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Scientific Paper

ACUTE TOXICITY AND CHOLINESTERASE INHIBITION IN CHICKS DOSED ORALLY WITH ORGANOPHOSPHATE INSECTICIDES

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Acute toxic effects of three commonly used insecticidal preparations of the organophosphates chlorpyrifos, diazinon, and dichlorvos were examined in mixed breed broiler chicks, and cholinesterase activity in plasma and brain were measured. The acute (24 h) oral median lethal doses (LD_{50}) of chlorpyrifos, diazinon, and dichlorvos were 10.79 mg kg^{-1}, 6.32 mg kg^{-1}, and 6.30 mg kg^{-1}, respectively, as determined by the up-and-down method in chicks. Signs of cholinergic toxicosis in the chicks appeared within two hours after dosing, and they included salivation, lacrimation, gasping, frequent defecation, drooping of wings, tremors, convulsions, and recumbency before death. Halving the oral LD_{50} of chlorpyrifos (5 mg kg^{-1}), diazinon (3 mg kg^{-1}), and dichlorvos (3 mg kg^{-1}) caused immobility and wing drooping, but not the clinical signs of cholinergic toxicity. However, at full LD_{50} doses of these insecticides, chicks showed clinical signs of cholinergic toxicity similar to those seen in the LD_{50} experiments. Two out of six chicks died within two hours after treatment with LD_{50} doses of chlorpyrifos and dichlorvos, whereas LD_{50} dosing with diazinon caused death in three out of six chicks. Compared to control values, the insecticides reduced plasma and whole brain cholinesterase activities by 29 % to 84 % and 18 % to 77 %, respectively, depending on the dose. The decrease in plasma cholinesterase correlated well (r = 0.82) with that of the brain. These data suggest that organophosphate insecticides administered orally at LD_{50} doses induce clinical signs of cholinergic poisoning and concurrently reduce brain and plasma cholinesterase activities in chicks.

KEY WORDS: *anticholinesterases, chlorpyrifos, cholinergic toxicity, diazinon, dichlorvos, LD_{50}*

Organophosphate (OP) insecticides are widely used in public health, veterinary practice and agriculture (1,2). They are common in the Middle East, including Iraq. Their main mechanism of toxic action in mammals and birds is to inhibit the target enzyme cholinesterase (ChE), which leads to accumulation of acetylcholine at the nerve terminals and neuromuscular junctions, and to cholinergic overstimulation manifested as muscarinic, nicotinic, and central nervous system effects (3-5). The most important diagnostic or biomarker endpoint of OP exposure and poisoning is decreased ChE activity in the blood (erythrocytes, plasma or serum) and other tissues, brain in particular (3-8). Birds have no ChE activity in erythrocytes; therefore, the extent of their exposure to and/or OP poisoning is based on ChE activity in plasma and nervous tissue (3, 9-11).

The toxicity of OP insecticides in birds is generally associated with lower plasma or brain ChE activities (9, 12-14). Decreases in ChE activity to 50 % or less of the normal is accepted as confirmation of exposure or diagnosis of acute poisoning with these insecticides (3, 15, 16). Various reports indicate close association between reduced blood or brain ChE activity and acute

toxicosis induced by doses close to or higher than the median lethal doses (LD_{50}) of OP insecticides in rodents and birds (3, 9, 16-19).

Chicks have already been used in experimental models of acute or subchronic OP poisoning (17-21). Assessing ChE inhibition profiles in OP poisoned chickens is a widely accepted standard for monitoring toxicosis and lethality (17, 22-24). The aim of this study was to further examine the acute toxicity of three commonly used preparations of OP insecticides chlorpyrifos [*O,O*-diethyl-*O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate], diazinon [*O,O*-diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)phosphorothioate] and dichlorvos (2,2-dichlorovinyl dimethyl phosphate) in chicks. Chlorpyrifos and diazinon inhibit ChE activity via their active metabolites (25-27), whereas dichlorvos directly inhibits the enzyme (25). Another aim of this study was to see if there is a correlation between plasma and brain cholinesterase activity in chicks dosed with OP insecticides. This will further enhance our understanding of the clinical response of chicks acutely poisoned with OP insecticides, as this animal species is frequently used in the evaluation of acute OP toxicity (17-24, 28, 29).

MATERIALS AND METHODS

Animals

Mixed breed broiler chicks of either sex (7 to 15 days old) were used in the study. They were kept in batches of 20 to 30 chicks at a time in a room at a temperature of 30 °C to 34 °C with constant lighting. Floor litter consisted of wood shavings; water and feed were given *ad libitum*.

Organophosphate insecticides

We used commercial preparations of three OP insecticides which are common in veterinary practice and public health (1, 2). The commercial insecticidal solutions of chlorpyrifos (50 %, Acichlore, VAPCO, Amman, Jordan), diazinon (60 % Diazinon-60EC, VAPCO), and dichlorvos (55 %, SAFA DDVP55EC, Kalite Yonetim, Turkey) were further diluted in distilled water to obtain the desired concentrations for oral dosing by a gavage needle (29) in a volume of 5 mL kg^{-1} body weight. The solutions were freshly prepared before use, and all doses of the OP insecticides were based on the active ingredients of the compounds.

Table 1 *24-hour median lethal dose (LD_{50}) of organophosphate insecticides determined for chicks using the up-and-down method*

Variable	Result
Chlorpyrifos	
LD_{50}	10.79 mg kg^{-1}, orally
Initial dose	20 mg kg^{-1}, orally
Last dose	10 mg kg^{-1}, orally
Number of chicks used	5 (XOXXX)*
Increase or decrease in dose	5 mg kg^{-1}, orally
Diazinon	
LD_{50}	6.32 mg kg^{-1}, orally
Initial dose	15 mg kg^{-1}, orally
Last dose	10 mg kg^{-1}, orally
Number of chicks used	6 (XXOXOX)*
Increase or decrease in dose	5 mg kg^{-1}, orally
Dichlorvos	
LD_{50}	6.30 mg kg^{-1}, orally
Initial dose	15 mg kg^{-1}, orally
Last dose	10 mg kg^{-1}, orally
Number of chicks used	6 (XXOXOX)*
Increase or decrease in dose	5 mg kg^{-1}, orally

**X = death; O = survival.*

Determination of LD_{50} of OP insecticides

The acute (24 h) LD_{50} of each OP insecticide was determined in chicks using the up-and-down method (30). The chicks were individually observed for the appearance of clinical signs of cholinergic toxicosis for two hours after OP dosing, and the 24-hour lethality was recorded.

Acute toxicity of OP insecticides

After determining the LD_{50} for each OP insecticide in the previous experiment, we randomised other chicks into three control and six OP groups of six birds each. Control chicks received distilled water at 5 mL kg^{-1} by gavage needle. OP groups received either half or full LD_{50} single oral doses (corrected to the nearest mg kg^{-1}) of the insecticides: chlorpyrifos at 5 mg kg^{-1} (half the LD_{50} dose) or 11 mg kg^{-1} (full LD_{50} dose) and diazinon and dichlorvos at 3 mg kg^{-1} (half the LD_{50} dose) or 6 mg kg^{-1} (full LD_{50} dose) each. We observed the chicks for the signs of cholinergic toxicosis and death within two hours after OP treatment.

Effects of OP insecticides on plasma and brain ChE activities

Two hours after each OP treatment, the chicks were euthanised to determine plasma and whole brain ChE activity. The brains of chicks which died within the two hours from poisoning were also included in ChE determination. However, we did not take blood samples of the dead chicks because of clotting problems. All brain and plasma samples were kept at -20 °C pending ChE analysis within one week. The whole brain was homogenised with a glass homogeniser in an ice bath in a pH 8.1 phosphate barbital buffer at 3 mL per 100 mg wet weight (19, 31, 32). An electrometric method described before in chickens (18, 19) and other birds (31, 32) was used to determine ChE activity in the plasma and brain samples. The reaction mixture contained 3 mL of distilled water, 0.2 mL of plasma or brain homogenate, and 3 mL of pH 8.1 solution of barbital-phosphate buffer (31, 32). The pH of the mixture (pH1) was measured using a pH meter with a glass electrode (Hanna Instruments, Romania). Then 0.10 mL of the substrate 7.5 % acetylthiocholine iodide (Merck, Germany) was added to the mixture, which was incubated at 37 °C for 30 min. At the end of the incubation period, the pH of the reaction mixture (pH2) was measured. Enzyme activity was calculated as follows:

ChE activity in units of ΔpH = (pH1 – pH2) - ΔpH of the blank

The blank contained no plasma or brain. The barbital-phosphate buffer solution consisted of 1.24 g sodium barbital (BDH, U.K.), 0.63 g potassium

Table 2 *Inhibition of plasma and whole brain cholinesterase activities in chicks dosed orally with organophosphate insecticides*

Organophosphate treatment	Plasma		Whole brain	
	ΔpH	inhibition %	ΔpH	inhibition %
Control (without treatment)	0.49±0.029		0.22±0.022	
Chlorpyrifos / mg kg^{-1}				
5	0.35±0.017	29	0.15±0.036	32
11	0.12±0.054*	76	0.05±0.012*	77
Diazinon / mg kg^{-1}				
3	0.31±0.024*	37	0.18±0.017	18
6	0.21±0.031*	57	0.10±0.014*	55
Dichlorvos / mg kg^{-1}				
3	0.25±0.027*	49	0.09±0.021*	59
6	0.08±0.013*	84	0.05±0.013*	77

Cholinesterase activity values are expressed as mean ± SE; n=18 chicks in the control group (pooled) and 6 chicks in each organophosphate treatment group. Cholinesterase activity was determined two hours after the insecticide dosing. The number of dead chicks treated with the higher dose of chlorpyrifos, diazinon, and dichlorvos were 2, 2, and 3, respectively, and their plasma cholinesterases were not determined.
** Significantly different from the control value, $p<0.05$.*

dihydrogen phosphate (Merck), and 35.07 g sodium chloride (BDH) dissolved in one litre of distilled water (31, 32).

The percentage of ChE inhibition was calculated as follows:

% ChE inhibition= [ChE activity (without OP)-ChE activity (with OP)/ ChE activity (without OP)] x 100

ChE activity values from the three control groups were pooled so that the final number of chicks in the control group was 18. Data as multiple means were subjected to the analysis of variance followed by Tukey's test (33). The level of statistical significance was set at $p<0.05$. The calculation of correlation coefficient (r) between the percentages of brain and plasma ChE inhibitions (using 29 inhibition values of plasma and corresponding brain ChEs of OP-treated chicks) was based on the regression line for both variables (33).

RESULTS

The acute (24 h) oral LD_{50} of chlorpyrifos, diazinon and dichlorvos in chicks, determined by the up-and-down method, was 10.79 mg kg^{-1}, 6.32 mg kg^{-1}, and 6.30 mg kg^{-1}, respectively (Table 1). The signs of cholinergic toxicosis appeared within two hours after dosing and they included salivation, lacrimation, gasping, frequent defecation, drooping of wings, tremors, convulsions, and recumbency before death.

Oral treatment of chicks with half the LD_{50} dose of chlorpyrifos (5 mg kg^{-1}), diazinon (3 mg kg^{-1}), and dichlorvos (3 mg kg^{-1}) caused immobility and wing drooping, but not the clinical signs of cholinergic toxicity seen in the LD_{50} experiment with these insecticides. However, at full LD_{50} doses, these OP insecticides caused clinical signs similar to those seen in the LD_{50} experiment. Two out of six chicks died within two hours after treatment with either chlorpyrifos and dichlorvos, whereas full LD_{50} dose of diazinon caused death in three out of 6 chicks. Compared to control animals, the OP insecticides reduced plasma and whole brain ChE activities by (29 to 84) % and (18 to 77) %, respectively, depending on the dose applied (Table 2). Correlation analysis showed that the inhibition of brain ChE activity correlated well (r=0.82) with that of the plasma ChE activity (Figure 1).

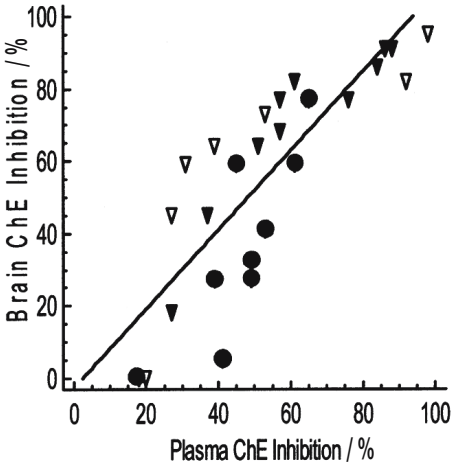

Figure 1 *Correlation between inhibition (%) of brain and plasma cholinesterase (ChE) activities in organophosphate-treated chicks (n=29); r = 0.82. Open triangles=chlorpyrifos, closed circles=diazinon and closed triangles=dichlorvos. See Table 1 for details of treatment and ChE inhibition.*

DISCUSSION

Our findings of the acute toxicity (LD_{50}) of chlorpyrifos, dichlorvos, and diazinon in chicks are generally in agreement with their toxic effects reported by others (20, 24, 25, 34, 35). In male leghorn chicks the reported acute oral LD_{50} of chlorpyrifos was 32 mg kg^{-1} [range: (14 to 72) mg kg^{-1}] (34); whereas that of dichlorvos was 6.45 mg kg^{-1} [range (5.10 to 8.06) mg kg^{-1}] (35). The LD_{50} of diazinon in birds generally ranges between 2.75 mg kg^{-1} and 40.8 mg kg^{-1} (36). According to their oral 24-hour LD_{50}, these insecticides could be categorised as highly toxic in chicks (37, 38). This supports the classification of OP insecticides according to their toxic potentials (3, 25). Several studies have indicated that bird sensitivity to acute OP poisoning does not differ substantially from that of mammals (12, 24, 25).

In our study, OP insecticides induced in chicks the signs of poisoning characteristic of cholinergic overstimulation. They were in similar to those reported in chickens and other birds acutely poisoned with OP insecticides (9, 19, 22, 24). The main mechanism of toxic action of OP insecticides in the avian species, as well as in mammals, is ChE inhibition with subsequent development of nicotinic, muscarinic, and central nervous effects (3, 6, 24, 25). Dichlorvos directly inhibits the target ChE, whereas chlorpyrifos and diazinon act through their active oxon metabolites (24-26, 37, 39).

Reduced ChE activity is still a reliable indicator of OP poisoning and a biomarker of absorption of OP insecticides (3, 4, 6-10). In the avian species, ChE activities in the brain and plasma are frequently measured to assess the condition of exposure or poisoning (9, 10, 12-14, 18, 19, 22, 24). In this study, the decreases in ChE activity in plasma (29 % to 84 %) and brain (18 % to 77 %) were dose–dependent; full LD_{50} doses of the OP insecticides caused greater enzyme inhibition in plasma (57 % to 84 %) and brain (55 % to 77 %).

Inhibition of brain ChE correlated well (r=0.82) with that of plasma ChE (Figure 1). Several studies also reported a concurrent drop in plasma and brain ChE activities in chickens acutely poisoned with various dosing regimens of chlorpyrifos (19, 20), dichlorvos (23), and diazinon (40). One hour after acute oral administration in chickens, dichlorvos (7 mg kg^{-1}) was reported to cause concurrent decreases in brain and plasma ChE activities by 62 % and 29 %, respectively (19). Chlorpyrifos also reduced both brain (43 % to 69 %) and plasma (40 % to 70 %) ChE activities in chicks two hours after acute oral administration at doses of 5 mg kg^{-1}, 10 mg kg^{-1}, and 20 mg kg^{-1} (20). Thirty minutes after dosing, diazinon at 10 mg kg^{-1} orally decreased brain and plasma ChE in chicks by 20 % and 79 %, respectively (40). A similar trend has been observed with brain and blood ChE activities following OP poisoning in rats (41, 42) and wild birds (9, 10). However, there are a number of exceptions to this generalisation (3, 6, 25, 43). Many factors may influence the extent of ChE inhibition in OP poisoning. These include the type of OP, its dose, route and duration of exposure, species involved, toxicokinetic aspects of the insecticide, tissues examined, or sampling time (3-6, 25, 33).

CONCLUSION

In conclusion, acute exposure of chicks to OP insecticides is associated with concurrent reductions in brain and plasma ChE activities and with signs of poisoning at doses close to their LD_{50} in the surviving birds. Further studies are needed to (re)evaluate the toxicity and ChE inhibition of other OP insecticides using chicks as a suitable animal model for acute OP toxicity studies.

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Sažetak

AKUTNA ORALNA TOKSIČNOST ORGANOFOSFORNIH INSEKTICIDA I INHIBICIJA KOLINESTERAZA U PILIĆA

Ispitano je akutno toksično djelovanje triju često rabljenih organofosfornih insekticida klorpirifosa, diazinona i diklorvosa u brojlera te je izmjerena aktivnost kolinesteraza u njihovoj plazmi i mozgu. Srednja letalna doza LD_{50} klorpirifosa iznosila je 10,79 mg kg^{-1}, diazinona 6,32 mg kg^{-1} te diklorvosa 6,30 mg kg^{-1}. Prvi su se znakovi kolinergičkoga sindroma u pilića javili unutar dva sata od oralne primjene, a obuhvaćali su slinjenje, suženje, teško disanje, učestalu defekaciju, obješena krila, drhtavicu, grčenje i nesposobnost stajanja uoči smrti. Oralna primjena polovice srednje letalne doze insekticida klorpirifosa (5 mg kg^{-1}), diazinona (3 mg kg^{-1}) i diklorvosa (3 mg kg^{-1}) dovela je do nepokretnosti i obješenih krila, ali bez kliničkih znakova kolinergičke toksičnosti koji su uočeni kod pokusa radi utvrđivanja srednje letalne doze (LD50). Međutim, doze ovih insekticida koje su odgovarale LD_{50}, dovele su do kliničkih znakova kolinergičke toksičnosti sličnih onima zamijećenim kod utvrđivanja LD_{50}. Dva od šest pilića uginula su unutar dva sata od primjene bilo klorpirifosa bilo diklorvosa u dozama koje su odgovarale LD_{50}, dok je diazinon u odgovarajućoj srednjoj letalnoj dozi uzrokovao smrt triju od šest pilića. U odnosu na kontrolne vrijednosti, insekticidi su doveli do smanjenja aktivnosti kolinesteraze koja je ovisila o dozi, a kretala se od 29 % do 84 % u plazmi te od 18 % do 77 % u mozgu. Pad aktivnosti kolinesteraze u plazmi dobro je korelirao s njezinim padom u mozgu (r=0,82). Ovi podaci upućuju na to da oralna primjena organofosfornih insekticida u dozama koje odgovaraju srednjoj letalnoj dozi dovode do znakova kolinergičkoga trovanja u pilića te do istodobnoga pada aktivnosti kolinesteraza u mozgu i plazmi.

KLJUČNE RIJEČI: *antikolinesteraze, diazinon, diklorvos, klorpirifos, kolinergička toksičnost, LD_{50}*

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