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ORGAN TOXICITY AND MECHANISMS

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Progression of Type I to Type II paralysis in acute organophosphorous poisoning: Is oxidative stress significant?

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Abstract Organophosphorous poisoning is a common method of deliberate self-harm in countries where the pesticides are readily available and can result in type I, II and/or III paralysis. The in-hospital morbidity and mortality of the poisoning are mostly associated with type II paralysis (intermediate syndrome). The aim of this study was to determine the role of oxidative stress in relation to the severity of poisoning and development of type II paralysis in patients suffering from acute organophosphate poisoning. This prospective study was carried out at the Christian Medical College Hospital. Thirty-two patients with acute organophosphorous poisoning, admitted in one medical unit over 17 months, were included in the study. They were clinically assessed for severity of poisoning and paralysis during the first 10 days of their hospitalisation. Temporal profiles of butyrylcholinesterase (BuChE) and oxidative stress parameters, for 4, 7 and 10 days of hospitalisation, were established in 25 of these patients. Type I and II paralysis were associated with severe poisoning. The majority of patients with type II paralysis had prior evidence of type I paralysis. The pattern of muscles that were paralysed in type I paralysis occurring alone and in type I paralysis proceeding to type II paralysis were similar. BuChE was significantly inhibited in all patients. Oxidative stress occurred in acute organophosphate poisoned patients and was greater in severe poisoning. The results suggest that type I paralysis may progress to type II paralysis in severely poisoned patients. They demonstrate early occurrence of oxidative stress in severe acute organophosphate poisoning. However, the development of type II paralysis is not associated with the level of oxidative stress. They suggest that mechanisms other than acetylcholine induced oxidative stress may be involved in the progression of type I to type II paralysis.

Keywords Butyrylcholinesterase · Organophosphate poisoning · Type I and type II paralysis continuum · Oxidative stress

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Introduction

Deliberate self-harm is an under-recognised public health problem in many countries of the developing world. Pesticides, especially the organophosphorous compounds, are common poisons used in deliberate self-harm. Organophosphorous pesticides are inhibitors of acetylcholinesterase (AChE) that can result in fatal neuromuscular paralysis if ingested or absorbed through the skin or mucous membrane. The occurrence and severity of paralysis is determined by the dose of organophosphate exposure. Acute organophosphorous poisoning (OPP) may cause three types of neuromuscular weakness: (1) type I paralysis—muscle weakness occurring at admission or within the first day associated with cholinergic signs. (2) Type II paralysis or intermediate syndrome, a delayed muscle weakness characterised by cranial, proximal limb, trunk and respiratory muscle weakness occurring 48–72 h after the poisoning, following the cholinergic phase. Complete recovery from type II paralysis usually takes 4–15 days. (3) Type III paralysis, a polyneuropathy occurring 1–4 weeks after ingestion of specific organophosphates (Wadia et al. 1974; Senanayake and Karalliedde.1987; Karalliedde 1999).

The in-hospital morbidity and mortality of organophosphate poisoning due to deliberate self-harm in India are mainly associated with type II paralysis (Khan et al. 2001; Samuel et al. 1995). There are no specific treatments to prevent or treat type II paralysis as its pathophysiology is not known. It would be necessary to elucidate mechanisms that underlie the development of type II paralysis if rational therapeutic interventions are to be considered.

In an earlier study, we found that severely poisoned patients who developed type I paralysis often went on to develop type II paralysis (Mathew et al. 2003). The same group of muscles, extraocular, facial, neck, shoulder and hip, were affected in both type I and II paralysis. These muscles were weak for a mean of 0.13–3 days in patients who only developed type I paralysis and for a mean of 3.21–11.29 days in those who also developed type II paralysis. It, therefore, appeared that type II paralysis was a continuum of type I paralysis in severe acute OPP.

Severe, persistent inhibition of AChE is postulated to underlie the development of type II paralysis (De Bleecker et al. 1993). Muscle injury and cellular oxidative stress occur in acute OPP and are correlated to increasing severity of poisoning and development of type II paralysis. They reflect inhibition of AChE (Mathew et al. 2003; Dandapani et al. 2003). AChE inhibition does not, however, account for the near absence of muscarinic signs in type II paralysis or the absence of paralysis in most patients despite severe, prolonged inhibition of AChE.

Although oxidative stress is not specific to organophosphate poisoning and can occur with other classes of pesticides, studies have shown increased oxidative stress and damage in both acute and chronic OPP in humans (Banerjee et al. 1999; Vidyasagar et al. 2004; Ranjbar et al. 2002). Increased oxidative stress has been associated with adverse outcome in acute OPP (Vidyasagar et al. 2004). Acute OPP also results in oxidative damage that occurs independent of AChE inhibition as is seen in renal tubular cell injury of OPP (Kovaic 2003; Poovala et al. 1998). The objective of our study was to examine the role of oxidative stress in the progression of type I to type II paralysis in acute OPP.

Methods

Patients

Thirty-two acute organophosphate poisoned patients admitted to hospital under one medical unit between May 2002 and September 2003 were included, with informed consent, in this study approved by the Institutional Research Committee. Their inclusion criteria, assessment of severity of poisoning at admission (Namba et al. 1971) and medical management were as given earlier (Khan et al. 2001).

A case of organophosphate poisoning was defined by two of the following three criteria: (1) history of organophosphate poison consumption (as ascertained by bottle/packet brought with the patient), (2) signs of organophosphate poisoning (at least one of the following three signs: bronchorrhea/increased salivation; miosis; fasciculations). (3) Low butyrylcholinesterase (BuChE) activity ($< 50\%$ of normal activity).

Organophosphate poisoned patients were seen in the emergency department and subsequently assessed daily for the first 10 days of hospitalisation. Assessment was done clinically for severity of poisoning [by the Namba scale of poisoning (mild/moderate/severe grades)] (Namba et al. 1971) (Table 1), presence of type II paralysis (intermediate syndrome), coma (assessed by the Glasgow coma scale), daily atropine requirement, ventilatory requirement and the development of respiratory infection. Daily muscle assessments included evaluation for facial, extraocular and neck muscle weakness and hip and shoulder muscle power, according to the Medical Research Council (UK) grading (1978) and the measurement of Forced Vital Capacity. The patients were managed according to standard clinical protocols. None of the patients were administered oximes during the period of the study. Oximes have been withdrawn from the management protocol of acute organophosphate poisoned patients in our institution after the results of two randomised controlled trials (Johnson et al. 1996; Cherian et al. 1997).

The guideline for atropinisation was as follows: injection atropine was administered by intravenous bolus in the emergency room to achieve a heart rate of 110/min, and then continued by infusion to maintain the same heart rate on the first day, a rate of 100/min on the second day, 90/min on the third day, 80/min on the fourth day and subsequently tapered.

Type I paralysis was defined as the occurrence of muscle weakness at admission or in the first 24 h accompanied by cholinergic signs.

Type II paralysis (intermediate syndrome) was defined as proximal muscle paralysis of grade 3 or less, 72 h after ingestion of organophosphate with or without requirement of mechanical ventilation, along with extraocular, neck and respiratory muscle weakness. The duration of intermediate syndrome was defined as the time taken to regain grade 4 muscle power in hip and shoulder muscles.

The MRC grading of muscle power was used for shoulder, elbow, hip, knee and ankle: 0, complete paralysis; 1, flicker of movement; 2, movement possible when gravity eliminated; 3, movement possible against gravity but not against any further resistance; 4, movement possible against partial resistance; 5, movement possible against full resistance or normal muscle power. Neck muscle weakness was assessed by the ability to lift the head off the bed and hand muscle weakness by the intensity of handgrip perceived by the examiner. In the ventilated patient, muscle assessments were performed in the supine position and the sitting position when possible. Assessments were performed when the patient was awake and conscious. If the patient could not be

Table 1 Grades of organophosphate poisoning (Namba et al. 1971)

Mild poisoning	The patient can walk but complains of fatigue, headache, dizziness, numbness of extremities, nausea and vomiting, excessive sweating and salivation, tightness in chest, abdominal cramps or diarrhoea. Serum BuChE is 20–50% of normal value.
Moderate poisoning	The patient cannot walk and there is generalised weakness, difficulty in talking, muscular fasciculations, miosis and severe symptoms of mild poisoning. Serum BuChE is 10–20% of normal value.
Severe poisoning	Unconsciousness, marked miosis and loss of papillary reflex to light, muscular fasciculations, flaccid paralysis, secretions from the mouth and nose, moist rates in lungs, respiratory difficulty and cyanosis. Serum BuChE $<10\%$ normal values.

aroused, then sedation was temporarily discontinued for a few hours till the patient was awake. If the patient was not cooperative for detailed muscle assessments, this was noted in the proforma.

Cholinergic symptoms were assessed at admission by the presence of sweating, vomiting, diarrhoea, salivation, wet lungs, fasciculations, miosis, bradycardia and urinary incontinence. The duration of cholinergic crises was assessed by the disappearance of the cholinergic signs.

ICU complications were defined as follows: (1) ventilator associated pneumonia—new onset pneumonia occurring after 72 h of hospitalisation in a ventilated patient characterised by fever, purulent tracheobronchial secretions and patch on the chest radiograph; (2) exposure keratitis—circum corneal congestion or loss of lustre of the cornea and corneal xerosis or corneal ulcer.

Coma was assessed by the Glasgow coma scale. The patient's consciousness was impaired if the score was less than 15 in a non-ventilated patient and less than 10 in a ventilated patient.

Laboratory methods

Blood was collected from most patients once a day and in all others as permitted by the patient up to 10 days of hospitalisation and once from 18 healthy controls with informed consent. Plasma, leucocytes and red blood cell membranes were separated and isolated in all samples (Dandapani et al. 2003). BuChE in plasma was estimated by the method of Ellman et al. (1961) using butyrylthiocholine as substrate.

Erythrocyte membranes were assayed for lipid peroxidation by measuring malondialdehyde formed using the thiobarbituric acid reaction (Ohkawa et al. 1979), conjugated dienes by their absorption at 233 nm (Chan and Levett 1977) and protein thiols by their reaction with 5′5′dithiobis (2-nitrobenzoic acid) (Habeeb 1972).

Leucocytes were assayed for catalase by measuring the breakdown of H_2O_2 at 240 nm (Aebi 1984), glutathione reductase by following the oxidation of NADPH at 340 nm in the presence of oxidised glutathione (Racker 1955) and xanthine oxidase and xanthine dehydrogenase by measuring uric acid production at 295 nm (Parks et al. 1988).

Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard.

Statistical analysis

Of the 32 patients, the analysis of oxidative stress includes only patients who could be followed up and excludes 6 patients who died early in the course of poisoning and 1 patient from whom serial blood samples could not be obtained.

Comparisons at admission were made between all OPP patients and 18 healthy controls from whom blood was obtained once.

To analyse the biochemical response over time, the area under the curve (AUC) was calculated for 4, 7 and 10 days of hospitalisation, as a summary statistic for all patients for BuChE and each oxidative parameter. The AUC is calculated by adding the areas under the curve between each pair of consecutive observations. It is the product of the time difference and the average of the two measurements (Mathews et al. 1990). These temporal profiles of BuChE and oxidative stress were compared between mild/moderately and severely poisoned patients and between all patients who developed and did not develop type II paralysis for 4 days of hospitalisation by the Mann–Whitney U test. They were also compared for 7 and 10 days of hospitalisation. Quantitative parameters between mildly poisoned patents who did not develop type II paralysis, severely poisoned patients who did not develop type II paralysis and severely poisoned patients who developed type II paralysis were analyzed using the Kruskal–Wallis test. For all tests $P<0.05$ was considered statistically significant. Statistical analysis was carried out using SPSS 11.0 software.

Table 2 Clinical profile of acute OPP patients

	Number		
	Total	Type II paralysis	
		Present	Absent
Number of patients	32[a]	6	21
Patients with mild/moderate poisoning	12	0	12
Patients with severe poisoning	20[a]	6	9
Patients with fasciculations	12[b]	4	7
Patients requiring mechanical ventilation	19	6	13
Patients requiring tracheostomy	6	5	1
Patients died	6[a]	1	

[a]Five patients could not be classified
[b]One patient could not be classified

Table 3 Profile of muscle weakness in type I and II paralysis in patients of acute OPP

Case	Paralysis	Weakness in muscles of									Minimum power grade	Duration of mechanical ventilation (days)	
		Face	Extra ocular	Neck	Shoulder	Elbow	Hand	Hip	Knee	Ankle			
TYPE I PARALYSIS ONLY												Duration of type I paralysis (days)	
1	Type I	+	+	+	+	+	+	+	+	+	0	3	3
2	Type I			+	+	+	+	+	+	+	3	3	5
3	Type I	+		+	+	+	+	+	+	+	2	2	3
4	Type I			+	+	+	+	+	+	+	3	2	6
TYPE II PARALYSIS ONLY												Duration of type II paralysis (days)	
5	Type I	+	+	+	+	+	+	+	+	+	2		
	Type II	+	+	+	+	+	+	+	+	+	2	17	28
6	Type I			+	+	+	+	+	+	+	2		
	Type II			+	+	+	+	+	+	+	2	6	14
7	Type I	CA	CA	CA	CA	CA	CA	CA	CA	CA	CA		
	Type II			+	+	+	+	+	+	+	2	8	7
8	Type I	+	+	+	+	+	+	+	+	+	CA		
	Type II	+	+	+	+	+	+	+	+	+	CA	12	15
9	Type I		+	+	+	+	+	+	+	+	CA		
	Type II			+	+	+	+	+	+	+	2	21	24
10	Type I	CA	CA	CA	CA	CA	CA	CA	CA	CA	CA		
	Type II	+	+	+	+	+	+	+	+	+	3	8	11

Cases 1–4 only developed type I paralysis. Cases 5–10 also developed type II paralysis
Type I paralysis represents muscle weakness in first 72 h of poisoning. Type II paralysis represents muscle weakness after 72 h
CA Muscle power could not be assessed due to sedation, altered consciousness or lack of cooperation
+ weakness present

Results

Baseline clinical characteristics of acute OPP patients

The mean age of the 32 patients was 30.8 years and the male–female ratio 7:1. In 28 patients, the ingestion was due to deliberate self-harm and in 4 the cause was uncertain. In 25 patients, all three diagnostic criteria were fulfilled and in the rest, two criteria were present. Twelve patients (37.5%) were admitted with mild/moderate poisoning and 20 (62.5%) with severe poisoning (Table 2). The mean lag time between ingestion of the poison and presentation to hospital was 7.02 h. Fasciculations were noted in 12 patients (37.5%) (Table 2). Cholinergic symptoms and signs present at admission included sweating (3.1%), vomiting (53.1%), diarrhoea (21.9%), miosis (50%), bradycardia (21.9%), respiratory distress (50%) and urinary incontinence (12.5%).

The ingested compounds were identified in 75% of cases. These included Phosphamidon (5), Monocrotophos (5), Methylparathion (4), Quinalphos (3), Triazophos (2), Dimethoate (2), Fenthion (1), Prophenophos (1), Chlorpyrifos (1).

Treatment and outcome

Eighteen patients were admitted in the medical intensive care unit, (ICU) of whom 94.4% were severely poisoned and 14 in the ward of whom 85.7% were mild/moderately poisoned. Sixteen patients had altered consciousness at admission and mean duration of altered sensorium was 2.6 days. The average duration of ICU care for those requiring it was 9.8 days and the average duration of hospitalisation for all patients was 9.2 days.

The average duration of cholinergic signs was 1.72 days, the average duration of atropine administration 2.62 days and the average dose of atropine was 102.2 mg ($\pm$266 mg). Nineteen patients (59.4%) required mechanical ventilation (mean duration 8.89 days) all of whom were severely poisoned (Table 2).

Twenty-seven patients could be assessed for paralysis, of whom six severely poisoned patients developed type II paralysis. The mean duration of type II paralysis was 12 days.

The medical complications that occurred in the ICU were: ventilator associated pneumonia (8), exposure keratitis (1), pneumothorax (2), acute renal failure due to rhabdomyolysis (1), tube dislocation (1), refractory shock (1) and unresponsive coma (1). Six severely poisoned patients required tracheostomy and six severely poisoned patients died (Table 2). The causes of death were: severe poisoning (3), rhabdomyolysis and acute renal failure (1), nosocomial pneumonia (2).

Type I and type II paralysis continuum

None of the patients with mild or moderate poisoning developed type I or type II paralysis. Among the severely

Table 4 Butyrylcholinesterase and oxidative stress in acute OPP patients on day 1 of hospitalisation

	Butyryl cholinesterase (U/l) (Mean ± SD)	Lipid peroxidation [U/mg protein (Mean ± SD)]	Conjugated dienes [U/mg protein (Mean ± SD)]	Protein thiols [U/mg protein (Mean ± SD)]	Xanthine dehydrogenase [U/mg protein (Mean ± SD)]	Xanthine oxidase [U/mg protein (Mean ± SD)]	Catalase [U/mg protein (Mean ± SD)]	Glutathione reductase [U/mg protein (Mean ± SD)]
Controls	5,489 ± 1,340	0.56 ± 0.405	19.04 ± 22.7	9.1 ± 4.45	146 ± 102	7.82 ± 6.85	0.52 ± 0.42	37.86 ± 30.96
All OPP	465 ± 790	2.68 ± 4.74	78.47 ± 144	22.59 ± 26.85	15 ± 23	45.42 ± 76.84	0.22 ± 0.18	7.65 ± 6.79
P	< 0.01	< 0.01	< 0.01	0.203	< 0.01	< 0.05	< 0.01	< 0.01

poisoned patients, five did not develop type I or type II paralysis, four developed type I but not type II paralysis and of six who developed type II paralysis, four had definite evidence of type I paralysis and two could not be adequately assessed for the presence of type I paralysis. Five patients were not classified as muscle power could not be assessed at admission because of coma and death before recovery from coma (Table 3).

The four patients who developed only type I paralysis had neck, shoulder, elbow, hip, knee and ankle weakness, the minimum weakness grade varied from 0 to 3, the duration of weakness from 2 to 3 days and the duration of mechanical ventilation from 3 to 6 days.

The six patients who developed type II paralysis had neck, shoulder, elbow, hip, knee and ankle weakness, the minimum weakness grade varied from 2 to 3, the duration of intermediate syndrome from 6 to 21 days and the duration of mechanical ventilation from 7 to 28 days. Of the six patients, four had similar distribution of weakness in the first 72 h. In two cases, accurate muscle assessments in the first 72 h were not possible because of altered consciousness, sedation and lack of cooperation from the patients.

Oxidative stress

Twenty-five patients were studied for oxidative stress. Among these 25 patients, 12 were mild/moderately poisoned, none of whom developed type II paralysis, and 13 were severely poisoned, 4 of whom developed type II paralysis.

Oxidative stress was compared between patient groups for different periods of hospitalisation of 4, 7 and 10 days. These specific time periods were chosen based on the duration of hospitalisation of different clinical groups and was done so as to control for prolonged immobilisation which itself can influence muscle injury and oxidative stress. Four days to equalise for immobilisation between all patients: 7 days to cover in-hospital stay of severely poisoned patients: 10 days to account for hospitalisation of severely poisoned patients who developed type II paralysis.

Butyrylcholinesterase was significantly inhibited in all OPP patients at admission (Table 4) and levels did not differ between patients of different severity of poisoning or between those who developed or did not develop type II paralysis over 4, 7 or 10 days of hospitalisation (Tables 5, 6).

A significant increase at admission, in lipid peroxidation, conjugated dienes and of xanthine dehydrogenase and xanthine oxidase and inhibition of catalase and glutathione reductase, was noted in all OPP patients compared to healthy controls (Table 4).

The temporal profiles of oxidative stress (AUC) for the first 4 days of hospitalisation did not differ significantly between mild/moderately and severely poisoned patients (Table 5) or between patients who developed or did not develop type II paralysis (Table 6). However the

Table 5 Temporal profiles of butyrylcholinesterase and oxidative stress for severity of acute OPP

OPP	Butyryl cholinesterase	Lipid peroxidation	Conjugated dienes	Protein thiols	Xanthine dehydrogenase	Xanthine oxidase	Catalase	Glutathione reductase
	U/l (Mean ± SD)	**AUC for 4 days of hospitalisation** [U/mg protein (Mean ± SD)]						
Mild/moderate	1,579 ± 1,802	3.35 ± 2.53	137 ± 166	53.7 ± 54.6	31.5 ± 32.2	68.7 ± 83.5	0.55 ± 0.24	14.6 ± 15.12
Severe	1,009 ± 1,311	7.76 ± 7.55	422 ± 712	80.8 ± 47.8	53.4 ± 80.7	117 ± 192	0.93 ± 0.92	36.6 ± 28.8
P	0.574	0.137	0.406	0.152	0.654	0.574	0.437	0.181
		AUC for 7 days of hospitalisation [U/mg protein (Mean ± SD)]						
Mild/moderate	1,579 ± 1,802	3.35 ± 2.53	137 ± 166	53.7 ± 54.6	31.5 ± 32.2	68.7 ± 83.5	0.55 ± 0.24	14.6 ± 15.12
Severe	2,975 ± 3,668	9.62 ± 8.12	809 ± 1,464	162 ± 161	112 ± 116	168 ± 219	1.53 ± 1.29	93.3 ± 44.6
P	0.437	0.06	< 0.05	< 0.01	0.099	0.123	< 0.05	< 0.01
		AUC for 10 days of hospitalisation [U/mg protein (Mean ± SD)]						
Mild/moderate	1,579 ± 1,802	3.46 ± 2.50	137 ± 166	53.7 ± 54.67	45.4 ± 29.8	68.7 ± 83.5	0.55 ± 0.24	58.32 ± 69.39
Severe	4,728 ± 6,704	8.08 ± 8.47	1,090 ± 2,094	233 ± 294	164 ± 187	219 ± 255	2.04 ± 1.78	75.25 ± 83.00
P	0.247	0.087	< 0.05	< 0.01	0.808	0.087	< 0.01	0.445

temporal profiles of oxidative stress (for conjugated dienes, protein thiols and catalase) for 7 and 10 days of hospitalisation were significantly increased in severe poisoning compared to mild/moderate poisoning (Table 5) and (for conjugated dienes, protein thiols, xanthine dehydrogenase and catalase) in patients who developed type II paralysis compared to those who did not (Table 6).

Analysis of variance between groups showed oxidative stress did not differ over 4 days of hospitalisation between mildly poisoned patents who did not develop type II paralysis, severely poisoned patients who did not develop type II paralysis and severely poisoned patients who developed type II paralysis. However the AUC of protein thiols, xanthine dehydrogenase and catalase were significantly different between the three groups over 7 and 10 days of hospitalisation, and for conjugated dienes and xanthine oxidase over 10 days only (Table 7).

Discussion

In this study, 18.75% (6 of 32) of patients developed type II paralysis. Earlier studies have shown rates of type II paralysis of 38.1 and 45.8% (De Silva et al. 1992), 18% (Wadia et al. 1974) and 42% (De Bleecker et al. 1993). Therefore, the rate of development of type II paralysis although on the lower side is comparable to other studies. The high rate of type II paralysis in our earlier studies of 72% (Khan et al. 2001), 68% (Mathew et al. 2003) and 84.2% (Dandapani et al. 2003) is probably due to the inclusion of only ICU admitted patients with severe poisoning. In this study, 43.8% of patients were non-ICU patients most of whom had mild/moderate poisoning. The impact of withdrawal of pralidoxime on the outcome of acute OPP and rate of type II paralysis at our centre is not clear.

The dose of organophosphate exposure and severity of poisoning were earlier shown to be the most important factors in determining the occurrence of type II paralysis in OPP (Khan et al. 2001). In the current studies, type I and type II paralysis did not occur in mild to moderate poisoning, however, this is not an invariable finding in other studies. In severe poisoning, about a third of the patients did not develop paralysis, a third developed type I paralysis which recovered in 72 h and a third developed type I paralysis which persisted beyond 72 h as type II paralysis. The pattern of muscles that were paralysed in type I paralysis occurring alone and in type I paralysis proceeding to type II paralysis were similar. These results suggest that type I and II paralysis

Table 6 Temporal profiles of butyrylcholinesterase and oxidative stress in development of type II paralysis of acute OPP

OPP	Butyryl cholinesterase	Lipid peroxidation	Conjugated dienes	Protein thiols	Xanthine dehydrogenase	Xanthine oxidase	Catalase	Glutathione reductase
	U/l (Mean ± SD)	**Type II paralysis AUC for 4 days of hospitalisation** [U/mg protein (Mean ± SD)]						
Negative	1,340 ± 1,647	4.91 ± 5.54	141 ± 155	56.4 ± 143.4	40.3 ± 65.1	87.3 ± 151	0.64 ± 0.40	26.5 ± 25.3
Positive	981 ± 1,113	9.49 ± 7.99	1,040 ± 1,111	127 ± 57.9	48.8 ± 33.8	129 ± 157	1.32 ± 1.52	15.2 ± 21.6
P	0.803	0.203	0.262	0.068	0.237	0.203	0.496	0.513
		Type II paralysis AUC for 7 days of hospitalization [U/mg protein (Mean ± SD)]						
Negative	1,966 ± 2,616	5.55 ± 5.67	179 ± 199	71.9 ± 53.7	50.8 ± 85.0	96.8 ± 153	0.87 ± 0.86	39.0 ± 45.9
Positive	4,086 ± 4,374	12.96 ± 9.3	2,100 ± 2,286	311 ± 237	152 ± 77.7	244 ± 241	2.06 ± 1.50	116 ± 1.78
P	0.113	0.096	< 0.05	< 0.01	< 0.05	0.067	< 0.05	0.103
		Type II paralysis AUC for 10 days of hospitalisation [U/mg protein (Mean ± SD)]						
Negative	2,451 ± 3,971	6.11 ± 6.85	185 ± 199	78.23 ± 64.59	111 ± 140	99.3 ± 152	0.96 ± 1.04	77.6 ± 76.7
Positive	8,324 ± 8,002	3.52 ± 3.05	2,733 ± 3,249	477 ± 431	4.89 ± 6.12	353 ± 295	3.07 ± 2.03	11.35 ± 5.60
P	< 0.05	0.402	< 0.05	< 0.01	< 0.05	< 0.05	< 0.05	0.41

Table 7 A comparison of temporal profiles of oxidative stress between clinical groups of acute organophosphate poisoned patients

OPP	Butyryl cholinesterase	Lipid peroxidation	Conjugated dienes	Protein thiols	Xanthine dehydrogenase	Xanthine oxidase	Catalase	Glutathione reductase
	U/l (Mean ± SD)	**AUC for 4 days of hospitalisation** [U/mg protein (Mean ± SD)]						
Mild/moderate type II negative	1,579 ± 1,802	3.35 ± 2.53	137 ± 166	53.7 ± 54.6	31.5 ± 32.2	68.7 ± 83.5	0.55 ± 0.24	14.6 ± 15.12
Severe type II negative	1,021 ± 1,454	6.85 ± 7.80	147 ± 149	59.9 ± 24.1	56.5 ± 104	129 ± 157	1.32 ± 1.52	15.2 ± 21.6
Severe type II positive	981 ± 11,13	9.49 ± 7.99	1,040 ± 1,111	127 ± 57.9	48.8 ± 33.8	129 ± 157	0.75 ± 0.54	47.2 ± 27.9
P	0.697	0.256	0.455	0.134	0.449	0.410	0.661	0.095
		AUC for 7 days of hospitalisation [U/mg protein (Mean ± SD)]						
Mild/moderate type II negative	1,579 ± 1,802	3.35 ± 2.53	137 ± 166	53.7 ± 54.6	31.5 ± 32.2	68.7 ± 83.5	0.55 ± 0.24	14.6 ± 15.12
Severe type II negative	2,482 ± 3,481	8.34 ± 7.52	235 ± 234	96.0 ± 44.3	86.4 ± 136	134 ± 215	1.29 ± 1.20	81.7 ± 52.7
Severe type II positive	4,086 ± 4,374	12.96 ± 9.3	2,100 ± 2,286	311 ± 237	152 ± 77.7	244 ± 241	2.06 ± 1.50	116 ± 1.78
P	0.262	0.081	0.059	< 0.01	< 0.05	0.109	< 0.05	< 0.05
		AUC for 10 days of hospitalisation [U/mg protein (Mean ± SD)]						
Mild/moderate type II negative	1,579 ± 1,802	3.46 ± 2.50	137 ± 166	53.7 ± 54.67	45.4 ± 29.8	68.7 ± 83.5	0.55 ± 0.24	58.32 ± 69.39
Severe type II negative	3,614 ± 5,683	9.65 ± 9.17	248 ± 231	110 ± 65	244 ± 182	140 ± 213	1.51 ± 1.43	100 ± 86
Severe type II positive	8,324 ± 8,002	3.52 ± 3.05	2,733 ± 3,249	477 ± 431	4.89 ± 6.12	353 ± 295	3.07 ± 2.03	11.35 ± 5.60
P	0.081	0.059	< 0.05	< 0.05	< 0.05	0.05	< 0.01	0.245

may not be distinctly separate clinical entities but an overlapping clinical continuum (Mathew et al. 2003). They also suggest that apart from the severity of poisoning there are other factors that lead to the development of type II paralysis.

Our earlier work shows that muscle injury occurs with acute OPP and is correlated to the severity of poisoning and the occurrence and severity of type II paralysis (Mathew et al. 2003). This indicates that AChE inhibition contributes to muscle injury in acute OPP. Hence there is a potential role of acetylcholine induced muscle injury in type I paralysis and progression to type II paralysis.

However, the near absence of muscarinic signs in the "persisting muscle weakness" of type II paralysis and that all patients with severe and persistent inhibition of AChE do not develop type II paralysis, suggests that in addition to AChE inhibition other mechanisms also contribute to the development of the paralysis.

In this study, oxidative damage was found to occur early in the course of acute OPP, arising from increased levels of superoxide and hydroxy radicals through induction of the pro-oxidant enzymes, xanthine dehydrogenase/xanthine oxidase, and insufficient ROS scavenging (by catalase as well as glutathione reductase). Ranjbar et al. (2005) have also shown oxidative stress to occur early in acute OPP, arising from depletion of antioxidant substrates.

Oxidative stress, shown in our study to occur in acute OPP, was greater in more severe poisoning but was not associated with the development of type II paralysis. Although oxidative damage was significantly greater in severely poisoned patients who developed type II paralysis at 7 and 10 days, compared to mildly or severely poisoned patients who did not develop type II paralysis, this difference was nullified when the role of immobilisation in contributing to oxidative stress was considered. Oxidative stress did not differ between OPP patients of different severity and paralysis when compared over equal days of immobility (the first 4 days of hospitalisation).

The severity of poisoning in OPP is determined by the extent of AChE inhibition (Mathew et al. 2003). Although BuChE levels do not truly reflect AChE levels, the degree of BuChE inhibition noted probably indicates significant AChE inhibition. These studies suggest that in acute OPP, oxidative damage arises from inhibition of AChE and implicate mechanisms that do not generate oxidative stress in the development of type II paralysis.

In this study, we could not demonstrate an association between muscle fasciculations and oxidative damage. This is in contrast to animal studies which have demonstrated relationship between organophosphate induced fasciculations, oxidative damage and muscle necrosis (Yang and Dettbarn 1998).

The results of this study support our earlier suggestion that organophosphate induced AChE inhibition initiates a process of muscle injury (Khan et al. 2001) that may partly be mediated through oxidative stress by

induction of pro-oxidant enzymes and inhibition of free radical scavenging enzymes. The similar levels of oxidative damage in all patients despite their severity of poisoning, that occurs early in the poisoning, suggests the activation of cellular protective mechanisms in patients who do not develop type II paralysis. Such protection may include heat shock proteins that are induced in OPP and protect against oxidative damage (Bagchi et al. 1996; Yang et al. 2002). Patients who do not express critical levels of heat shock proteins early in the poisoning may not be sufficiently protected and develop type II paralysis. This is currently under study.

In conclusion, in addition to the dose of exposure and severity of organophosphate poisoning, which reflect AChE inhibition, other factors may determine why some patients recover from type I paralysis and others progress to develop type II paralysis. Laboratory data support this clinical observation and suggest mechanisms other than acetylcholine induced oxidative stress may be involved in the progression of type I to type II paralysis.

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