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Effects of Oximes and Atropine on Acute Phosphamidon Intoxication in *Bubalus bubalis*

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Owing to their widespread application in agriculture and veterinary medicine, organophosphate (OP) insecticides are frequently involved in livestock poisonings in many cases with fatal consequences (Singh 1981; Kimura et al. 1988; Smith and Lewis 1988; Pritchard 1989). Of several causes, farm animal species including buffaloes may encounter OP poisoning by ingestion of insecticide sprayed fodder (Singh 1981; Hatch 1988). Although specific treatment of OP intoxication includes atropine sulfate (ATS) to counteract the muscarinic effects and oximes to reactivate cholinesterase (ChE) enzyme, the success of therapy varies from one OP insecticide to another and with the animal species involved (Gupta 1984; Srivastava 1984; Sandhu 1985; Hatch 1988). OP insecticide phosphamidon is widely employed to control insect pests on various crops including forages and has potential to induce severe intoxication in buffaloes (Jha 1987; Awal et al. 1988). However, there are, as yet, no data to establish the efficacy of oximes and ATS in phosphamidon intoxication in buffalo species. The present study was, therefore, undertaken to investigate the effectiveness of diacetyl monoxime (DAM) and 2-pyridine aldoxime methochloride (2-PAM) in conjunction with ATS to counteract acute toxicity and inactivation of circulating esterases induced by phosphamidon in buffalo calves.

MATERIALS AND METHODS

Healthy male buffalo calves weighing between 70 and 120 kg, purchased from the local market, were acclimatized to the departmental animal house conditions for

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14 days before being used in this investigation. Animals were maintained on green fodder, wheat straw and water was provided ad libitum. All the calves were fasted for 18-20 hr prior to administration of phosphamidon in a single dose of 80 mg/kg body wt. The requisite quantity of phosphamidon (0,0-dimethyl-0-(2-chloro-2-(diethylcarbamoyl)-1-methylvinyl) phosphate, dimecron; 85% w/w; Hindustan Ciba Geigy Ltd., Bombay) diluted with 100 ml of tap water was given by drenching (p.o.). The insecticide-poisoned animals were randomly divided into 3 groups and were treated with antidotes within 15-20 min of phosphamidon administration. Animals of groups I, II and III were given ATS (Sigma Chemical Co., St. Louis, MO), DAM (Sisco Research Labs., Bombay) plus ATS and 2-PAM (Aldrich Chemical Co., Milwaukee, WI) plus ATS, respectively. These drugs were administered by intravenous (i.v.) and intramuscular (i.m.) routes. The details of dosages and schedule of administration of antidotes are presented in Table 1.

All the treated animals were observed closely for the appearance of toxic signs and for any protection against insecticide-induced lethality. Blood samples were taken from the jugular vein of each calf into heparinized and nonheparinized tubes at predetermined time intervals to investigate the influence of different antidotal treatments on phosphamidon-inhibited esterases. Plasma was separated from the heparinized blood by centrifugation at 3000 rpm for 15 min. The ChE activity in erythrocytes and plasma was determined according to the method of Voss and Sachsse (1970) as modified by Moroi et al. (1976). Serum carboxylesterase (CarbE) was determined by the method of Mendoza et al. (1971). Data were evaluated statistically using Student's t-test and $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Table 1 presents the results on the effects of different antidotal treatments on phosphamidon-induced clinical toxicity and lethality. The rationale for the selection of dosages of antidotes has been recently described (Srivastava 1984; Malik and Srivastava 1987). Antidotes were administered within 15-20 min after insecticide exposure and at this time, all the animals exhibited toxic signs which were characterized by frothy salivation, excitement, muscle fasciculations, tremors, incoordination and respiratory distress. The time course and dose-response relationship for acute phosphamidon toxicosis in buffalo calves revealed that the dose of phosphamidon (80 mg/kg body wt) used in the present study was capable of producing 100%

Table 1. Effects of oximes and atropine sulfate on phosphamidon (80 mg/kg, p.o.)-induced toxic signs and lethality in buffalo calves

Group	Drug[a]	No. of animals used	Treatment regimen	Time of disappearance of toxic signs after initial treatment (min)	No. of animals survived	Time of death after phosphamidon administration (hr)
I	ATS	3	0.5 mg/kg (1/4 i.v. and 3/4 i.m.) followed by 0.5 mg/kg, i.m. at 5-6, 12-14, 24-26 and 48 hr	10-15	0	4-120
II	DAM	3	30 mg/kg, i.v. followed by 15 mg/kg, i.m. at 12 hr	10-15	0	15-76
	+ ATS		+ 0.5 mg/kg (1/4 i.v. and 3/4 i.m.) followed by 0.5 mg/kg, i.m. at 5-6, 14-16 and 48 hr			
III	2-PAM	3	30 mg/kg, i.v. followed by 15 mg/kg, i.m. at 4 and 8 hr	10-15	0	26-42
	+ATS		+ 0.5 mg/kg (1/4 i.v. and 3/4 i.m.) followed by 0.5 mg/kg, i.m. at 6-8, 14-15 and 25-26 hr			

[a]Drugs were given 15-20 min after administration of phosphamidon

Table 2. Effects of oximes and atropine sulfate on blood cholinesterases of buffalo calves intoxicated with phosphamidon (80 mg/kg, p.o.)

Drug[a]	Time after phosphamidon administration (hr)						
	0	0.25	1	4	12	24	72
	Erythrocyte cholinesterase (nmol acetylthiocholine hydrolyzed/min/mL)						
ATS	2228±37	1915±57[b]	1619±89[b]	1549±87[b]	1332[c]	1410[d]	1201[d]
DAM+ATS	2228±14	1941±82[b]	1497±79[b]	1688±51[b]	1358±30[b]	1279[c]	1044[d]
2-PAM+ATS	2124±17	1810±35[b]	1480±46[b]	1163±17[b]	1010±17[b]	975±35[b]	
	Plasma cholinesterase (nmol acetylthiocholine hydrolyzed/min/mL)						
ATS	167±1.2	147±2.9[b]	127±4.9[b]	120±8.6[b]	115[c]	126[d]	113[d]
DAM+ATS	162±1.2	138±3.7[b]	100±2.0[b]	123±8.0[b]	102±2.4[b]	104[c]	96[d]
2-PAM+ATS	164±1.5	146±1.5[b]	115±1.4[b]	113±2.5[b]	107±1.4[b]	106±1.4[b]	

Values given are mean±SE of 3 animals unless otherwise stated

[a]Drugs were given 15-20 min after administration of phosphamidon. For details, see Table 1

[b]Significant difference when compared with 0 hr value of the same animals ($P < 0.05$)

[c]Value is mean of 2 animals

[d]Value is of 1 animal

mortality within 2-9 hr (Awal et al. 1988). Obviously, the dose was lethal because it cannot be eliminated before it completely inactivated sensitive esterases. Administration of antidotes abolished the toxic signs within 10-15 min but there was reappearance of these signs necessitating the repeated dosing with oximes and ATS. Although ATS, DAM plus ATS and 2-PAM plus ATS increased the survival time to 4-120, 15-76 and 26-42 hr, respectively as compared to 2-9 hr in untreated calves (Awal et al. 1988), none of the treatments employed was eventually effective in combating phosphamidon-induced lethality.

Phosphamidon caused significant ($P < 0.05$) inactivation of erythrocyte ChE (13-15%) and plasma ChE (11-15%) within 15 min (Table 2). At this time, serum CarbE was also significantly ($P < 0.05$) inhibited to the extent of 11-17% (Table 3). Since ATS has no potential to reactivate phosphorylated-ChE and acts solely by its muscarinic cholinolytic action (Taylor 1991), it was administered to prevent symptoms and the rate of reactivation of esterases was measured with or without oximes. There was no significant ($P > 0.05$) reduction in the ChE and CarbE inhibition in animals treated

Table 3. Effects of oximes and atropine sulfate on serum carboxylesterase of buffalo calves intoxicated with phosphamidon (80 mg/kg,p.o.)

Drug[a]	Time after phosphamidon administration (hr)						
	0	0.25	1	4	12	24	72
ATS	117 ±2.6	104 ±3.0[b]	91 ±4.0[b]	83 ±6.9[b]	72[c]	72[d]	72[d]
DAM +ATS	104 ±4.0	86 ±4.7[b]	65 ±4.0[b]	81 ±1.3[b]	63 ±5.5[b]	72[c]	76[d]
2-PAM +ATS	115 ±1.8	96 ±1.9[b]	67 ±3.2[b]	76 ±1.9[b]	65 ±1.9[b]	59 ±1.8[b]	

Values given are expressed as nmol indophenol formed/min/mL and represent the mean±SE of 3 animals unless otherwise stated.

[a]Drugs were given 15-20 min after administration of phosphamidon. For details, see Table 1

[b]Significant difference when compared with 0 hr value of the same animals ($P < 0.05$)

[c]Value is mean of 2 animals

[d]Value is of 1 animal

with DAM plus ATS and 2-PAM plus ATS as compared to those given ATS alone. The esterase activities remained significantly ($P < 0.05$) inhibited in all the treated groups at various time intervals. It is thus apparent that the degree of acute exposure to OP is critical. Various factors such as the physiology of the animal, the reactivation rate by oximes and the inhibition rate by OP determine the amount of excess OP the animal is capable of handling per unit of time. The OP- inactivated esterases may be regenerated by oximes during treatment, but sufficient OP is usually present to further inhibit these reactivated enzymes. It is, therefore, important that therapy must be carried out until all the OP is hydrolyzed and eliminated and sufficient esterases are present for the sustenance of life of intoxicated animal.

It is desirable to employ oxime reactivators in conjunction with ATS as the combination produces far more satisfactory results than does either drug alone (Hatch 1988; Taylor 1991). Oximes and ATS have been used to combat acute OP intoxication in buffaloes with varying success. DAM alone or in conjunction with ATS has been reported to cause marked reactivation of phosphorylated-esterases and to completely antagonize the acute toxicity induced by 100% lethal dose of fenitrothion (Srivastava 1984). The combined therapy was, however, of no benefit against monocrotophos intoxication (Sandhu 1985). Conversely, a widely employed oxime 2-PAM was found to accentuate toxicity of malathion in buffalo calves (Gupta 1984).

An early administration of ChE-reactivating oxime has been emphasized for combating OP intoxication, since with some OP compounds aging of the phosphorylated-ChE occurs and the aged enzyme becomes refractory to dephosphorylation by oximes (Taylor 1991). In the present study, although antidotal therapy was instituted within 15-20 min after insecticide administration, no significant ($P > 0.05$) reactivation of phosphamidon-inhibited esterases was seen in animals treated with either DAM plus ATS or 2-PAM plus ATS. These results suggest that phosphamidon is one of the most hazardous OP insecticides for buffaloes.

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