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NOVEL CLINICAL TOXICOLOGY AND PHARMACOLOGY OF INSECTICIDES SELF POISONING

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Abstract:
Self-poisoning from organophosphorus insecticides is a huge global health issue that kills over 100,000 people each year. It's a multi-organ disorder including the inhibition of cholinesterases and maybe other enzymes, as well as the effects of ingested solvents in excessive concentrations. Variability in lipophilicity, speed of activation, speed and effectiveness of acetylcholinesterase inhibition, and chemical groups connected to the phosphorus leads in varying poisoning onset, severity, clinical toxidrome, and case fatality among organophosphorus insecticides. The current treatment is only somewhat effective, focusing on reactivating acetylcholinesterase and counteracting the effects of too much acetylcholine on muscarinic receptors. Rapid titration of atropine during resuscitation can save lives and can be done without oxygen. The therapeutic role of oximes is unknown. Small trials of novel antidotes have been conducted, but the wide range of poisoning makes interpretation challenging. To test therapies in suitably powered research, more work is required.

Key words: *Toxicology, Pharmacology, Insecticides self poisoning, Review*

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INTRODUCTION:

Organophosphorus (OP) insecticide self-poisoning or attempted suicide is the most important global form of acute poisoning, affecting over one million people each year and killing around 100,000. The introduction of these insecticides into global agricultural during the Green Revolution in the 1960s [1] brought them into poor rural communities that were completely unprepared to use or store them properly. Easy access to highly hazardous pesticides that are fatal after ingestion of small amounts transformed previously nonlethal self-poisoning into suicides. Suicide rates in countries such as Sri Lanka exploded as health care systems were simply unable to deal with these fast-acting poisons [2].

Prevention of OP self-poisoning will need to be multifaceted, involving regulation to remove the most hazardous pesticides from agricultural practice; the improved use and storage of pesticides, particularly by small-scale farmers; and improved medical management. Data from Sri Lanka and Bangladesh have shown that bans of the most highly hazardous OP insecticides [e.g., World Health Organization (WHO) Class I toxicity compounds such as methyl parathion and monocrotophos] have resulted in remarkable reductions in overall suicide rates without overtly affecting agricultural yield or costs . In Sri Lanka's case, these bans, in combination with bans of endosulfan and paraquat, have saved an estimated 93,000 lives over 20 years at a direct regulatory cost of USD 1.4 per life-year saved. Many thousands of lives will be saved if such bans of highly hazardous OP insecticides are implemented worldwide [3].

Unfortunately, even where the highly hazardous Class I OPs have been banned, the case fatality for self-poisoning with WHO Class II insecticides such as dimethoate remains high, with 10–20% of people dying . More effective treatments are urgently required. Although many millions of people have died from OP insecticide self-poisoning since the 1960s, the subject has gained relatively little attention. Instead, almost all research is focused on OP nerve agent chemical weapons such as sarin. Immense effort and funds go into finding novel antidotes for OP nerve agents, despite the relative paucity of cases and the huge human cost of insecticide self-poisoning[4].

TOXICOLOGY AND CLINICAL COURSE:

The pivotal mechanism of OP insecticide toxicity is inhibition of acetylcholinesterase (AChE) at cholinergic synapses across the central nervous system and autonomic nervous system, as well as at the neuromuscular junction (NMJ). An inability to break down acetylcholine results in overstimulation of muscarinic and nicotinic receptors and clinical features that include excess sweating, salivation, bronchospasm, bronchorrhea (pulmonary edema), bradycardia and hypotension, NMJ dysfunction, and reduced consciousness [5] .

Patients die from respiratory failure due to a combination of a lack of central respiratory drive, NMJ dysfunction, and hypoxia from bronchorrhea. Patients who survive long enough to be hospitalized will need resuscitation with oxygen, fluids, and a muscarinic receptor antagonist (typically atropine). Many comatose patients require intubation and ventilation to maintain respiratory function.

The timing of acute respiratory failure, and therefore the likelihood of surviving to reach medical care, varies according to the dose and particular OP insecticide ingested. Patients ingesting very large doses or highly toxic pesticides will more rapidly inhibit a clinically significant proportion of their AChE and exhibit features earlier. Lipid solubility and the need for conversion to an active poison (see the section titled Organophosphorus Insecticide Chemistry) also likely affect the time to onset. Out-of-hospital cardiorespiratory arrest in most parts of the world will result in the patient's death; in a Korean study, in a location with an effective emergency ambulance system, only 22% and 10% of insecticide-poisoned patients with an out-of-hospital cardiac arrest survived to admission and discharge, respectively[6] .

Patients who reach a hospital in time to be intubated and ventilated may still die from OP poisoning . High-dose dimethoate self-poisoning often results in death from cardiovascular shock that is resistant to atropine and vasopressors , which may be due in part to the effect of solvents in combination with the OP active ingredient (see the section titled Solvent and Ethanol Coingestants). In addition, patients who become unconscious before hospital presentation may aspirate their stomach contents, resulting in aspiration pneumonia and/or acute respiratory distress syndrome, or they may suffer hypoxic brain injury from which they do not recover[7].

Wadia et al. [8] and then Senanayake &Karalliedde [9] described a delayed respiratory failure, called type II respiratory failure or intermediate syndrome, respectively, occurring in conscious patients that seems to be due to NMJ dysfunction of particularly proximal muscles. This respiratory failure contrasts with that which occurs earlier in unconscious patients, where a loss of central respiratory drive is

likely to be a major component. Patients with late respiratory failure (occurring after 24 h) often require ventilation for many days [in one study, for a median of 284 h compared to just 45 h for those intubated before 24 h], leaving them at high risk of complications from immobility and mechanical ventilation. This delayed respiratory failure may occur after resolution of the acute cholinergic crisis, as originally described. It can also occur at the same time as the acute cholinergic syndrome and reduced consciousness; some patients effectively wake up from the central effects of the OP insecticide but continue to have a respiratory paralysis due to peripheral effects.

OP insecticides also inhibit the plasma enzyme butyrylcholinesterase (BuChE) however, this inhibition appears to have no substantial clinical effect beyond a potential small benefit from stoichiometric binding to the OP insecticide in plasma, lowering its concentration. There is also marked variation in the degree to which particular OP insecticides inhibit BuChE versus AChE [10] (26), suggesting that BuChE inhibition is not a good marker of AChE inhibition, unless the specific OP is known.

NOVEL TOXICOLOGY:

Casida&Quistad [11] have reported OP inhibition of a range of enzymes across multiple body systems in animal models. This inhibition, where investigated, does not appear to be important for acute rodent OP toxicity. Their role has not yet been studied in poisoned humans, and it is possible that some may be of clinical relevance.

The OP insecticide chlorpyrifos potently inhibits brain monoacylglycerol (MAG) lipase activity, a key degrading enzyme of the endogenous endocannabinoid system. MAG lipase inhibition results in raised concentration of the cannabinoid agonist 2-arachidonoylglycerol (2-AG) in the brain, which is associated with hypomobility in rodents. A second endocannabinoiddegrading enzyme, fatty acid amide hydrolase (FAAH), is also inhibited by the OP insecticide profenofos, but its inhibition does not correlate with clinical effects . Chlorpyrifos is a common WHO Class II OP insecticide responsible for many deaths worldwide. Similar to all OP poisoning, chlorpyrifos is acutely associated with coma and paralysis; it is possible that raised 2-AG has a role in the coma. Human studies are required but will be complex due to the difficulty of measuring the activity of an enzyme (i.e., MAG lipase) that does not occur in the blood. Plasma cannabinoid concentrations can be measured, and increased concentrations in acute poisoning could be suggestive of reduced breakdown. However, plasma activity may not necessarily correlate with activity in the brain, the presumed site of key activity [12] .

Mode of intoxication:

Intoxication may occur following absorption via the gastrointestinal system, respiratory tract or skin. Deliberate or accidental ingestion is a common mode of poisoning. Deliberate ingestion, which is usually of suicidal intent, is common in developing countries[13] where these agents are relatively more readily available and cheaper compared with the more sophisticated agents used for suicidal purposes in developed countries.

Inhalation may occur when spraying is carried out under improper conditions. Intoxication by inhalation may occur also during chemical warfare and following accidents during storage, particularly when stocks catch fire. Absorption through skin may occur in handlers and sprayers. Exposure during normal operational production and use depends mainly on the quality of personal clothing and the physical state of the insecticides.

Metabolism:

After absorption, OP insecticides and their metabolites distribute quickly in all tissues, maximum concentrations usually being reached in the liver and kidney. Lipophilic compounds may reach high concentrations in neural and other lipid-rich tissues. Plasma half-life after a single administration ranges from a few minutes to a few hours, depending on the compound and route of administration.Metabolism occurs principally by oxidation, hydrolysis by esterases and transfer of portions of the molecule to glutathione. Most thiophosphorus compounds with P=S, such as parathion, are converted to the biologically active oxon, paraoxon (P=O), by microsomes in the liver.
Detoxification of OP insecticides occurs either by biochemical modification of their structure of by linkage to binding sites without toxicological significance. Malathion is metabolized to inactive products more rapidly in higher animals than in insects; consequently it is less dangerous to man, other mammals and birds in insecticidal concentrations. Elimination of OP compounds and their metabolites occurs mainly via urine and faeces. Urinary and faecal excretion is usually rapid, 80-90% of most compounds being eliminated within 48 h. A very small proportion of OP compounds and their active forms (oxons) are eliminated unchanged in the urine. Some compounds (e.g. fenthion, fenitrothion)

are known to persist in the body for longer periods[14] .

MECHANISM OF ACTION AND TOXICOLOGY:

OP insecticides exert their biological actions mainly by inhibition of enzymes. Esterases are the target responsible for OP toxicity to insects and mammals, the toxicity depending primarily on the power of the OP compound to inhibit the esterases.

Inhibition of acetyI cholinesterase (AChE):

AChE is responsible for hydrolytic cleavage of acetylcholine (ACh) to choline and acetic acid. ACh acts as neurotransmitter for all preganglionic autonomic fibres, all post-ganglionic parasympathetic fibres and a few post-ganglionic sympathetic fibres. Moreover, ACh is the neurohumoral transmitter at the skeletal muscle motor endplates and some interneuronal synapses in the central nervous system (CNS). Synaptic conduction is mediated by the pre-synaptic release of ACh into the interneuronal space. The arrangement of the synapse is such that ACh comes into contact with AChE before the post-synaptic receptor sites. ACh which is not degraded by AChE binds to the post-synaptic receptors, resulting in generation of an excitatory post-synaptic potential and propagation of the impulse.

The transient nature of this event is caused by degradation of ACh by AChE. AChE is an enzyme located mainly in the nervous system and in the motor end-plates of skeletal muscle. When AChE is inhibited, the post-synaptic cholinergic transmission time is extended and this results in protracted cholinergic overstimulation.

Neuropathy target esterase inhibition:

Delayed neurotoxic action of OP insecticides is independent of AChE inhibition, but related to the phosphorylation of a specific esteratic enzyme in the nervous tissue[15-16] which has been termed "neurotoxic esterase" or "neuropathy target esterase" (NTE) [17-18] . The initial biochemical reaction is phosphorylation of NTE. The essential second step responsible for the neuropathy (organophosphate-induced delayed polyneuropathy (OPIDP)) is the transformation of the phosphorylated enzyme to an aged form. If compounds such as phosphinates and carbamates link to NTE before contact with a neuropathic OP, ageing does not occur, preventing the development of a neuropathy[19] .

Inhibition of other enzymes:

A number of other enzymes including Upases, trypsin and chymotrypsin are phosphorylated by OP insecticides. The rate of reaction with these enzymes is generally slower than with AChE, and the clinical consequences of these reactions are not known as yet [20] .

Myopathic effects:

Muscular weakness following OP intoxication in animals had been known to experimental scientists for many years, the earliest observations being made by Carey. Paralysis appearing within 24 h of poisoning and lasting a few days or weeks had been described in hens. Myopathic changes were described in the diaphragm, gastrocnemius and psoas muscles of the rat following sublethal doses of OP compounds such as DFP, tabun and paraoxon . Necrotic changes have been shown to begin and to be most extensive in the region of the motor endplate . The peak of necrosis was seen at 1-3 days; at 7 days, signs of muscle recovery were evident. Full recovery occurred within 2-3 weeks. This myopathy was different from the delayed neuropathy which began at nerve terminals approximately 3 weeks after exposure to DFP when muscle contractile strength was returning to normal [21].

Muscle paralysis occurring in man after apparent recovery from the cholinergic crisis but before the expected onset of the delayed polyneuropathy has been identified by us recently as the " Intermediate Syndrome" (IMS) [22]. Onset, progression and recovery of muscle weakness in the IMS corresponds closely to the sequence of myopathic changes observed in animal experiments and is distinguishable from the muscle weakness that follows the delayed polyneuropathy which sets in 2-4 weeks after poisoning. The diaphragm was affected most severely in most instances in the animal experiments and this finding is consistent with the cardinal feature of respiratory failure in the IMS.

Carcinogenicity and teratogenicity:

As OP agents are alkylating agents, it has been implied that they may be responsible for rautagenic and carcinogenic effects. However, animal studies to date have not shown dose-related carcinogenic effects and the evidence suggests that these compounds are unlikely to persist long enough in vivo to exert any alkylating effect they may possess .
OP poisoning and pregnancy In experimental animals, OP poisoning during pregnancy causes pre-natal and post-natal death and congenital abnormalities, viz. vertebral deformities, limb defects, polydactyly, intestinal herniae, cleft palate and hydroureter . Following OP intoxication during the third month of human pregnancy, abortion has been performed as continuation of pregnancy was

considered hazardous. However, successful management of the poisoning during the second and third trimesters may allow the pregnancy to continue to term unaffected[23] and result in the delivery of normal, healthy babies.

CLINICAL MANIFESTATIONS:

Cholinergic phase Cholinergic manifestations are summarized best under three categories based on the site of cumulation of ACh: muscarinic (all post-ganglionic nerve endings), nicotinic (autonomic ganglia and skeletal muscle endplates) and central (synapses in the CNS).

These symptoms may arise in varying combinations. Their severity and time of onset depend on the chemical composition of the agent and the mode of intoxication. Following massive ingestion, symptoms arise within minutes. Death has occurred within 5 min after ingestion of concentrated TEPP. However, in most instances symptoms appear within 30 min of exposure and almost always in less than 12 h.

The most serious manifestation and the usual cause of death is respiratory failure which results from weakness of the muscles of ventilation and depression of the respiratory centre, aggravated by excessive tracheobronchial secretions and bronchospasm. Loss of consciousness in severe intoxication and the accompanying vomiting predisposes to aspiration of gastric contents into the lungs. Bradycardia may be severe and may progress to heart block. The clinical presentation is often one of acute medical emergency which requires urgent cardiorespiratory resuscitation.

Intermediate syndrome (IMS):

After recovery from the cholinergic crisis, but before the expected onset of the delayed polyneuropathy, some patients develop a state of muscle paralysis which we have described recently as the "Intermediate Syndrome". The syndrome is of acute onset, often seen 24—96 h after poisoning, affecting conscious patients without fasciculations or other cholinergic manifestations. The cardinal feature of the syndrome is muscle weakness affecting predominantly the proximal limb muscles and neck flexors. Muscles innervated by motor cranial nerves III—VII and X are affected also in different combinations. The syndrome carries a risk of death, because of respiratory paralysis, if not recognized early and treated adequately. The agents commonly responsible are fenthion, monocrotophos and dimethoate.

Respiratory insufficiency develops over approximately 6 h. Initially, the patient uses accessory muscles of ventilation. There is increase in ventilatory rate, sweating, restlessness and later cyanosis. Unattended, the patient soon becomes unconscious and death follows.

Delayed polyneuropathy (OPIDP):

Most reports of OPIDP involve intoxication with non-insecticidal OP agents. These include an early report in the 19th century when phosphocreosote was used to treat pulmonary tuberculosis . In the 1930s more than 50000 U.S. citizens became paralysed after drinking Jamaica ginger contaminated with TOCP. An outbreak involving approximately 10000 persons occurred in Morocco in 1959 after a mixture of olive oil and aircraft lubricating oil was sold as food . Several other outbreaks have occurred in Durban, Fiji, Vietnam and Sri Lanka, where the poisoning was traced in most instances to accidental contamination or adulteration of cooking oils with mineral oils . There have been reports of OPIDP with insecticides mipafox, leptophos, trichlophon, trichlornat, chlorpyrifos and methamidophos[24] .

Behavioural effects:

Behavioural changes have been documented following acute or chronic OP poisoning. These symptoms may take months to regress. In human subjects exposed to OP agents to an extent sufficient to depress plasma or erythrocyte ChE, some or all of the following observations have been made:

(1) Impairment of vigilance, information processing, psychomotor speed and memory.

(2) Poor performance and perception of speech.

(3) Increased tendency to depression, anxiety and irritability.

(4) A tendency to faster frequencies and higher voltages in EEG records.

The EEG abnormalities were related positively to the level of AChE inhibition during the initial stages of intoxication .

DIAGNOSIS:

Acute cholinergic crisis Clinical diagnosis is relatively easy and is based on the characteristic symptoms and signs, and the history of exposure to OP agent. Helpful signs include miosis and muscle fasciculations. Excessive secretions in the mouth and respiratory tract, sweating and lachrymation are other useful signs. History may be denied in attempts at suicide or unavailable in patients who are found unconscious. In these situations the pungent garlic-like odour in breath, vomitus or faeces may suggest OP intoxication.

The response to atropine therapy may also be a useful aid to diagnosis. Patients with OP poisoning show tolerance to atropine and failure to produce signs of atropinization (mydriasis, tachycardia, flushing of skin, dryness of mouth and skin) with 1-2 mg i.v. indicates OP poisoning.

Intermediate syndrome (IMS):
The diagnosis is clinical and should be suspected when a patient who is recovering from the cholinergic crisis develops respiratory difficulty. The presence of muscle weakness in the absence of muscle fasciculations and other cholinergic features differentiates it from cholinergic crisis. The early onset of muscle weakness distinguishes the IMS from the delayed polyneuropathy which appears 2-3 weeks after poisoning. There is also an obvious contrast between the distribution of muscular weakness in the two conditions .

Delayed polyneuropathy:
History of intoxication with OP agents and the time of onset and distribution of muscle weakness differentiate OPIDP from other causes of acute polyneuropathy.

Cholinesterase inhibition:
AChE is present in human erythrocytes (RBC) and is the same as the enzyme present in the target synapses. Thus changing concentrations of AChE in RBC are assumed to mirror the effects in the target organs, provided the OP agent has equal access to blood and synapses. In acute poisoning, high inhibition of RBC-AChE might not correlate with severity of the symptoms. Plasma contains a related enzyme, pseudocholinesterase, which has no known physiological function. The sensitivities of AChE and of pseudo-ChE to OP agents differ . Thus the use of whole blood samples in analysis gives only an approximate estimate of the activity of RBC-AChE. However, in many field situations and in clinical practice, procedures using whole blood are more practical than those using separated RBC. Physiological variations in blood concentrations of ChE occur both within and between individuals[25] .

OP agents in blood and urine:
Measurement of OP compounds in plasma or of metabolites in urine is a more sensitive indicator of exposure than measurement of ChE activity in the blood. The information derived from these two methods differs, as excretion of OP agents occurs rapidly, whilst enzyme activity recovers more slowly. Thus ChE observations, RBC-ChE in particular, provide a summary of the physiological effect of exposure, whereas blood and urine assays of OP agents or their metabolites provide accurate quantifiable data relating to the time course of intoxication. Although the analysis of urine for concentrations of intact pesticides and their metabolites is useful, because of rapid hydrolysis of OP agents by the body, it is often not possible to detect the parent compound, except in cases of severe intentional poisoning[26] .

ORGANOPHOSPHORUS INSECTICIDE CHEMISTRY:

Many hundreds of OP insecticides were developed and introduced into global agriculture in the twentieth century. They vary in multiple important ways, including the degree of lipid solubility, the alkyl groups attached to the phosphorus, the rate of activation (conversion from thion to oxon), and the rate of AChE inhibition. These differences result in marked variation in toxicity, the speed of onset, and the clinical syndrome after ingestion[27]. Fortunately, a smaller range of compounds is typically used for agriculture in any one area, reducing the variation in self-poisoning seen among patients.

OP insecticides vary widely in their lipid solubility. Some OP insecticides are relatively hydrophilic with log Kow (log P) values <1.0 [e.g., dimethoate (0.76) and trichlorfon (0.51)], while others are highly lipophilic with high log Kow values [e.g., chlorpyrifos (5.05), dichlofenthion (5.14), and profenofos (4.56)]. Lipophilicity markedly affects the volume of distribution, the acuteness of toxicity, and both the duration and recrudescence of toxicity, as shown in rat studies of trichlorfon and dichlofenthion. Poisoning with lipophilic insecticides results in relatively minor early clinical features, recurrence of toxicity, delayed respiratory failure, and prolonged cholinesterase inhibition due to sustained delivery from fat stores to the systemic circulation [28]. Poisoning with hydrophilic OP insecticides often produces relatively acute poisoning with rapid resolution if the patient survives .

In addition, the quantity of patient fat affects the outcome of poisoning with lipophilic OP insecticides. A Korean study of overweight patients [body mass index (BMI) > 25] showed a longer duration of ventilation, intensive care, and hospital admission after poisoning with highly lipophilic OP insecticides compared to nonlipophilic OP insecticides [29]. This difference between highly lipophilic and nonlipophilic OP insecticides did not occur in patients with a BMI of 25 or less.

Many OP insecticides are propoisons (i.e., thions) , with a P==S structure that must be converted to a P==O (or oxon) structure to obtain effective cholinesterase inhibition. Thion OPs are activated by cytochrome P450 (CYP450) enzymes in the liver and intestinal mucosa. The precise CYP450s responsible vary according to the concentration of OP. For example, at low concentrations, chlorpyrifos, diazinon, parathion, and malathion are all metabolized and activated in vitro by CYP1A2 and 2B6 . However, at the higher concentrations likely to occur from self-poisoning, CYP3A4 becomes dominant. The CYP450 enzymes involved in the metabolism of active oxons to inactive metabolites are less clear. The rates of conversion may determine the speed of inhibition and speed of onset of clinical features. However, this does not appear to always be a key rate-limiting step since a highly potent thion such as parathion, which must be converted in vivo to paraoxon, can induce clinical features, including coma and respiratory arrest, within 15–30 min of ingestion[30] .

The speed of AChE inhibition itself may be a more important factor. In vitro studies have shown widely differing rates of inhibition by oxons, with fenthion, for example, being a slow inhibitor of AChE. This relatively slow inhibition of AChE by fenthion and its slow conversion to fenthion oxon, more than its high lipid solubility (producing low extracellular fluid concentrations), may account for the much-delayed toxicity of fenthion compared to other lipidsolublethion OP insecticides such as chlorpyrifos.

Binding to, and the inhibition of, AChE results in the production of either dimethoxyphosphorylated or diethoxy-phosphorylated AChE, irrespective of the actual OP insecticide involved. A few are S-alkyl OP insecticides in which one of the alkyl groups is attached to the phosphorus via a sulfur atom. This chemistry has major implications for the speed of aging and therefore the efficacy of oxime treatment. Although the splitting of the choline–enzyme bond in normal acetylcholine metabolism is completed within microseconds, the severing of the OP compound–enzyme bond is prolonged. The half-life of this reaction depends on the chemistry of the substituted phosphate. The in vitro half-life for spontaneous reactivation of human AChE inhibited by dimethoxy OPs is 0.7–0.86 h, and 31–57 h for diethoxy inhibition[31] . Spontaneous reactivation is therefore quicker with dimethoxy OPs; however, this is only clinically relevant in patients with more moderate OP toxicity because the reactivated AChE is simply reinhibited again in patients with high OP concentrations.

Dimethoxy-phosphorylated AChE ages faster than diethoxy-phosphorylated AChE, meaning that it rapidly becomes unresponsive to oximes with a half-life of 3.7 h (versus 33 h for diethoxy). A delay of 4 h to oxime therapy will mean that 50% of AChE is already irreversibly inhibited. Oxime therapy may be effective for several days with diethoxy OP insecticides. Aging of AChE inhibited by S-alkyl OP insecticides appears to occur very quickly, allowing no response to oximes, even if given early[32] .

OP insecticide toxicity may also be increased in the bottle, before ingestion, by chemical reactions resulting from storage at warm temperatures. In a large Pakistani epidemic, the conversion of malathion to the more toxic compound isomalathion in the bottle correlated with increased toxicity. Increased toxicity has also been noted after the prolonged storage in warm conditions of diazinon and dimethoate .

CLASSICAL TREATMENT OF ORGANOPHOSPHORUS INSECTICIDE POISONING:

The primary cause of death after anticholinesterase poisoning is respiratory failure and hypoxemia resulting from muscarinic effects on the cardiovascular and pulmonary systems (i.e., bronchospasm, bronchorrhea, aspiration, bradydysrhythmias, or hypotension), nicotinic effects on skeletal muscles (i.e., weakness and paralysis), loss of central respiratory drive, and seizures (rare).

Therefore, initial treatment for a patient exposed to OP compounds should be directed at ensuring an adequate airway, oxygenation, and ventilation and at stabilizing cardiorespiratory function by reversing excessive muscarinic effects . Once the patient is stable, the administration of an AChE-reactivating oxime drug, such as pralidoxime or obidoxime, can be considered along with the need for skin and/or gastric decontamination[33].

Atropine:

The use of atropine in OP insecticide poisoning has been accepted practice since the 1950s however, the preferred regimen has been clarified only recently. Atropine must be administered until the features of the acute cholinergic syndrome have settled: In particular, the bronchorrhea must be resolved and the lungs clear (while being aware of focal consolidation due to aspiration that will not resolve with atropine), the heart rate adequate (around 80 beats/min), and the

blood pressure adequate (a systolic blood pressure greater than 80–90 mm Hg). This status is referred to as being atropinized. Unfortunately, such doses of atropine do not counter the central loss of respiratory drive or NMJ dysfunction; therefore, most severely poisoned patients require intubation and mechanical ventilation[34].

A systematic review of treatment guidelines in 2002–2003 found 33 different recommendations for administering atropine to resuscitate an OP insecticide–poisoned patient. Most recommended a range of fixed atropine doses (e.g., 2–5 mg) given every 5–15 min without titration to effect. Comparing the time to give 23.4 mg, the median dose in a Sri Lankan cohort of patients, the different regimens took from 8 to 1,380 min. Several regimens took more than 4 h to give sufficient atropine to stabilize patients. Administration of the high doses needed by some patients required many hours, while leaving the patients dangerously unstable. In the systematic review, one regimen stood out—that of Cynthia Aaron , who recommended giving 1–2 mg initially and then doubling the dose every 5 min in the absence of a response. This regimen took only 15 to 20 min to give 23.4 mg, could give much higher doses quickly if required, and was titrated to effect.

This regimen was incorporated into a clinical guideline and is now recommended in the majority of guidelines worldwide following a randomized controlled trial (RCT) performed in Bangladesh. This RCT tested the standard therapy (2–5 mg every 10–15 min, followed by an infusion) with Aaron's regimen (1.8 to 3 mg every 5 min, doubled until atropinization occurred, followed by an infusion) in 156 patients with acute OP insecticide self-poisoning. Most importantly, the doubling dose regimen resulted in an 84% reduction in the mean time to atropinization, from 152 min to 24 min, with only a modest increase in total dose of atropine required. This much faster resuscitation was associated with a fall in case fatality from 22.5% to 8.0% and a reduction in the proportion of patients showing atropine toxicity from 28.4% to 12%.

After initial loading, atropine should be continued as an infusion titrated against cholinergic features. The infused dose can often be reduced to around 1 mg/h after several hours . Patients should thereafter be carefully and frequently observed for evidence of (a) deteriorating neurologic function and potential paralysis requiring ventilation and (b) either recurrent cholinergic signs suggestive of inadequate atropine dosing or atropine toxicity indicative of a need to reduce atropine dosing[35].

One concern in the rural Asian district hospitals where the majority of patients are seen is the intermittent supply of oxygen. For many years, guidelines have indicated that patients should not receive atropine until hypoxia has been treated with oxygen due to the risk of inducing ventricular tachydysrhythmias . Unfortunately, many of these hospitals do not have easy access to oxygen. At the same time, atropine is effective at treating hypoxia by reversing bronchorrhea and bronchospasm. A review of the data cited in the guidelines revealed just two case reports, of debatable relevance, of cardiotoxicity associated with atropine . A large Sri Lankan case series in which patients received atropine on admission, whether oxygen was available or not, demonstrated no evidence of fatal atropine-induced dysrhythmias in such patients[36] .

Overall, these guidelines have caused unnecessary confusion, impeded good clinical care, and are not evidence based. Atropine can be given, if clinically necessary, before oxygen becomes available.

Oximes:

In the 1950s, groups in the United States and United Kingdom developed pralidoxime, a drug that reactivated AChE inhibited by OP compounds. Initially, it was used for occupational poisoning with high-potency (WHO Class I) diethoxy OP insecticides such as parathion. For such poisonings with diluted insecticide, solvents and other coformulants are not relevant. Patients treated with 1 g of pralidoxime showed good reactivation of red blood cell AChE and clinical recovery, leading to recommendations that it be used for all OP insecticide–poisoned patients .

However, clinicians quickly recognized that much larger doses than 1 g might be needed for patients with intentional overdoses, who drink large quantities of OP in combination with solvents, particularly for less toxic WHO Class II pesticides such as malathion where large quantities need to be ingested to elicit moderate-severe poisoning. Recommended doses of 0.5 g/h after a loading dose for such high-dose poisoning. However, this advice did not appear in guidelines and most patients over the following decades received 1 g every 6 h for 1–2 days.

An observational study performed in 1991 reported that the absence of pralidoxime for 6 months in Sri Lanka was not associated with worse outcomes, suggesting a lack of clinical effect. Advocates responded that higher doses, akin to Namba et al.'s high-dose regimen, should be given to all patients . WHO guidelines that were published in 2000

reinforced the view. However, in vitro studies with human red blood cell AChE and clinical studies of AChE inhibition indicate that the reactivation by oximes of AChE inhibited by dimethoxy or S-alkyl OP insecticides is much less effective than the reactivation of diethoxy OP insecticides (see the section titled Organophosphorus Insecticide Chemistry) [37].

Treatment with oximes of poisoning with the dimethoxy thion OP dimethoate additionally seems to be even less effective than expected . This may be due to the production of isodimethoate in the pesticide bottle, when stored in hot conditions, before ingestion by the patient, producing an S-alkyl OP that is resistant to oximes.

These findings indicate that oximes are unlikely to be effective for many patients who are poisoned by dimethoxy or S-alkyl OP insecticides. Oximes may also be ineffective for all OP insecticide poisoning cases if the concentration of pesticide in the body is very high, overwhelming the capacity of oximes to reactivate AChE[38].

Systematic reviews of clinical trials of pralidoxime, including high-dose regimens (infusions of 0.5 g/h after a loading dose), compared with placebo support the idea that it does not prevent death or intubation (Tables 1 and 2) or shorten the duration of ventilation. Of note, several of these studies included high-dose infusions for up to 7 days without benefit, indicating that inadequate dosing is not responsible for the lack of effect. The lack of efficacy may be due to the very large doses of OP insecticide ingested during self-harm. For example, a typical ingested dose of 100 mL of a 40% parathion formulation contains 40 g of active ingredient. This equals 666 mg/kg parathion for a 60-kg adult, a dose 51-fold greater than the rat oral median lethal dose for parathion of 13 mg/kg . The clinically tolerated doses of oxime may be unable to counter such huge doses, with any reactivated oxime simply being rapidly inhibited again by the high blood OP concentration.

An RCT of 200 OP-poisoned patients compared two pralidoxime regimens [2 g loading dose over 30 min followed by either 1 g pralidoxime (over 1 h) every 4 h for 2 days (total dose 14 g) or an infusion of 1 g/h for 2 days (total dose 50 g)], showing decreased mortality. It is unclear how these data complement the placebo-controlled data, especially since the patients were less severely poisoned than in other studies and a large proportion were intubated at baseline and cared for in an intensive care unit [66% versus, for example, 17% in a Sri Lankan RCT].

Some groups have reported a benefit by titrating pralidoxime dosing against BuChE reactivation . However, OP insecticides inhibit BuChE to variable degrees , and AChE inhibition may differ from BuChE inhibition. A pralidoxime 2 g dose variably reactivated BuChE in patients who were poisoned by two WHO Class II diethoxy OP insecticides, chlorpyrifos and quinalphos; however, unlike AChE reactivation, the BuChE reactivation was not sustained. This dose did not reactivate BuChE inhibited by the dimethoxy OPs dimethoate or fenthion at all. A pralidoxime 1 g dose produced no reactivation with any OP insecticides. This suggests that titrating pralidoxime dosing against BuChE reactivation is unlikely to be effective, unless perhaps the particular OP ingested and its pharmacodynamics are known.

Although pralidoxime has been the key oxime used worldwide since the 1960s, there are other more potent oximes such as obidoxime and trimedoxime . Lower concentrations of these oximes are required to reactivate human red blood cell AChE ex vivo, however, aging still occurs, indicating the need for early therapy. Although careful observational data have been reported for obidoxime treatment of OP insecticide self-poisoned patients showing clinical improvement, there are no clinical trial data showing clinical effectiveness.

Benzodiazepines:

Benzodiazepine γ-aminobutyric acid (GABA) agonists such as diazepam or midazolam are recommended to settle agitation, prevent or treat seizures, and reduce fasciculations. Animal studies of OP nerve agent poisoning indicate that they can also prevent neuronal damage , however, the relevance of this pathology to human poisoning remains unclear. Although they are a classically described feature of OP insecticide poisoning , overt seizures are uncommon (1–3%) in hospitalized adult patients, perhaps due to effective atropinization. Seizures may be more common in children . No clinical trials have assessed whether benzodiazepine administration in OP insecticide self-poisoning offers clinical benefit [39] .

NOVEL TREATMENTS FOR ORGANOPHOSPHORUS INSECTICIDE POISONING:

Current therapy is based on two treatments that were first reported in the 1950s. Many more possible therapies have been tested in animals; a few have made it into small, inadequately powered clinical studies . There is an urgent need to find additional

treatments that can complement and augment the often inadequate current therapy[40].

Magnesium Sulfate and Calcium Channel Blockade:

The use of magnesium or calcium channel blockers (CCBs) such as nifedipine in OP compound poisoning has long been advocated . The precise mechanism of how this intervention might work remains unclear. Calcium is required in the presynaptic terminus for the exocytosis of acetylcholine to occur. Interruption of the calcium flow through channels by magnesium or CCBs may be sufficient to reduce the synaptic concentration of acetylcholine. OP insecticides also inhibit Ca2+ ATPase, the enzyme responsible for removing cytosolic Ca2+ [41] (105). Rat studies suggest that CCBs reactivate OP-inhibited Ca2+ ATPase, decreasing intracellular Ca2+ concentrations and theoretically reducing acetylcholine release.

Administration of CCBs or magnesium to rodents before or soon after OP exposure, in addition to atropine and/or oxime, reduces mortality. A nonrandomized Iranian clinical study of 4 g magnesium sulfate (MgSO4) in acute OP poisoning during 2003–2004 suggested that it was effective in reducing mortality and length of hospital stay. A total of eight clinical studies or trials have now been performed (441 patients; 239 patients receivingMgSO4, 202 control patients; MgSO4 doses up to 26 g/day), all of small-to-modest size and with marked risk of bias. The pooled odds ratios for MgSO4 for mortality and need for intubation and ventilation for all eight studies were 0.55 [95% confidence interval (CI), 0.32–0.94] and 0.52 (95% CI, 0.34–0.79), respectively . This result suggests that this intervention might be beneficial, but it is far from definitive due to the size of the RCTs and the risk of bias. A large RCT is required to provide clear evidence[42].

Sodium Bicarbonate for Plasma Alkalinization:

OP insecticide poisoning often causes a metabolic and respiratory acidosis due to hypotension, hypoxia, and hypoventilation. This usually settles with fluid resuscitation, oxygen, atropinization, and mechanical ventilation. However, clinicians have proposed that sodium bicarbonate should be used as an antidote for OP insecticide poisoning to alkalinize the plasma , as is done routinely to treat sodium channel blockade in tricyclic antidepressant poisoning. The proposed mechanism of effect is poorly defined but may include enhanced pesticide clearance from the body, improved efficacy of oximes, and a direct effect on NMJ function. A systematic review of the literature identified only five low-quality clinical studies that together suggested a possible benefit from plasma alkalinization[43] .

However, attempts to study the approach in Sri Lankan district hospitals with few intensive care resources indicated difficulty in resource-poor hospitals that were inexperienced in giving bicarbonate . Further studies are required to understand whether it benefits patients and how it could be used in low-income countries that see the majority of patients.

Salbutamol:

OP insecticide poisoning is characterized by bronchorrhea and noncardiogenic pulmonary edema, which hinder oxygen exchange. Adequate atropinization turns off the fluid production, but it does not increase the removal of fluid from alveoli. A complementary therapy that increases fluid removal from alveoli could speed up the return of effective oxygen exchange and resuscitation. Salbutamol accelerates alveolar fluid clearance by enhancing salt and water transfer across alveolar and distal airways. It may also reverse OP insecticide–induced bronchoconstriction, thereby improving respiratory mechanics by decreasing airflow resistance and peak airway pressures as well as increasing dynamic compliance [44] .

A small phase II dose-response study was performed in a resource-poor hospital in Bangladesh to explore the effects of nebulized salbutamol. OP insecticide–poisoned patients (n = 75) requiring atropine for cholinergic features received a single 2.5 or 5 mg dose of salbutamol, or saline placebo, and their peripheral blood oxygen saturations were monitored every minute for 60 min. A mild tachycardia occurred in response to the higher dose of salbutamol, suggesting absorption. Oxygen saturations did not improve with the salbutamol. Indeed, recovery had already occurred by 20 min after placebo, and the higher dose was associated with a longer time to oxygen saturations that were consistently >95%. It is possible that this negative finding was due to the inevitable variation between patient groups seen in this small RCT; however, the results do not encourage additional studies in light of the other possible treatments.

Nicotinic Antagonists:

A key problem with OP insecticide poisoning is the NMJ dysfunction (intermediate syndrome) that may occur hours or days after exposure . The mechanism is uncertain; however, it occurs in the face of adequate atropinization, suggesting a

nonmuscariniceffect. The main mechanism proposed is overstimulation of post- and/or presynaptic nicotinic receptors at the NMJ. A competitive nicotinic blockade, with a drug such as rocuronium, may prevent this overstimulation and damage. Phase II studies are required to explore the best way to give nicotinic antagonists to OP-poisoned patients.

Lipid Emulsions:

Lipid emulsions have been widely recommended for acute poisoning with lipid-soluble poisons, although the evidence and rationale are weak for treatment of oral overdoses rather than intravenous local anesthetic overdoses . Many OP insecticides are lipid soluble, and it is possible that intravenous lipid emulsions may redistribute the poison, but they may also increase absorption from the gut, thereby increasing toxicity. A rodent study has suggested that there may be a benefit from this treatment . Similarly, an uncontrolled study of 40 patients, published in abstract form, also suggested some benefit compared to historical controls. However, a recent in vitro study has suggested that the lipid emulsion may actually stabilize the OP from degradation. More studies are required to identify whether the approach is associated with a benefit for certain OP insecticides and/or worse toxicity before it can be used outside a clinical trial.

Acetylcysteine:

Oxidative stress has been reported in OP insecticide poisoning, likely due to initial hypoxia and tissue hypoperfusion. However, some researchers have proposed that oxidative stress is causal for poor outcomes rather than being associated with severe poisoning . Acetylcysteine has been tested as a treatment for rodents and in small, underpowered RCTs [45]. Again, larger RCTs are required before its clinical use.

CONCLUSION:

Despite the fact that several antidotes have been proposed for the treatment of OP pesticide poisoning, only one antidote – the muscarinic receptor antagonist atropine – has clear evidence of efficacy and a clear function in management. Oximes are commonly used and suggested, however there is limited high-quality RCT evidence of their efficacy. To determine appropriate doses of these antidotes and the patient subgroups who might benefit from their delivery, clinical pharmacology research is required. The wide diversity of OP insecticides, which affects pharmacokinetics, period of toxicity start, and treatment response, will make identifying effective antidotes more difficult.

REFERENCES:

1. Casida JE, Quistad GB. 2005. Golden age of insecticide research: past, present, or future? Annu. Rev. Entomol. 43:1–16
2. Gunnell D, Fernando R, Hewagama M, Priyangika WDD, Konradsen F, Eddleston M. 2007. The impact of pesticide regulations on suicide in Sri Lanka. Int. J. Epidemiol. 36:1235–42
3. Gunnell D, Knipe D, Chang SS, Pearson M, Konradsen F, et al. 2017. Prevention of suicide with regulations aimed at restricting access to highly hazardous pesticides: a systematic review of the international evidence. Lancet Global Health 5:e1026–37
4. Buckley NA, Karalliedde L, Dawson A, Senanayake N, Eddleston M. 2004. Where is the evidence for the management of pesticide poisoning? Is clinical toxicology fiddling while the developing world burns? J. Toxicol. Clin. Toxicol. 42:113–16
5. Peter JV, Sudarsan TI, Moran JL. 2014. Clinical features of organophosphate poisoning: a review of different classification systems and approaches. Indian J. Crit. Care Med. 18:735–45
6. Park JH, Shin SD, Song KJ, Park CB, Ro YS, Kwak YH. 2012. Epidemiology and outcomes of poisoninginduced out-of-hospital cardiac arrest. Resuscitation 83:51–57
7. Aardema H, Meertens JH, Ligtenberg JJ, Peters-Polman OM, Tulleken JE, Zijlstra JG. 2008. Organophosphorus pesticide poisoning: cases and developments. Neth. J. Med. 66:149–53
8. Wadia RS, Sadagopan C, Amin RB, Sardesai HV. 1974. Neurological manifestations of organophosphate insecticide poisoning. J. Neurol. Neurosurg. Psychiatry 37:841–47
9. Senanayake N, Karalliedde L. 1987. Neurotoxic effects of organophosphate insecticides: an intermediate syndrome. N. Engl. J. Med. 316:761–63
10. Eddleston M, Eyer P, Worek F, Sheriff MHR, Buckley NA. 2008. Predicting outcome using butyrylcholinesterase activity in organophosphorus pesticide self-poisoning. QJM 101:467–74
11. Casida JE, Quistad GB. 2005. Serine hydrolase targets of organophosphorus toxicants. Chem. Biol. Interact. 157–158:277–83
12. Quistad GB, Klintenberg R, Caboni P, Liang SN, Casida JE. 2006. Monoacylglycerol lipase inhibition by organophosphorus compounds leads to elevation of brain 2-arachidonoylglycerol and the associated hypomotility in mice. Toxicol. Appl. Pharmacol. 211:78–83.

13. Davies JE. Changing profile of pesticide poisoning. New England Journal of Medicine 1987; 316: 807-808
14. Maroni M. Review of toxicological properties and biotransformation of organophosphorus esters. In: WHO Manual of Analytical Methods. Cremona: WHO Collaborating Centre for Occupational Health, 1985; 3-39
15. Johnson MK. A phosphorylation site in brain and the delayed neurotoxic effect of some organophosphorus compounds. Biochemical Journal 1969; 111: 487^95.
16. Johnson MK. The delayed neurotoxic effect of some organophosphorus compounds: Identification of the phosphorylation site as an esterase. Biochemical Journal 1969; 114: 711-717.
17. Johnson MK. The delayed neuropathy caused by some organophosphorus esters: mechanism and challenge. Critical Reviews in Toxicology 1975; 3: 289-316.
18. Johnson MK. The target for initiation of delayed neurotoxicity by organophosphorus esters: biochemical studies and toxicological applications. Reviews in Biochemical Toxicology 1982; 4: 141.
19. Johnson MK, Lauwerys R. Protection by some carbamates against the delayed neurotoxic effects of diisopropyl phosphorofluoridate. Nature (London) 1969; 222: 1066-1067.
20. Hayes WJ jr. Pesticides Studied in Man. Baltimore: Williams & Wilkins, 1982; 284-113.
21. Salpeter MM, Kasprzak H, Feng H, Fertuck H. Endplates after esterase inactivation in vivo: correlation between esterase concentration, functional response and fine structure. Journal of Neurocytology 1979; 8: 95-115
22. Senanayake N, Karalliedde L. Neurotoxic effects of organophosphorus insecticides: an intermediate syndrome. New England Journal of Medicine 1987; 316: 761-763.
23. Karalliedde L, Senanayake N, Ariaratnam A. Acute organophosphorus insecticide poisoning during pregnancy. Human Toxicology 1988; 7: 363-36.
24. Smith HV, Spalding JMK. Outbreak of paralysis in Morocco due to ortho-cresyl phosphate poisoning. Lancet 1959;2: 1019-1021.
25. WHO/ILO/UNEP, Environmental Health Criteria-63. Organophosphorus Insecticides: A General Introduction. Geneva: World Health Organisation (WHO) 1986, 17-111.
26. Minton NA, Murray VSG. A review of organophosphate poisoning. Medical Toxicology 1988; 3: 350-375
27. Eddleston M, Buckley NA, Eyer P, Dawson AH. 2008. Medical management of acute organophosphorus pesticide poisoning. Lancet 371:597–607
28. Davies JE, Barquet A, Freed VH, Haque R, Morgade C, et al. 1975. Human pesticide poisonings by a fat soluble organophosphate pesticide. Arch. Environ. Health 30:608–13
29. Lee DH, Jung KY, Choi YH, Cheon YJ. 2014. Body mass index as a prognostic factor in organophosphate-poisoned patients. Am. J. Emerg. Med. 32:693–
30. Eyer F, Meischner V, Kiderlen D, Thiermann H, Worek F, et al. 2003. Human parathion poisoning. A toxicokinetic analysis. Toxicol. Rev. 22:143–63
31. Eyer P. 2003. The role of oximes in the management of organophosphorus pesticide poisoning. Toxicol. Rev. 22:165–90
32. Eddleston M, Worek F, Eyer P, Thiermann H, von Meyer L, et al. 2009. Poisoning with the S-alkyl organophosphorus insecticides profenofos and prothiofos. QJM 102:785–92
33. Eddleston M, Dawson A, Karalliedde L, Dissanayake W, Hittarage A, et al. 2004. Early management after self-poisoning with an organophosphorus or carbamate pesticide—a treatment protocol for junior doctors. Crit. Care 8:R391–97
34. Durham WF, Hayes WJ Jr. 1962. Organic phosphorus poisoning and its therapy. With special reference to modes of action and compounds that reactivate inhibited cholinesterase. Arch. Environ. Health 5:21–47
35. Perera PM, Shahmy S, Gawarammana I, Dawson AH. 2008. Comparison of two commonly practiced atropinization regimens in acute organophosphorus and carbamate poisoning, doubling doses vs. ad hoc: a prospective observational study. Hum. Exp. Toxicol. 27:513–18
36. Konickx LA, Bingham K, Eddleston M. 2014. Is oxygen required before atropine administration in organophosphorus or carbamate pesticide poisoning?—A cohort study. Clin. Toxicol. 52:531–37
37. Worek F, Backer M, Thiermann H, Szinicz L, Mast U, et al. 1997. Reappraisal of indications and limitations of oxime therapy in organophosphate poisoning. Hum. Exp. Toxicol. 16:466–72
38. Worek F, Thiermann H, Wille T. 2016. Oximes in organophosphate poisoning: 60 years of hope and despair. Chem. Biol. Interact. 259:93–98
39. Panda M, Hutin YJ, Ramachandran V, Murhekar M. 2009. A fatal waterborne outbreak of

pesticide poisoning caused by damaged pipelines, Sindhikela, Bolangir, Orissa, India, 2008. J. Toxicol. 2009:692496
40. Peter JV, Moran JL, Pichamuthu K, Chacko B. 2008. Adjuncts and alternatives to oxime therapy in organophosphate poisoning—is there evidence of benefit in human poisoning? A review. Anaesth. Intensive Care 36:339–50
41. Choudhary S, Gill KD. 2001. Protective effect of nimodipine on dichlorvos-induced delayed neurotoxicity in rat brain. Biochem. Pharmacol. 62:1265–72
42. 106. Brvar M, Chan MY, Dawson AH, Ribchester RR, Eddleston M. 2018. Magnesium and calcium channel blocking drugs as antidotes for acute organophosphorus insecticide poisoning—a systematic review and meta-analysis. Clin. Toxicol. 56:725–36
43. Roberts D, Buckley NA. 2005. Alkalinisation for organophosphorus pesticide poisoning. Cochrane Database Syst. Rev. 2005(1):CD004897
44. Morina P, Herrera M, Venegas J, Mora D, Rodriguez M, Pino E. 1997. Effects of nebulized salbutamol ˜ on respiratory mechanics in adult respiratory distress syndrome. Intensive Care Med. 23:58–64
45. Shadnia S, Ashrafivand S, Mostafalou S, Abdollahi M. 2011. N-acetylcysteine a novel treatment for acute human organophosphate poisoning. Int. J. Pharmacol. 7:732–35.