Oximes in acute organophosphorus pesticide poisoning: a systematic review of clinical trials

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Summary

Background: Acute organophosphorus (OP) pesticide poisoning is widespread in the developing world. Standard treatment involves the administration of intravenous atropine and an oxime to counter acetylcholinesterase inhibition at the synapse, but the usefulness of oximes is uncertain.

Aim: To assess the evidence on the use of oximes in OP poisoning.

Design: Systematic review.

Methods: We searched Medline, Embase, and Cochrane databases (last check 01/02/02) for ‘organophosphate’ or ‘oxime’ together with ‘poisoning’ or ‘overdose’. We cross-referenced from other articles, and contacted experts to identify unpublished studies. A Web search engine [www.google.com] was also used, with the keywords ‘organophosphate’, ‘oxime’, and ‘trial’ (last check 01/02/02).

Results: We found two randomized controlled trials (RCTs) involving 182 patients treated with pralidoxime. The RCTs found no benefit with pralidoxime, and have been used to argue that pralidoxime should not be used in OP poisoning.

Discussion: The RCT authors must be congratulated for attempting important studies in a difficult environment. However, their studies did not take into account recently clarified issues regarding outcome, and their methodology is unclear. A generalized statement that pralidoxime should not be used in OP poisoning is not supported by the published results. Oximes may well be irrelevant in the overwhelming self-poisoning typical of the tropics, but a large RCT comparing the current WHO-recommended pralidoxime regimen (>30 mg/kg bolus followed by >8 mg/kg/h infusion) with placebo is needed for a definitive answer. Such a study should be designed to identify any patient subgroups that might benefit from oximes.

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Introduction

Deliberate self-poisoning has reached epidemic proportions in parts of the developing world, where the toxicity of available poisons and sparse medical facilities ensure a high fatality rate.\textsuperscript{1,2} Many deaths are due to organophosphorus (OP) pesticides and occur in the young, economically-active age group.\textsuperscript{2–5} Fatality rates of 20% are common, and the WHO has estimated that 200,000 people die each year from pesticide poisoning\textsuperscript{6} (although the accuracy of these figures is keenly debated\textsuperscript{7}).

Unfortunately, the widespread use of OP pesticides in the developing world’s agricultural communities will make reduction of deaths by primary prevention a difficult task.

OP pesticides inhibit acetylcholinesterase (AChE) at muscarinic and nicotinic synapses by depositing a phosphoryl group at the enzyme’s active site (reaction 1, Figure 1); this results in an accumulation of acetylcholine and uncontrolled activation of cholinergic synapses. Standard therapy involves
In trials of pralidoxime, the identification of the poison taken, in particular its dimethyl/diethyl status, is therefore essential. Occurs more rapidly with dimethylated pesticides, pralidoxime is only useful before about 12 h with dimethylated enzyme.

The regeneration of active AChE by exerting a nucleophilic attack on the phosphoryl group, transferring it from the enzyme to the absorbing pesticide. The use of high doses of atropine is well established, the use of oximes more controversial.

Oximes reactivate acetylcholinesterase by removing the phosphoryl group (reaction 2, Figure 1). Pralidoxime is the oxime most often used world-wide, and occurs in two common forms: pralidoxime chloride (2-PAM; molecular weight 173; used world-wide) and mesylate (P2S; MW 232; used in the UK). The great majority of its effects are on the peripheral nervous system, since its lipid solubility is low and entry into the CNS limited. Atropine works at muscarinic synapses, competitively antagonizing the accumulated acetylcholine. The main therapeutic effect of pralidoxime is predicted to be recovery of neuromuscular transmission at nicotinic synapses.

In vitro experiments have shown that oximes are effective reactivators of human AChE inhibited by OP compounds. In some situations, however, reactivation of inhibited AChE by oximes will likely be absent or limited, for example where there is: (i) poor affinity for the particular OP-AChE complex; (ii) insufficient dose or duration of treatment; (iii) persistence of the OP within the patient and therefore rapid re-inhibition of newly reactivated enzyme; and (iv) ageing of the inhibited AChE (reaction 3, Figure 1; references 9, 13–15).

In 1961, Sundvall reported that the minimum effective plasma concentration of P2S was 4 mg/l in cats poisoned with a quaternary analogue of the nerve agent sarin. This result has been uncritically extrapolated to all oxime and OP interactions, but it is now clear that the degree of reactivation is dependent on the specific identity and concentrations of both oxime and OP.

Figure 1. Reaction of organophosphorus pesticides with acetylcholinesterase. A dimethylphosphorylated organophosphorus pesticide (methylparaoxon) inhibits acetylcholinesterase (AChE) by phosphorylating the serine hydroxyl group at the enzyme’s active site (reaction 1). This reaction occurs very quickly. Active AChE is subsequently regenerated by a hydroxyl ion attacking the phosphorylated serine residue, removing the phosphate moiety and releasing active enzyme (reaction 2). This regenerative process, however, is much slower than inhibition, requiring hours to days to occur (spontaneous reactivation $t_{1/2} \approx 0.7$ h for dimethyl and 31 h for diethyl compounds).

While in the inactive state, the enzyme is prone to ‘ageing’ (reaction 3) in which one alkyl side chain of the phosphoryl moiety is removed non-enzymatically, leaving a hydroxyl group in its place. ‘Aged’ AChE with its negatively charged phosphate can no longer be attacked by a negatively charged nucleophile, i.e. OH or an oximate group, and regeneration is no longer possible. This reaction occurs considerably faster with enzymes that have been inhibited by dimethylated pesticides ($t_{1/2} \approx 3.7$ h) than those inhibited by diethylated pesticides ($t_{1/2} \approx 31$ h).

The slower the regenerative process, the greater the quantity of inactive AChE available for ageing. Pralidoxime catalyses the regeneration of active AChE by exerting a nucleophilic attack on the phosphoryl group, transferring it from the enzyme to itself. By speeding up reaction 2, it reduces the quantity of inactive AChE available for ageing. However, because ageing occurs more rapidly with dimethylated pesticides, pralidoxime is only useful before about 12 h with dimethylated enzyme.

In trials of pralidoxime, the identification of the poison taken, in particular its dimethyl/diethyl status, is therefore essential.

(Data from references 14 and 18).
Figure 2. Influence of poison concentration and delay of obidoxime therapy on the reactivation of erythrocyte AChE. Erythrocyte AChE was determined according to Worek et al.\textsuperscript{42} and referred to the haemoglobin content of blood (mU/µmol HbFe); the normal value is about 600 mU/µmol Hb. Activity \textit{in vivo} demonstrates the activity of diluted (1 : 100) venous blood of the patient; activity \textit{in vitro} demonstrates its activity after reactivation with a supratherapeutic dose of
When these issues were debated in the mid-1990s, no RCTs of oximes in OP poisoning had been published. We have therefore carried out a systematic search for such RCTs to look for evidence of oximes producing clinical benefit in OP-poisoned patients.

Methods

We carried out a systematic search for clinical trials by searching Medline, Embase, and Cochrane databases (last checked 01/02/02), cross-referencing from other articles, and contacting experts in the field to identify unpublished studies. All articles that were selected with the text words ‘organophosphate’ or ‘oxime’ together with ‘poisoning’ or ‘overdose’ were examined. Articles that could possibly be randomized clinical trials were retrieved to determine if this was the case. The web was also searched using [www.google.com] and the Keywords ‘organophosphates’, ‘oxime’, and ‘trial’ (last checked 01/02/02).

A request (with a draft of this manuscript) was sent to authors of published RCTs to complete a CONSORT statement to clarify the methodology used. Unfortunately, we did not receive any responses.

Results

The initial standard database searches located only two RCTs. More ad hoc methods, such as the review of references cited in recent review articles and web searching of the Journal of Association of Physicians of India located two further trials. In total, we found two published RCTs (Table 1), and one paper and one meeting abstract that described two further small clinical trials. All identified studies assessed pralidoxime; clinical trials of obidoxime or other oximes have not been reported.

RCT 1

The first trial, in 72 patients, was done between August 1991 and December 1992 at the Christian Medical College in Vellore, India. There was no untreated control group. A 1 g bolus of pralidoxime (termed ‘low dose’) was compared with 12 g given as a reducing infusion over 4 days without a loading dose (termed ‘high dose’). This RCT reported an increased mortality rate (22% vs. 14%; OR 1.77, 95%CI 0.52–6.0) and increased requirement for ventilation (67% vs. 47%; OR 2.04, 95%CI 0.78–5.3) among patients who received the infusion, compared to those who received the bolus dose. The authors argued that ‘high-dose’ pralidoxime was therefore ‘associated with a worse outcome’ and should have ‘no role in the routine management of patients with OP poisoning’.

RCT 2

The dates of the second trial are not given in either publication. Following on from RCT 1, it compared ‘high-dose’ pralidoxime (i.e. 12 g by continuous infusion without loading dose) with placebo saline infusion in 110 patients. Although
<table>
<thead>
<tr>
<th></th>
<th>RCT 1 (Aug 91–Dec 92)</th>
<th>RCT 2 (Dates uncertain)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Controls ('Low-dose')</td>
<td>Cases ('High-dose')</td>
</tr>
<tr>
<td>( n )</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Dose (estimated/kg—see legend)</td>
<td>Bolus: 22/mg/kg dose</td>
<td>No bolus loading dose</td>
</tr>
<tr>
<td></td>
<td>No infusion</td>
<td>Day 1: 5.6 mg/kg/h</td>
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<td></td>
<td></td>
<td>Day 2: 2.8 mg/kg/h</td>
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<td></td>
<td></td>
<td>Day 3: 1.8 mg/kg/h</td>
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<td></td>
<td></td>
<td>Day 4: 0.9 mg/kg/h</td>
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<tr>
<td>Pesticides</td>
<td></td>
<td></td>
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<tr>
<td>Dimethyl OPs</td>
<td>56%</td>
<td>61%</td>
</tr>
<tr>
<td>Diethyl OPs</td>
<td>25%</td>
<td>22%</td>
</tr>
<tr>
<td>Unknown</td>
<td>19%</td>
<td>17%</td>
</tr>
<tr>
<td>Time from ingestion to start of Rx</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 6 ) h</td>
<td>8</td>
<td>3</td>
</tr>
<tr>
<td>( 6–12 ) h</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>( 12–24 ) h</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>( 24–36 ) h</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>( 36–48 ) h</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Pseudocholinesterase levels</td>
<td>338.9 (260.5)</td>
<td>441.3 (450.3)</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deaths</td>
<td>5 (14%)</td>
<td>8 (22%)</td>
</tr>
<tr>
<td>Ventilated</td>
<td>17 (47%)</td>
<td>24 (67%)</td>
</tr>
<tr>
<td>Intermediate syndrome</td>
<td>13 (36%)</td>
<td>20 (56%)</td>
</tr>
</tbody>
</table>

Both RCTs were performed at the Christian Medical College, Vellore. Patients recruited to the trial were on the medical ITU with a history and signs/symptoms suggestive of organophosphorus (OP) poisoning plus a pseudocholinesterase level < 50% of normal; the number of patients excluded was not stated. Randomization was in blocks of four; methods of concealment to reduce bias were not stated. Patient weight was not stated; to estimate quantities of pralidoxime given to patients as mg/kg, an estimated mean weight of 45 kg was used (patients in a poisoning RCT in Sri Lanka had a mean weight of 48.2 kg (SD 8.9)).
the published details are incomplete, it appears that a different dosage regimen to that of RCT 1 was used: 12 g over 3 days (estimated 3.7 mg/kg/h for a 45 kg patient). The ‘high-dose’ regimen was associated with a significantly higher risk of death (29% vs. 5%; OR 7.1, 95% CI 1.9–26.0) and requirement for ventilation (67% vs. 40%; OR 3.1, 95% CI 1.4–6.7). The authors concluded that 2PAM ‘has no role in the management of patients with organophosphorus poisoning and … does more harm than good’.

**Trial 3**

A trial of pralidoxime was carried out in 34 patients in Tehran, Iran, during the early 1990s.33 Seventeen patients received atropine alone, while 17 received 600–800 mg pralidoxime every 4–8 h for 4 days in addition to atropine. The pralidoxime dose was based on the patient’s condition. Methods of allocation and concealment are not stated, but recent discussion with Professor Balali-Mood (Mashad, Iran) has indicated that it was not randomized.

All patients presented within 10 h of pesticide ingestion; 35% of control patients ingested diethylphosphorylated OPs compared to 29% of intervention patients. Pralidoxime use resulted in no significant reduction in the number of patients requiring ventilation (41% vs. 47%; OR 0.79, 95% CI 0.20–3.0) or the number of patients dying (18% vs. 18%; OR 1.0, 95% CI 0.17–5.8). The authors concluded that atropine alone should be used in the treatment of acute OP poisoning.

**Trial 4**

A fourth study has been published in abstract format only.34 Twenty OP-poisoned patients were included in this trial of two vials or four vials of pralidoxime iodide, but neither trial design nor results are apparent from the abstract. We have been unable to elicit any response from writing to the author. It seems unlikely that conclusive evidence will result from such a small trial.

**Discussion**

There are two published RCTs of pralidoxime in 182 patients with acute OP poisoning. These studies have since been used to argue that oximes should not be used in acute OP poisoning.35 The authors must be congratulated for attempting important studies in such a difficult environment. However, the studies did not take into account recently clarified issues important for outcome, and the published methodology is unclear. Therefore such a generalized statement cannot be justified from the published results and we believe that the evidence for or against the use of oximes is not yet established.

Most importantly, the studies did not evaluate the current WHO-sponsored recommendations for pralidoxime therapy (at least 30 mg/kg bolus followed by > 8 mg/kg/h infusion). Further, they were published before the widespread adoption of the CONSORT guidelines for the reporting of RCTs28 and it is very difficult to determine the methodology of the trials from the published record.

It is likely that the ‘high-dose’ regimen of pralidoxime used in Vellore did not produce an effective plasma concentration. Pharmacokinetic studies have shown that 1 g given over 30 min to patients with a mean weight of 72 kg (SD 8.5) falls below a plasma concentration of 4 mg/l within 1.5 h.36 The weight of the Indian study participants is not given in either paper; however, an estimated mean weight of 45 kg (see table legend) would only increase the effective concentration time by a factor of ~2. In the group receiving the infusion only, it appears doubtful whether an estimated initial dose of 2.8 mg/kg over the first 30 mins would ever give a plasma level above 4 mg/l. Furthermore, recent studies have suggested that even 4 mg/l may actually be insufficient for many pesticides.

An alternative interpretation of this study’s results would therefore be that a loading dose of pralidoxime is required to reach an effective plasma concentration and that a bolus dose alone, while producing an effective concentration for only several hours, offers some benefit.

However, the worse outcome seen in patients who received pralidoxime in RCT 2 suggests that the pralidoxime infusion harms patients. An alternative explanation is that features of the RCT itself led to this result. Neither power calculations nor stopping rules are presented in the published papers. It is not clear why the trial was stopped at 110 patients. Could the differences have been due to chance and might they have equalized out with time?

Sicker patients might also have been randomized to the intervention arm of RCT 2, which had much reduced mean pseudocholinesterase levels at baseline. No information on masking is given, and a block size of four was used in both studies. As pointed out by Schulz,37 such a small non-varying block size can often be unravelled: if treatment assignment becomes known after allocation, a sequence can be easily discerned from the pattern of past assignments, giving the risk of selection bias, even if concealment has been adequate.

Whether pesticides had dimethyl or diethyl groups was also not controlled for—this is
important if only diethylphosphorylated AChEs respond to pralidoxime after 12 h.\textsuperscript{14,38} The information is given for RCT 1 (Table 1) but not RCT 2. In this latter study, it would be important to control for both the form of OP ingested and the time post-ingestion that therapy started. Deaths may have occurred in patients ingesting dimethyllated compounds who presented after 12 h, when pralidoxime would not be expected to work.

The reports of the two small studies from Iran and north India provide too few details of trial design for any conclusions to be safely drawn.

**Conclusions**

Detailed observational clinical studies suggest that oximes encourage AChE regeneration.\textsuperscript{13–15} Animal data consistently show a marked positive effect of oximes on survival.\textsuperscript{39} Physicians in India, China and Vietnam have recently reported improved outcomes in uncontrolled studies with high doses of pralidoxime (references 40, 41, and Drs Pham Due and Nguyen Thi Du, Hanoi, personal communication). However, no clinical trials have formally assessed the efficacy of high-dose pralidoxime.

Two small RCTs have looked at either lower doses: 1 g bolus doses of pralidoxime or 12 g given by infusion over 3–4 days. Increased mortality was found with patients receiving the infusion of pralidoxime without a loading dose compared to either placebo or a loading dose alone. The authors of these studies have stated that pralidoxime should not be used for OP poisoning. However, we do not believe that the results of these RCTs can be used to make such a generalized comment and the effectiveness of pralidoxime in our view is still undecided.

Although the studies are methodologically weak, it is quite possible that their conclusions are correct. There are many factors present in self-poisoning cases in South Asia that would reduce the effectiveness of pralidoxime. A study from Sri Lanka showed that around 70% of OP poisoned patients had ingested dimethyllated compounds;\textsuperscript{23} this situation may well be similar in other parts of the tropics and since many patients present more than 12 h after the poisoning, it may be too late for oximes. In addition, the suicidal dose is often large and the pesticide persistent for several days, resulting in repeated inhibition of any newly reactivated AChE.

We believe that a large high-quality RCT comparing the current WHO-recommended regimen with placebo is required to definitively assess the value of pralidoxime in acute OP poisoning. Such a trial may well confirm the Vellore group’s findings.

Randomization should be stratified according to baseline severity, time to presentation, and class of OP pesticide taken (diethyl or dimethyl), with predefined sub-group hypotheses. Because of the importance of ageing in determining the usefulness of oximes, red blood cell AChE activity and the potential for ex-vivo reactivation will have to be measured for such a study to be fully interpretable. Only after such a study has been completed will it be possible to determine whether OP-poisoned patients benefit from oxime therapy.

**Conflict of interest**

The authors have recently been funded by the Wellcome Trust, UK, to perform a large randomized controlled trial of pralidoxime in patients with acute OP pesticide poisoning.

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**References**


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