Pharmacological agents in the prophylaxis/treatment of organophosphorous pesticide intoxication

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Organophosphorus pesticide poisoning causes tens of thousands of deaths each year across the world. Poisoning includes acute cholinergic crisis as a result of AChE inhibition, intermediate syndrome (IMS) due to neuromuscular necrosis and organophosphate-induced delayed neuropathy (OPIDN) due to inhibition of neuropathy target esterase (NTE). Standard treatment for acute poisoning involves administration of intravenous atropine, oxime 2-PAM to counter AChE inhibition and diazepam for CNS protection. However clinical trials showed ineffectiveness of the standard therapy regimen. Although new oximes that can reactivate both peripheral and cerebral AChE and other prophylactic agents such as human serum butyrylcholinesterase (Hu BChE), sodium bicarbonate, huperzine A (a reversible ChE inhibitor) with imidazenil (a GABA\(_A\) receptor modulator) have been proved effective in animal models, systematic clinical trials in patients are warranted. For IMS which is non-responsive to standard therapy, supportive therapy specifically artificial respiration followed by recovery is indicated. For OPIDN which has a different target (NTE) than AChE, standard therapy is ineffective. However neuroprotective drugs such as corticosteroids proved partially effective. Pretreatment with protease inhibitor PMSF has been shown to protect the aging of NTE and prevent the development of delayed symptoms in hens. Since the biology of NTE is being explored, new pharmacological agents should be developed in future. OP pesticide poisoning is a serious condition that needs rapid diagnosis and treatment. Since respiratory failure is the major reason for mortality, artificial respiration, careful monitoring, appropriate treatment and early recognition of OP pesticide poisoning may decrease the mortality rate among these patients.

**Keywords:** Antidote, OPIDN, Organophosphorus pesticide, Prophylatic agents

**Introduction**

Organophosphorus (OP) pesticides were first synthesized and discovered by German chemist Gerhard Schrader at company IG Farben in the 1930s. After World War II, American companies began synthesizing organophosphate pesticides in large quantities. Parathion was among the first marketed, followed by Malathion. The popularity of these insecticides increased after many of the organochlorine insecticides like DDT, dieldrin, and heptachlor were banned in the 1970s. In 1944 two well-known organophosphorus insecticides, diethyl-p-nitrophenylphosphate (paraoxon) and diethyl-p-nitrophenylthiophosphate (parathion) were synthesized.

Currently more than 100 different OP pesticides are used mainly as insecticides in agriculture and gardening\(^1\). The commonly used OP pesticides are given in Table 1. The number of intoxications with organophosphorus pesticides (OPs) is estimated at some 3 millions per year, and the number of deaths and casualties some 300,000 per year worldwide\(^1\). In developing countries, the most common cause of pesticide poisonings is due to occupational exposure. However, a review of poisoning studies in developing countries reveals that pesticide poisonings associated with high mortality rates are usually the result of self-poisoning. Particularly, a great number of victims were present in rural regions due to the widespread availability of acutely toxic pesticides used in agriculture\(^3\). While some of these intoxications may be attributed to accidental exposure, the majority of

<table>
<thead>
<tr>
<th>Pesticides</th>
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<tr>
<td>Chlorpyrifos*</td>
<td>Chlorpyrifos-methyl</td>
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<tr>
<td>Diazinon</td>
<td>Dichlorvos*</td>
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<tr>
<td>Dimethoate</td>
<td>Disulfoton</td>
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<tr>
<td>Fenithion</td>
<td>Fenthion*</td>
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<tr>
<td>Glyphosate</td>
<td>Isocarbophos</td>
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<tr>
<td>Isofenphos-methyl</td>
<td>Leptothis*</td>
</tr>
<tr>
<td>Malathion</td>
<td>Methamidophos*</td>
</tr>
<tr>
<td>Mipafos*</td>
<td>Omethoate*</td>
</tr>
<tr>
<td>Paraoxon</td>
<td>Parathion*</td>
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<tr>
<td>Parathion-methyl</td>
<td>Phenthoate</td>
</tr>
<tr>
<td>Phorate</td>
<td>Trichlorfon*</td>
</tr>
<tr>
<td>Trichlorphos</td>
<td>Tri-O-cresyl phosphate*</td>
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\(^*\)Pesticides known to induce OPIDN.
Casualties result from suicidal attempts with ingestion of multiple lethal doses. Therefore, the indiscriminate use of these pesticides results in a toxicity risk to non-target organisms and environmental pollution. This communication reviews the current understanding and future perspectives of the management and pharmacological treatment of human poisoning with OP pesticides.

**Mechanism of action of organophosphorus pesticides**

The basic chemical structure of all OP pesticides is depicted in Fig. 1. The structural diversity of OP pesticides is due to different substituents at the phosphorus atom. Substituents at R1 and R2 are alkyl, alkoxy, alkylthio or amino groups, and substituent at X is a labile acyl residue (halide, cyano, phenol or thiol group), known as the leaving group. The reactivity of OP pesticides varies upon chemical structure. Electrophilicity of the phosphorus atom is crucial for the biological actions. OP pesticides that have double bonds between phosphorus and oxygen atoms (P=O) are highly electrophilic at the phosphorus atom and accordingly are highly reactive. Groups that enhance the reactivity of the phosphorus atom are nitro, cyano, halogen, ketone and carboxylic ester. Deactivating groups include hydroxyl and carboxylic acid.

Organophosphates share a common mode of action, exerting their toxic effects primarily via acetylcholinesterase (AChE) inhibition. Cholinesterases are hydrolytic enzymes, the butyrylcholinesterase (BuChE) and AChE are both blocked by OP pesticides. Although both cholinesterases hydrolyze acetylcholine (ACh) and other choline esters, they differ in their relative affinity for some substrates. A single enzyme molecule is capable of hydrolyzing $6 \times 10^5$ molecules of acetylcholine (ACh)/min. Muscle and nerve AChE are only present in the synaptic cleft and cannot be measured directly. Since erythrocyte AChE has a similar structure as the synaptic enzyme, it appears to be a suitable parameter to reflect various reactions at the synaptic site. Therefore, its measurement is of high value for therapeutic management, especially oxime administration, during the course of intoxication.

**Clinical presentation of poisoning with organophosphorus pesticides**

Exposure to OP pesticides can induce four different clinical syndromes:

1. Acute cholinergic crisis as a result of AChE inhibition
2. Intermediate syndrome (IMS) whose underlying mechanism(s) is still unclear
3. Organophosphate-induced delayed neuropathy (OPIDN) that has been explained by the inhibition of neuropathy target esterase
4. Chronic organophosphate induced neuropsychiatric disorder due to long-term low-level exposure.

Acute cholinergic crisis that immediately follows exposure to OP pesticides includes hyperstimulation of muscarinic receptors (e.g., bradycardia, bronchoconstriction, bronchorrhoea, hypotension, increased gastrointestinal motility, abdominal cramps, miosis, hyper salivation), nicotinic receptors (e.g., hypertension, tachycardia, fibrillation, fasciculation, necrosis of striated muscles), and both central muscarinic and nicotinic receptors (e.g., tremor, movement in coordination, seizures, central depression of respiration, coma, death). Although the immediate cause of death could also be cardiac arrest.
or heart failure, central respiratory failure due to loss of respiratory drive is by far the most frequent cause of death after intoxication with OP pesticides.

**Antidotes in the treatment of acute poisoning with organophosphorus pesticides**

The current view of the treatment of OP pesticide poisoning includes three strategies:

1. **Anticholinergic drug** (e.g., atropine)
2. **Cholinesterase-reactivating agents** (e.g., oximes)
3. **Anticonvulsant drugs** (e.g., benzodiazepines)

**Anticholinergic drug** (e.g., atropine)

The development of treatment against OP pesticide poisoning started with recognition of the efficacy of atropine as an antidote for their parasympathomimetic effects. This is mainly due to the fact that atropine, as an antimuscarinic drug, is not capable of counteracting effects provoked by nicotinic hyperstimulation, while at the same time, possesses only limited antimuscarinic action in the central nervous system (CNS). Also, convulsions can be blocked by atropine only for a very limited time after exposure to OP pesticides since other transmitter systems have become involved in cholinergic overstimulation in the brain (e.g., -amino butyric acid, glutamate). Nevertheless, atropine is the initial drug of choice in acute organophosphate poisoning.

**Cholinesterase-reactivating agents** (e.g., oximes)

The first successful destruction of phosphylated AChE was accomplished by hydroxylamine in 1951. In the following years, development of specific antidotes such as pralidoxime (PAM-2), the first pyridinium oxime was developed. Pralidoxime reactivates the phosphylated enzyme about a million times faster than hydroxylamine. PAM-2 was first used against parathion poisoning in Japan. Further investigations in this area resulted in synthesis of bispyridinium oximes: trimedoxime (TMB-4), obidoxime (LüH-6), HI-6, and HL6-7. PAM-2 (pyridinium-2-aldoloxime) and TMB-4 Cl2 [1, 3-Bis (4-hydroxyiminomethyl-1-pyridinium) propane dichloride] were synthesized in the United States. LüH-6 Cl2 a 1, 3-Bis (4-hydroxyiminomethyl-1-pyridinium) 2-oxapropylamine dichloride was synthesized in Germany and introduced into the medical practice. An oxime HI-6 Cl2 [1-(2-hydroxyiminomethyl-1-pyridinium)-3-(4-carbamoyl-1-pyridinium)-2-oxapropylamine dichloride] was also synthesized by Germans. The most recent oxime of importance (after LüH-6 and HI-6) was HL6-7, synthesized by Germans. Its chemical name is 1-(4-(aminocarbonyl) pyridinium) methoxy) methyl)-2,4-bis (hydroxyimino)methyl) pyridinium diiodide. Quaternary pyridinium oximes do not cross the blood brain barrier hence unable to reactivate the central cholinesterase enzyme. However tertiary oximes such as monoisonitrosoacetone (MINA) and diacetyl-monoxime (DAM) have been shown effective in the reactivation of brain AChE and abolition of convulsions in OP pesticide intoxicated animals. Numerous attempts have also been made to improve the antidotal properties of the conventional mono- and bis-pyridinium mono (di)-oximes by modifying their structure. Indians have developed new derivatives of bispyridinium and 1-alkyl pyridinium oximes which reactivated both blood and brain AChE in DFP intoxicated animals. The efficacy of these new oximes is far better than 2-PAM and warrant for testing in patients in the clinic.

**Mechanism of action of oximes**

Reactivation of inhibited AChE by removal of the phosphyl moiety from the AChE active site serine is considered to be the primary mechanism of action for oximes. Clinical data obtained in organophosphorus pesticide-poisoned patients provide evidence for the validity of this assumption. The oxime is oriented proximally to exert a nucleophilic attack on the phosphorus of the enzyme-inhibitor complex. Intermediate in the reactivation is a complex between the phosphorylated enzyme and the reactivator. The enzyme-inhibitor-oxime complex is then split off, leaving the regenerated enzyme (Fig. 3). Mechanistic
studies of oxime (a reversible ligand) action have shown that it may bind to cholinesterases either at the catalytic site or at the allosteric site, or at both sites of the enzymes, thus explaining the mechanism of protection afforded by oxime administration. Therefore the stereo chemical arrangements of oximes can play an important role in the difference in their therapeutic efficacy. The direct pharmacological effects such as direct reaction with OP pesticides, anticholinergic and sympathomimetic effects and a decrease in the amount of liberated acetylcholine into the synaptic cleft, may also be relevant for the interpretation of antidotal potency of oximes. It is possible to administer oximes prophylactically before the poisoning occurs. Oximes protect cholinesterases from phosphorylation by OP pesticides. Oximes may be able to reactivate the enzyme before a significant amount of aging to occur. This approach has been tried by the British who used P2S tablets as a pretreatment drug. But due to short elimination half-lives of the oximes make them inappropriate for prophylactic use. Another prophylactic approach is a combination of cholinesterase and an oxime pre-treatment that has been explored as a pseudo-catalytic bioscavenger, such that the catalytic activity of inhibited cholinesterase can rapidly and continuously be restored in the presence of an oxime.

Anticonvulsant drugs (e.g., benzodiazepines)

The most important anticonvulsant in present use is diazepam. The combination of atropine and diazepam is more effective than atropine or oxime alone in reducing mortality. Basically, the benzodiazepines potentiate the action of the inhibitory neurotransmitter γ-aminobutyric acid at its receptors. In the cholinergic nervous system, diazepam probably decreases the synaptic release of ACh. The main consequence of the action of benzodiazepines in CNS is hyper polarization of neurons which makes them significantly less susceptible to cholinergically-induced depolarization. The ultimate result is cessation of propagation of convulsions. Diazepam is of benefit in organophosphate poisoned patients by reducing anxiety, restlessness and muscle fasciculations, terminating convulsions, and reducing morbidity and mortality when used in conjunction with atropine and oxime. Diazepam should be given to patients poisoned with OP pesticides whenever convulsions or pronounced muscle fasciculation are present. In severe poisoning, diazepam administration should be considered even before these complications develop. Sivilotti et al. have used multiple centrally acting antidotes (diazepam, xylazine, morphine and ketamine) in animals intoxicated with OP pesticide and observed protection from severe poisoning. These findings suggest new possibilities for prophylaxis or therapy against OP pesticide poisoning.

Other prophylactic agents

Several adjunct and alternative therapies have been explored in animal and human studies other than standard therapy and summarized in Table 2. In animal studies sodium bicarbonate addition to standard therapy improved the protective efficacy against dichlorvos poisoning. NMDA receptor antagonist dizocilpine alone or in combination with atropine blocked the convulsion caused by acute dose of dichlorvos in mice. Oxidative stress seems to be one of the important components of the mechanism of OP pesticide toxicity. Animal studies have shown protective effects of antioxidants such as N-acetylcysteine and melatonin against acute OP pesticide-induced oxidative tissue damage. Interleukin-10, a cytoprotective agent protected the peripheral tissue damage in rats caused by poisoning with OP pesticide. Efficacy of an adenosine A1 receptor agonist phenylisopropyl adenosine (PIA) against standard therapy in rat intoxicated with OP pesticide showed partial protection in the abolition of acute symptoms and tissue damage. Pibiri et al. have shown that a combination of huperzine A (a reversible ChE inhibitor) with imidazenil (a GABA_A receptor modulator) is a prophylactic, potent, and safe therapeutic strategy to overcome OP toxicity. Clinical studies in humans have been conducted using scopolamine, a competitive inhibitor of acetylcholine which can cross the blood-brain barrier and prove effective against chlorpyrifos.

![Table 2](https://example.com/table2.png)

<table>
<thead>
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<th>Agents</th>
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<tr>
<td>Clonidine*</td>
<td>Dizocilpine</td>
</tr>
<tr>
<td>Human serum butyrylcholinesterase (Hu BChE)*</td>
<td>Huperzine A</td>
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<tr>
<td>Imidazenil</td>
<td>Interleukin-10</td>
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<tr>
<td>Ketamine</td>
<td>Magnesium sulfate*</td>
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<td>Melatonin</td>
<td>Morphine</td>
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<tr>
<td>N-acetylcysteine</td>
<td>Phenylisopropyl adenosine (PIA)</td>
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<tr>
<td>Scopolamine*</td>
<td>Sodium bicarbonate</td>
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<td>Xylazine</td>
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*Used clinically in humans
poisoning, specifically CNS toxicity. A phase II clinical trial was conducted using clonidine in patients within acute OP pesticide poisoning prove effective at moderate doses but hypotension was associated with high dose as a side effect. Beneficial effect of magnesium sulfate at a dose of 4g/day concurrent with standard therapy, in OP acute human poisoning has been reported. Several studies over the last two decades have demonstrated that exogenously administered human serum butyrylcholinesterase (Hu BChE) can be used prophylactically to counteract the toxicity of OP pesticides. A recent report suggests the suitability of human butyrylcholinesterase as therapeutic marker and pseudo catalytic scavenger in organophosphate poisoning by a kinetic analysis. Since oxime-induced reactivation of cholinesterase is too slow to accomplish pseudo catalytic function, BChE would be effective as a stoichiometric scavenger.

Antidotes for OP pesticide-induced intermediate syndrome

Intermediate syndrome (IMS) has been considered as a major contributing factor of organophosphate-related morbidity and mortality because of its frequent occurrence and probable consequence of respiratory failure. Despite a high incidence, the pathophysiology that underlies IMS remains unclear. Proposed mechanisms of IMS include muscle necrosis, prolonged acetylcholinesterase inhibition, down regulation or desensitization of postsynaptic acetylcholine receptors, failure of postsynaptic acetylcholine release, and oxidative stress-related myopathy. The clinical manifestations of IMS typically occur within 24 to 96 h, affecting conscious patients without cholinergic signs, and involve the muscles of respiration, proximal limb muscles, neck flexors, and muscles innervated by motor cranial nerves. A 33 year old female ingested an unknown quantity of malathion in a suicide attempt. Cholinergic signs consistent with severe organophosphate intoxication developed and were treated within 6 h of ingestion. Intravenous atropine and a continuous infusion of pralidoxime (400 mg/h) were administered. Prolonged depression of plasma and red blood cell cholinesterases were documented. Despite an initial clinical improvement and the presence of plasma pralidoxime concentrations exceeding 4 µg/mL, the patient developed profound motor paralysis consistent with the diagnosis of IMS. The use of conventional antidotes atropine and oximes are ineffective in IMS patients. With appropriate therapy that commonly includes artificial respiration; complete recovery develops 5-18 days later. The treatment of IMS is mainly supportive.

Antidotes for organophosphate-induced delayed neuropathy (OPIDN)

Organophosphate-induced delayed neurotoxicity (OPIDN) or organophosphate-induced delayed polyneuropathy (OPIDP) is a toxicity syndrome caused by certain OP pesticides (Table 1). It is characterized by distal muscular weakness in the hand and feet, ataxia and paralysis due to degeneration of axons in the central and peripheral nervous system that appear about 4-21 days after OP pesticide exposure. The molecular target for OPIDN is considered to be an enzyme in the nervous system known as neuropathy target esterase (NTE). NTE is also present in peripheral lymphocytes and platelets which can be used as biochemical marker of OPIDN. The ability of a NTE inhibitor to cause OPIDN, besides its affinity for the enzyme, is related to its chemical structure and the residue left attached to the NTE. If such residues undergo the aging reaction i.e. the loss of an alkyl group bound to the enzyme, those OP pesticides usually have a high likelihood of causing OPIDN. Protection from neuropathic doses of OP inhibitors is
obtained when NTE is inhibited with nonageable inhibitors (Fig. 4). Promotion affects either the progression or expression of OPIDN after the initial biochemical effect on NTE. Some recent observations suggest that development of OPIDN in hens can be influenced by PMSF and methylprednisolone when they are given before or soon after neuropathic OP pesticides. Administration of PMSF prior to or following the administration of leptophos can significantly modify not only clinical signs of OPIDN but also changes of several biochemical indices accompanied by OPIDN. Furthermore, it is possible to expect that these biochemical indices can provide some valuable clues for exploring the modification of OPIDN by PMSF treatment\(^\text{74}\). Results of a recent study demonstrated that the pretreatment with PMSF could inhibit TOCP-induced NF degradation while it protected hens against the development of OPIDN, which suggested the inhibition of NF-associated protease in peripheral nerves that might be an underlying protective mechanism of PMSF against OPIDN\(^\text{75}\). Modification of OPIDN development has also been reported using calcium channel blockers such as verapamil and ganglioside mixture\(^\text{76-78}\). Because of the use of corticosteroids and vitamin B complex in the neurological diseases, these drugs have also been evaluated against OPIDN. It was observed that delayed neuropathy induced by OP pesticides could not be resisted completely by the treatment with prednisolone or vitamin B complex, but clinical signs of OPIDN and pathological changes in hens that received these two protective agents after OP pesticides were less severe than those in hens that received only OP pesticides\(^\text{79,80}\). There are controversial reports regarding use of corticosteroids against OPIDN in hens; it was beneficial against TOTP-induced delayed neurotoxicity but was ineffective against DFP-induced delayed neurotoxicity\(^\text{81,82}\).

Medical treatment (clinical trials) in patients with organophosphorus pesticides poisoning

A pathway that either increases the toxicity or decreases the toxicity of OP pesticides in humans is depicted in Fig. 5. Beneficial role and efficacy of oximes drugs in clinical practice especially those evaluated in controlled clinical trials against OP pesticides are described here. Patients (60) acutely poisoned with various organophosphorus insecticides were treated with atropine, diazepam and HI-6 (500 mg every 6 h, im) for 2 to 7 days, depending on the severity of the organophosphate poisoning. No adverse effects were noted in patients treated with oximes\(^\text{24}\). Nine patients intoxicated with organophosphorus insecticides were treated with PAM-2 methylsulphate (Contrathion) using a dose of 4.42 mg/kg as a bolus injection followed by continuous infusion (2.14 mg/kg/h)\(^\text{83}\). In a series of five case reports, LüH-6 (Toxogonin) 250 mg was given as an intravenous bolus followed by continuous infusion of 750 mg/24 h in cases of life-threatening parathion poisoning. This dose was effective, especially when the dose of parathion absorbed was relatively low\(^\text{25}\). In a clinical study of 63 patients poisoned with organophosphorus insecticides, patients were divided into three groups: one was treated with atropine only, while the other two received atropine and either PAM-2 or LüH-6. Three of the patients who received the LüH-6 combination therapy developed hepatitis and two of them died due to liver failure, which may indicate over dosage of LüH-6\(^\text{84}\). Clinical trials in India and Iran carried out in OP pesticide poisoning using pralidoxime were also not very much effective\(^\text{85-88}\). A systematic review of clinical trials by Eddleton et al.\(^\text{89}\) concluded that a large clinical trial is required to compare the current WHO-recommended pralidoxime regimen (>30 mg/kg bolus followed by >8 mg/kg/hr infusion) with placebo to determine definitively the role of oxime therapy in OP pesticide poisoning. A recent clinical trial concluded that despite clear reactivation of RBC AChE in diethyl OP pesticide poisoned patients, no
evidence of improved survival with 2-PAM or reduces need for intubation in patients with OP pesticide poisoning. Further studies of different dose regimen and different oximes are required.

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