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Poisoning with the S-Alkyl organophosphorus insecticides profenofos and prothiofos

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Summary

Background: Many organophosphorus (OP) insecticides have either two O-methyl or two O-ethyl groups attached to the phosphorus atom. This chemical structure affects their responsiveness to oxime-induced acetylcholinesterase (AChE) reactivation after poisoning. However, several OP insecticides are atypical and do not have these structures.

Aim: We aimed to describe the clinical course and responsiveness to therapy of people poisoned with two S-alkyl OP insecticides—profenofos and prothiofos.

Design: We set up a prospective cohort of patients with acute profenofos or prothiofos self-poisoning admitted to acute medical wards in two Sri Lankan district hospitals. Clinical observation was carried out throughout their inpatient stay; blood samples were taken in a subgroup for assay of cholinesterases and insecticide.

Results: Ninety-five patients poisoned with profenofos and 12 with prothiofos were recruited over 5 years. Median time to admission was 4 (IQR 3–7) h. Eleven patients poisoned with profenofos died (11/95; 11.6%, 95% CI 5.9–22); two prothiofos-poisoned patients required intubation. Both intubations and death occurred late compared with other OP insecticides. Prolonged ventilation was needed in those who survived—a median of 310 (IQR 154–349) h. Unexpectedly, red cell AChE activity on admission did not correlate with clinical severity—all patients had severe AChE inhibition (about 1% of normal) but most had only mild cholinergic features, were conscious, and did not require ventilatory support.

Conclusions: Compared with other commonly used OP insecticides, profenofos and prothiofos are of moderately severe toxicity, causing relatively delayed respiratory failure and death. There was no apparent response to oxime therapy. The lack of correlation between red cell AChE activity and clinical features suggests that this parameter may not always be a useful marker of synaptic AChE activity and severity after OP pesticide poisoning.
Introduction

Pesticide self-poisoning is a major global health problem, killing 250–350,000 people each year.\(^1,2\) Organophosphorus (OP) insecticide poisoning is a particularly severe problem, accounting for around 2/3 of deaths.\(^3\)

The majority of OP insecticides can be grouped according to their chemical structure as a diethoxy OP [with two O–C\(_2\)H\(_5\) groups attached to the phosphorus that binds to and inhibits acetylcholinesterase (AChE)] or a dimethoxy OP (with two O–CH\(_3\) groups) (see Figure 2 of Eddleston et al.\(^4\)). The identity of these alkyl groups has fundamental effects on the pharmacodynamics of poisoning and treatment, determining to a large extent whether oximes effectively reanimate OP-inhibited AChE.\(^4,5\)

However, a few OP insecticides do not fit into these categories, including profenofos and prothiofos (Figure 1). Both are highly lipid soluble, moderately toxic OP insecticides. Of note, they have an S-alkyl (S–C\(_3\)H\(_7\)) group attached to the phosphorus, in addition to the more typical O–C\(_2\)H\(_5\) group. Although in vitro studies with human red cells have been done,\(^6,7\) the consequences of this different structure on AChE inhibition and reactivation with oximes in vivo are unknown. We therefore prospectively studied patients with a history of S-alkyl OP poisoning to better understand its clinical course. Cholinesterase inhibition and re-activation with pralidoxime was studied in a subgroup of profenofos poisoned patients.

Materials and Methods

Patients

Study patients were consecutive patients admitted to two Sri Lankan hospitals with a history of acute poisoning with profenofos 50% emulsifiable concentrate (EC50) or prothiofos EC50, as indicated by the patient or relatives, the transferring doctor or the pesticide bottle. We have previously noted the ingestion history to be highly accurate.\(^4,8\) They formed part of a cohort from which patients were recruited to RCTs of routine activated charcoal administration (ISRCTN02920054) and high-dose pralidoxime (ISRCTN-55264358) that showed no effect.\(^9,10\) Ethics approval was obtained from the Oxfordshire Clinical Research Ethics Committee and Faculty of Medicine Ethics Committee, Colombo.

Patients were treated using a standard protocol that has been described earlier.\(^4,11\) They were resuscitated with fluids and atropine to raise the systolic blood pressure above 80 mmHg, the heart rate above 80 b.p.m. and clear the lungs of audible secretions and wheeze. Symptomatic patients who required atropine initially received pralidoxime chloride 1g q6h for 1–3 days. From May 2004, symptomatic patients requiring atropine were eligible for an RCT of high-dose pralidoxime chloride (2g loading dose over 20 min followed by 0.5 g/h for up to 7 days, or until atropine had not been required for 12–24 h) vs. saline placebo. Three profenofos poisoned patients were recruited; symptomatic patients not recruited to the trial received pralidoxime chloride 1g q6h for 1–3 days.

Figure 1. Characteristics and structure of (A) profenofos and (B) prothiofos.
Patients remained under the care of the consultant physicians. Management protocols were agreed between the ward doctors and study team. Decisions about intubation and transfer of patients to intensive care were made by the medical team independently of the study doctors. All decisions were made on the basis of the patient’s clinical condition and not the particular OP ingested.

All patients were seen regularly by full time study doctors at least every 3 h and more according to clinical need. Significant events (intubation, seizures and death) were recorded at the time of the event. Patients were also seen on a study ward round twice each day and their condition over the previous 12 h were recorded. We did not produce a Poison Severity Score routinely for patients; in a previous study, we noted that the Glasgow Coma Score (GCS) was as good as the Poison Severity Score in predicting outcome.12

Patients were first managed on the medical ward. Seriously ill patients, as judged by the ward’s medical staff on basis of GCS and cardiorespiratory function, were transferred to the intensive care unit (ICU) as beds became available. Each hospital had 2–8 ICU beds for medical patients; there was always difficulty in obtaining a bed. This article describes all patients with S-alkyl poisoning, whether they were admitted to ICU or not.

Criteria for intubation were tidal volume <180 ml/breath using a Wright’s respirometer, respiratory rate <10 breaths/min or failure of a Guedel airway to preserve airway patency. Arterial blood gases were not available to guide therapy; pulse oximetry was sometimes available. Hypotensive patients, not responding to atropine and fluid resuscitation, were treated with high doses of dopamine plus dobutamine as necessary by infusion pump. Norepinephrine and epinephrine infusions were not used; bolus epinephrine was administered for cardiac arrest.

**Toxicological analysis**

Blood samples were taken on admission, and at regular intervals thereafter, from patients recruited to the pralidoxime RCT and from patients admitted during two periods (9 May–10 July 2002 and 2–26 December 2002). Lab assay capacity limited the sample number that could be handled and determined the short period of sampling.

For red cell AChE measurement, 0.2 ml of EDTA blood was diluted at the bedside into 4 ml of cooled saline and rapidly frozen to −20°C. Red cell AChE activity is usually 600–700 mU/μmol Hb; a small study of Caucasians reported a mean of 651±18 mU/μmol Hb.14 We selected a value of 600 mU/μmol Hb to be the lower limit of normal for this study. Plasma was then separated from a second EDTA blood sample and frozen at −20°C. All analyses were done in Munich. AChE activity was assayed according to a modified Ellman method, using ethopropazine to inhibit butyrylcholinesterase (BuChE) activity.14 Re-activatability of AChE (ageing) and BuChE were assessed as described.5,14 A median BuChE value of 6000 mU/ml was taken as normal for this study.15

Profenofos was quantified in plasma by gas chromatography with a lower limit of quantitation of 0.05 nmol/ml plasma.

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Figure 2. (A) Timing of intubation; (B) duration of ventilation and (C) timing of death post-ingestion in profenofos and prothiofos poisoned patients. For 2C, patients were separated according to whether they were intubated (circles) or not (squares) before death.
Statistics
Data analysis was performed in Prism v4. Clinical characteristics were summarized using counts (percentages) for categorical variables and the median [interquartile range (IQR)] for non-normally distributed continuous variables.

Results
Between 31 March 2002 and 26 March 2007, 14,034 patients with acute poisoning were prospectively reviewed on admission to Anuradhapura and Polonnaruwa General Hospitals, Sri Lanka. One hundred and seven had ingested an S-alkyl OP insecticide—95 profenofos and 12 prothiofos.

The median age of the patients was 32 years (IQR 24–45); 82 (76.6%) were male. They presented a median of 4 h (IQR 3–7) post-ingestion. Fifty-seven (53.3%) had previously received atropine; of these patients 11 (10.3% of all patients) had received pralidoxime chloride in the transferring hospital. On admission to the secondary hospital, around one-third (37/107; 34.6%) were symptomatic, requiring atropine for cholinergic features. However, very few were severely poisoned with reduced consciousness—median GCS was high (15/15; IQR 15–15) with only 8/107 having a GCS <9.

Mortality and respiratory failure
Eleven patients poisoned with profenofos died (11/95; 11.6%, 95% CI 5.9–20); one prothiofos patient died (1/12; 8.3%, 95% CI 0.2–38). Deaths from profenofos poisoning occurred in a median of 29 (IQR 17–113) h post-ingestion (Figure 2C); none occurred within 11 h of ingestion. The death from prothiofos occurred 67 h post-ingestion.

Thirteen patients poisoned with profenofos required intubation for a low tidal volume (<180 ml/breath), bradypnoea, or failure of a Guedel airway to preserve airway patency (13/95; 13.7%, 95% CI 7.5–22). Two prothiofos-poisoned patients required intubation (2/12; 16.7% 95% CI 2.1–48). Intubation for profenofos poisoning occurred in a median of 9.2 (IQR 5.2–26) h post-ingestion (Figure 2A). Seven intubated patients with profenofos poisoning (7/13, 53.8%) survived. The two intubations for prothiofos poisoning occurred 11 and 30 h post-ingestion; one intubated patient survived. Of the seven profenofos-poisoned patients who survived, median duration of ventilation was 320 h (IQR 140–353) (Figure 2B). The intubated patient with prothiofos poisoning who survived was intubated for 300 h.

Cholinesterase inhibition and reactivation
Cholinesterases were assayed in 10 patients with profenofos poisoning (Figure 3). Proof of profenofos exposure was sought and found in five patients. Median plasma concentration in admission samples was 3.35 (range 0.67–7.31) μmol/l.

Red cell AChE was almost completely inhibited on admission, with a median activity of 7 (IQR 1–30) μU/μmol Hb—approximately 1% of normal activity (Figure 3A). Remarkably, unlike dimethoxy and diethoxy OP poisoning, there was no reactivation of red cell AChE [median activity at baseline: 25 (IQR 3.5–81) μU/μmol Hb; median activity at 1-h post-pralidoxime: 19 (IQR 8–33) μU/μmol Hb] in the four patients who received 1–2 g loading dose of pralidoxime.

This lack of response to oximes suggested that nearly all the inhibited red cell AChE was already aged on admission. In vitro assays with very high doses of obidoxime confirmed that the enzyme was aged; median non-aged red cell AChE at baseline was 25 (IQR 9–100) μU/μmol Hb (Figure 3B), indicating that >95% of red cell AChE was aged.

BuChE was similarly completely inhibited on admission. Median plasma BuChE on admission in the 10 patients was 33 (IQR 9–73) μU/ml, <1% of estimated normal activity. It started to rise after 2–3 days as inhibitory activity in plasma faded (Figure 3).

Despite such severe red cell AChE inhibition, these patients were very well on admission. Only two of the 10 had a GCS score less than 15/15 on admission; two patients had no symptoms of poisoning after receiving bolus doses of atropine in the transferring hospital. This is surprising considering the generally held view that red cell AChE activity is a good marker of neuromuscular function in OP poisoning.

Discussion
This study shows that the S-alkyl OP insecticides profenofos and prothiofos are likely to be of moderately severe toxicity as emulsifiable concentrates, lying between high toxicity OPs such as dimethoate and monocrotophos and lower toxicity OPs such as chlorpyrifos. However, most importantly and unexpectedly, many patients were well on admission and none required immediate intubation, despite (in profenofos poisoned patients) almost complete inhibition of red cell AChE activity. Few subsequently required intubation, and both intubation and death occurred late compared with other OPs. This is very different to chlorpyrifos and quinalphos poisoning in which red cell AChE is also...
severely inhibited on admission, but the majority of intubations occur at this time. The reason for this remarkable discordance between red cell AChE activity and clinical picture on presentation in profenofos poisoning is unclear. Clinical presentation results from AChE inhibition in tissues other than the blood, particularly the neuromuscular junction and CNS. It is possible that profenofos does not penetrate the CNS. One rat study has reported that severe blood cholinesterase inhibition was associated with only mild CNS AChE inhibition after profenofos poisoning. However, blood cholinesterase activity reported for control animals in this article was very low, suggesting methodological problems. The high partition coefficient for the OPs (Figure 1) should not preclude them from penetrating the CNS. Furthermore, the clinical severity of profenofos poisoning correlated with brain AChE activity in a study of chickens.

Another explanation may relate to differential toxicity of the chiral profenofos enantiomers. In vitro, the (+)-profenofos enantiomer inhibits AChE 23-fold more potently than the (−)-enantiomer, while the (−)-enantiomer is 23-fold more potent in vivo. The reason for this dichotomy was attributed to stereo-specific metabolism in vivo with preferential oxidative activation of the (−)-enantiomer to a more potent inhibitor of AChE than the non-activated (+)-enantiomer. This putative active metabolite has been tentatively assigned a phosphorothiolate S-oxide structure (R–S(O)–P(O)−). It has not been isolated because it rapidly converts to the phosphinyl-oxysulfenate (R–S–O–P(O)−), which is unlikely to be a phosphorylating agent. Hence, the putative highly active S-oxide metabolite resembles a fast-acting ‘hit-and-run’ compound.

AChE inhibited by the (+)-profenofos enantiomer or (−)-phosphorothiolate S-oxide shows very different post-inhibitory reactions. While the former yields an enzyme that undergoes rapid spontaneous and oxime-induced reactivation, AChE inhibited by the latter ages rapidly and is refractory to reactivation. If these rapid reactions occur in the blood under physiological conditions, we hypothesize that the (−)-profenofos S-oxide is used up in venous blood and does not appear in arterial blood. Consequently, neuronal AChE inhibition will result from the action of the (+)-enantiomer of profenofos. This compound, although a potent AChE inhibitor, shows rather rapid spontaneous reactivation that is similar to dimethoxyphosphoryl–AChE. Hence, inhibition of AChE will gradually

![Figure 3. Cholinesterase inhibition in 10 profenofos patients. Inhibition of both cholinesterases was nearly complete on admission; there was no response to loading doses of 1–2 g of pralidoxime chloride. Three patients subsequently died. (A) In vivo AChE activity; (B) Ex vivo AChE activity showing non-aged AChE; (C) BuChE activity; (D) AChE inhibitory activity in plasma, indicating presence of active OP. Few samples were available after 72 h. Mean ± SD.](image-url)
fade and residual AChE-activity may remain at a level that does not cause severe poisoning. This hypothesis may explain the presence of almost completely inhibited and aged red cell AChE in patients with mild cholinergic toxicity soon after profenofos ingestion. It also suggests that oximes may be clinically useful despite complete red cell AChE ageing. However, experimental proof is required to test this hypothesis. This should be possible by measuring profenofos enantiomers in matched venous and arterial blood samples from profenofos poisoned animals or humans, and measuring the neuromuscular function and the response to oximes.

Of note, our findings indicate that red cell AChE activity may not always be an accurate marker of synaptic function or OP poisoning severity, as we and others have proposed previously.16,17,27,28 The evidence for the usefulness of red cell AChE as a marker in OP pesticide poisoning is limited. An unpublished retrospective study in 16 patients demonstrated that red cell AChE correlated with plasma atropine concentration and presumably AChE activity in muscarinic synapses.29 A neurophysiological study in 34 patients showed that it correlated with patterns of neuromuscular transmission.28 Both studies included patients taking a variety of diethoxy and dimethoxy OPs. There is a lack of studies reporting a useful correlation between red cell AChE and clinical severity.

Neither profenofos nor prothiofos poisoning appears to be a major clinical problem. There are just four case reports, all fatal, in the literature.30–33 Three other papers have reported cases in India,18 Nepal34 and Taiwan.35 This case series is by far the biggest published to date. Acephate (CAS 30560-19-1) and methamidophos (CAS 10265-92-6) are the two other S-alkyl OPs, the latter a metabolite of the former. Whilst acephate poisoning appears to be uncommon, with relatively few case reports,18,36,37 methamidophos is highly toxic and has killed many people in Sri Lanka,38 Taiwan,35 and China.39–41 Although in vitro studies have been done,5,24 no in vivo human data have been published about cholinesterase inhibition with these S-alkyl OPs.

Limitations of this article include the small number of prothiofos-poisoned patients, lack of AChE activity data for these patients, and the lack of facilities for close monitoring of patients that might have allowed better description of cause of death. In addition, the number of profenofos-poisoned patients recruited to the RCT and therefore having their blood cholinesterase and plasma OP measured was small. However, the very low AChE activity in this group was very consistent and there appears no reason to think that they are unrepresentative of the whole case series.

Conclusion
Self-poisoning with the S-alkyl OPs profenofos and prothiofos is moderately dangerous when compared with other OP pesticides. Most importantly, red cell AChE activity in profenofos-poisoned patients is not re-activated by pralidoxime and did not correlate with clinical severity, suggesting that this marker may not be generally valid for OP poisoning. It will be useful now to find ways of measuring CNS AChE activity, alongside neuromuscular and red cell AChE activity, in animal models of OP poisoning and human patients to determine the relative role of each enzyme.

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