chicks in comparison with the control values (Table 3). The metoclopramide-treated chicks appeared sedated as they manifested drooping of the neck, immobility, closed eyelids, decreased distress squeaks and recumbency.

Dosing the chicks orally with diazinon at 10 mg/kg significantly inhibited the plasma cholinesterase activity by 79% and this inhibited the cholinesterase activity of the whole brain by 20%, although this was non-significant (Table 4). Metoclopramide pretreatment at 100 mg/kg, s.c. reduced the degree of cholinesterase inhibition caused by diazinon (10 mg/kg, p.o.) in the plasma and whole brain to 55% and 13%, respectively (Table 4).

Diazinon at 10 mg/kg, p.o. produced signs of cholinergic toxicosis in the chicks, and this included salivation, lacrimation, gasping and convulsions within 2 h, and the 2-h and 24-h lethalities were 88% and 100%, respectively (Table 5). Metoclopramide at the dose rates of 12.5-200 mg/kg, s.c. and given 15 min before diazinon (10 mg/kg, p.o.) variably decreased the occurrence of toxic manifestations in the chicks (Table 5). The highest dose of metoclopramide (200 mg/kg, s.c.) reduced the 2-h and 24-h lethalities from 88% and 100%, respectively, in the control group (diazinon only) to 75% each (Table 5). Correspondingly, all the metoclopramide-treated groups had low overall toxicity scores, and the highest dose of metoclopramide significantly reduced the score by 32% in comparison with the control group (diazinon only) (Fig. 1). Metoclopramide treatments did not significantly affect the latencies to the onset of signs of poisoning and the lethalities induced by diazinon (data not shown).

Discussion

Metoclopramide pretreatment reduced the diazinon-induced lethality in chicks, as was determined by the LD50 values, with a protective ratio of 1.8. This finding in a chick model of acute organophosphate poisoning confirms an

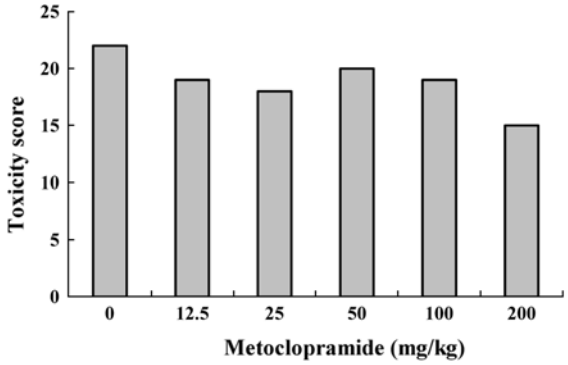

Fig. 1. Effects of metoclopramide pretreatments on the severity of toxicosis induced by diazinon (10 mg/kg, p.o.) in chicks.

earlier suggestion concerning the protective benefit of metoclopramide against organophosphate poisoning [22,23]. The mechanism of such a protective effect has been suggested to be associated with an inhibiting action of metoclopramide on the target cholinesterases in the nervous tissues, thus preventing further inhibition by the organophosphate [14,22,23]. Our *in vitro* and *in vivo* experiments on metoclopramide inhibition of the plasma and whole brain cholinesterase activities further support and expand the previous findings on the cholinesterase inhibitory benefits of metoclopramide, as was demonstrated in rats [14,23,24].

In this study, metoclopramide pretreatment reduced the cholinesterase inhibitory effects of diazinon by 24% in the plasma and by 7% in the whole brain. This finding supports the notion that the protective effect of metoclopramide on the cholinesterases could potentially contribute to the protective benefit of this drug against organophosphate poisoning [14,23,24]. Further, the protective benefit of metoclopramide appeared to also include the whole brain of the chicks. Nerve tissues, including the brain, are the main targets affected by organophosphate poisoning [4,16,26,28]. Organophosphates inhibit cholinesterase activity and cause accumulation of acetylcholine at the nerve endings and they subsequently induce cholinergic overstimulation that's manifested as muscarinic, nicotinic and central nervous system effects such as convulsions [4,16,26,28].

Diazinon at a dose of 10 mg/kg, p.o., which is close to its LD50 value, produced as expected signs and symptoms of organophosphate poisoning in the chicks along with inhibitory effects on the plasma and whole brain cholinesterases. However, as metoclopramide incompletely protected the enzyme, the drug only partially reduced the occurrence of signs of diazinon toxicosis as well as reducing lethality in the chicks. The single treatment with metoclopramide and its partial effectiveness suggest a need for reevaluating the protective effect of metoclopramide against organophosphate poisoning. Multiple metoclopramide pretreatments might also be taken into consideration.

The chicks treated with metoclopramide were found to be sedated as they manifested drooping of the head, immobility, closed eyelids, decreased distress squeaks and recumbency. Metoclopramide sedation in chicks has been recently reported [3] and the contribution of this sedation to the protective effect of the drug against diazinon-induced poisoning is not clear at the present time. The sedative effect of metoclopramide is probably not related to its anticholinesterase effect, as the drug is a dopamine D2-receptor antagonist [25]. Antidopaminergic drugs produce a state of sedation and tranquilization in birds and mammals [1,27].

In conclusion, metoclopramide was found to partially protect chicks against the acute toxicity of diazinon. Further studies are needed on the protective effect of metoclopramide against poisoning induced by other organophosphate insecticides.

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