

The effectiveness of oximes against organophosphate poisoning

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Abstract. Organophosphate compounds (OPs) have been used by human in many ways over years. However, the misuse of organophosphate has been very common because their notorious toxicity. For example, there has a lot of case that people and children handling OPs improperly resulting in casualty and suffering. The main purpose of this research is to understand the basic of treatment of oxime drugs against organophosphate poisoning based on the clinical data and raise awareness of OPs poisoning. It mainly consists of 3 parts about oxime treatment. The first part is introduction of mechanism of oxime treatment. It talks about the mechanism and the studies of effectiveness of oxime against OPs. Then the most concerning problem, aging, is discussed based on the studies of the behavior of OPs inhibited enzymes, which sometimes oxime is incapable to treat during the second part. Last part mainly talked about the solution that scientists have discovered during research that help oxime treatment to be more effective to deal with the problem.

1 Introduction

Organophosphate compounds (OPs), have been playing an important role in human utility for a long time. It is crucial for the body because of many components such as cell membrane, DNA, and ATP, which plays an important role in transferring energy throughout the body. It is also used in most of the pesticide, and herbicide to remove unwanted parties. However, organophosphates also are a concerning health problem due to its acute toxic effect. The death toll by organophosphate each year is relatively high. Not only people can be exposed through pesticide and herbicide. However, OPs also can fall into illegal and improper uses. Dichlorvos, a classic OPs and one of the most common herbicides in China, are often used in food fraudulence to mimic the real taste of food in China as the problem of fraudulent food products grow in China. This epidemic left a lot of people hospitalized and suffering. It also can be used by terrorists as a mass destruction weapon. The biggest case is the Tokyo subway Sarin incident, which left 14 deaths and 1050 injuries [1]. Therefore, many OPs have been banned as nerve agents by the Chemical Weapons Convention, which is signed by 193 countries. The main symptoms are miosis, respiratory distress, vomiting, and bradycardia [2]. Another problem caused by OPs according to poisoning is brain damage due to the inhibition

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of central neural system. For example, 61% of the patients were suffering from mild (22.2%), moderate (12%) to severe (26.8%) brain injury.

Knowing the mechanism that organophosphates affect the human body is crucial to understand the notorious toxicity of organophosphates. Organophosphates affect the body by interacting with a crucial enzyme called acetylcholinesterase, which is a neuro control unit [3]. The enzyme (AChE) breaks down the neurotransmitter called acetylcholine (ACh) by attaching the carbonyl group in ACh to the serine site in AChE and ammonium cation in ACh to the anion site in AChE and breaking down the neurotransmitter into acetate and choline by cleaving the ester bond of ACh to prevent the overflow of neurotransmitter in normal circumstance. When the organophosphate is introduced in the neuro system, the ability of AChE breaking down the neurotransmitter will be impaired by organophosphates because organophosphates can bind to serine site of AChE tightly and AChE cannot breakdown the ACh. Therefore, the amount of ACh will significantly increase and overflow the system due to the presence of OPs and thus it will cause neurological failure. Because it only needs 1:1 portion of organophosphate to disable AChE. The toxic and lethal dose of OPs to humans is usually very low, which is the reason why OPs poisoning raise society's concern. OPs are so toxic and effective to put down a person that it is banned by the Chemical Weapons Convention, an organization that is signed by 193 countries, as a deadly nerve agent.

The scientists have been working on finding the antidotes for a long time and it's still a developing topic nowadays. Some mechanisms and synergisms are still yet to be explained between certain drugs. However, scientists understand the basics of removal of OPs from AChE to reactivate the enzyme. Thus, it can be reactivated through many methods.

One of the most well-known drugs that relieve OPs poisoning is oxime. Within the oxime the most famous one is pralidoxime (2-PAM or PAM). It also has been practiced by clinics the most. After one of the biggest OPs poisoning accidents, Tokyo subway sarin attack, the patients were mostly treated by PAM with atropine. The number of patients was so large that the whole nation's supply of PAM was barely enough to be able to treat every patient. Therefore, the development of PAM is crucial for curing OPs poisoning. This research will systematically discuss the efficacy and therapeutic mechanisms of oximes in treating organophosphate poisoning.

2 Mechanism in action of oxime drugs against OPs poisoning

Understanding the mechanism of oxime drugs is a key point to develop the treatment against OPs poisoning. The common oxime is pralidoxime, asoxime, and diacetyl monoxime. The common characteristics of most oxime used is that all of oxime has either positive charge or significant partial positive charge such as carbonyl group. And oxime groups, which is an imine with a hydroxyl group on it and can be made by mixing ketone or aldehyde with hydroxylamine under acidic condition.

The first step of interaction with oxime and inhibited AChE is docking the positive or partial positive part of drugs to the unoccupied anionic site of AChE to secure the position of the reaction [4]. Because the oxime has partial negative charge on the hydroxyl group, it will be attracted to the phosphorus in phosphate that has partial positive charge. Therefore, a bond can form between the oxime and phosphate through an ester bond. And by doing that, the bond between the serine site in AChE will break and the AChE will be regenerate the functions of AChE to break down acetylcholine. The efficiency of oxime drugs against OPs poisoning has been proven to be from somewhat efficient to very efficient by many clinical cases. For instance, researchers had performed research in vitro by measuring the amount of reactivated AChE after giving the certain does of some of the most famous Oxime against pesticide paraoxon poisoning such as obidoxime, 2-PAM, asoxime chloride, HLo 7 [5].

Worek et al. added paraoxon with concentration of 160 nmol/l to 3 μ L of incubated AChE with concentration of 2.5 U/ml and tested the percentage of active AChE (paraoxon being $2.76 \pm 0.10\%$) after 30 minutes of exposure to OPs [5]. Then Worek et al. added amount from 10 μ M, 30 μ M, and 100 μ M of each oxime.

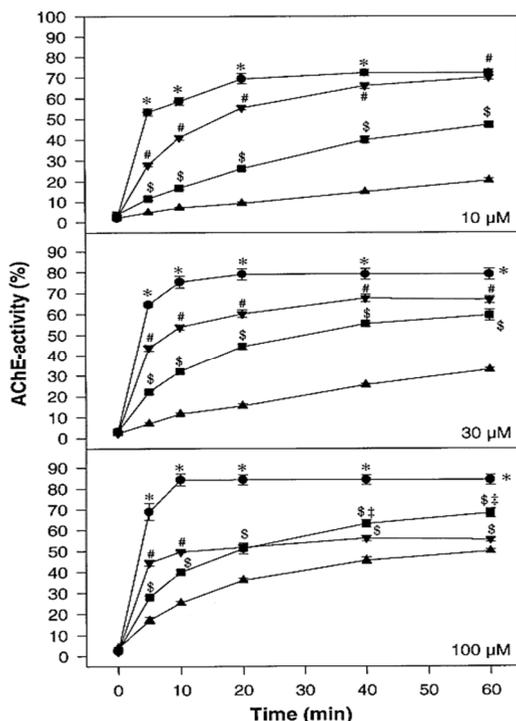


Fig. 1. The activities after oxime drugs were added against paraoxon poisoning: Obidoxime (circle), 2-PAM (square), Asoxime chloride (triangle up), and HLo 7 (triangle down) [5]

As shown in Fig. 1, the effectiveness of oxime is clearly proven that it can significantly reactivate the function from approximately 3% back to over 80% with a dose of 100 μ M of obidoxime against paraoxon. However, effectiveness of the drugs may vary based on the dose and type of oxime given, and the specific name of OPs. For example, test of asoxime chloride shown that it has almost no effect with the dose of 10 μ M after 60 minutes while obidoxime reactivated 70% of AChE with the same dose after only 20 minutes (Fig. 1). Asoxime chlorides were only proven to be effective if the dose were 100 μ M. Oxime will have different impact on different types of OPs due to aging process. It is important to study clinical cases of oxime treatment to give different types and different dose based on the types of OPs poisoning to maximize the survivability of patients.

3 Problems with oxime treatment

The effectiveness of oxime treatment can be very different from no effect to significant effect on reactivating the AChE. One of the well-known drawbacks of oxime drug is called aging. The aging effect can significantly affect the effectiveness of oxime treatment, which means oxime will not have any effect on aged AChE. Knowing the mechanism of aging is crucial for avoiding the aging process. Aging happens when OPs bind to AChE, and OPs can undergo an electrophilic aliphatic substitution with the alcohol group on the serine site of AChE with presence of water in equilibrium [6], which is similar with Fischer esterification.

It can kick alkyl or halogen group out and form a permanent bond with hydroxyl group on the serine site. The ester bond between the phosphorous and alkyl group can be hydrolyzed into an alcohol and an acid further with the nitrogen on 3 positions of imidazole in histidine to form a salt bridge with imidazole being protonated and the phosphate being an anion on the dealkylated oxygen site. The salt bridge formation of OPs and AChE cannot be removed by oxime drugs. Therefore, it is important to give patients proper treatment as soon as possible before inhibited AChE become aged.

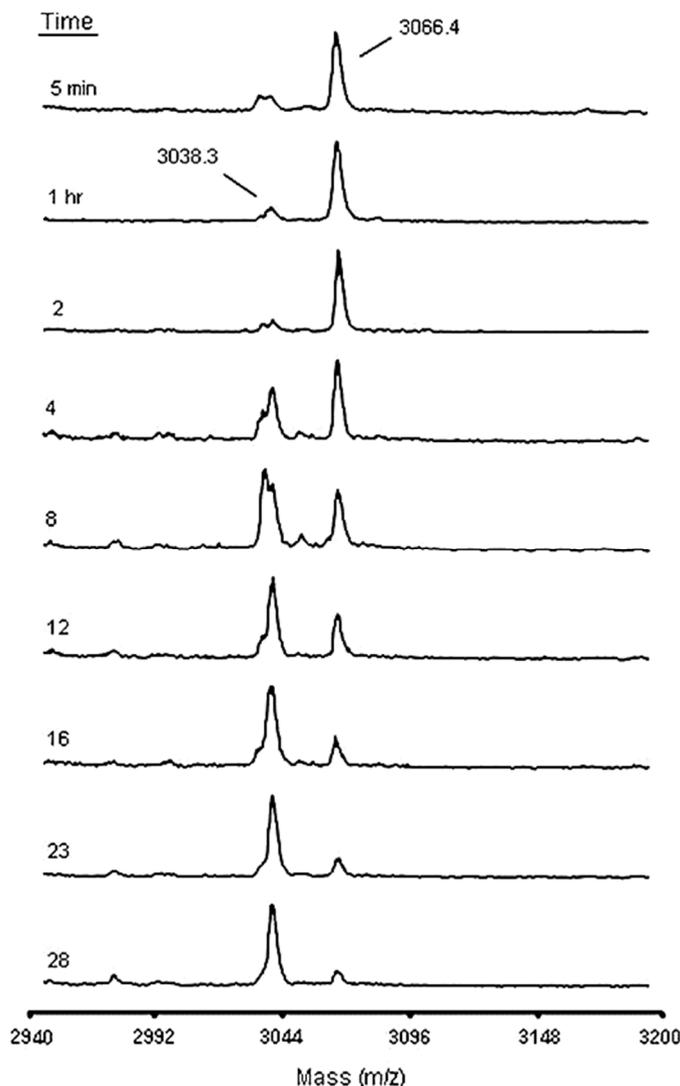


Fig. 2. The aging effect of echothiophate poisoning [7].

The process of aging is proven by many cases. Researchers analyzed human butylcholinesterase (BChE), which has same function with AChE, in vitro with concentration of 4.5 mg/ml by mass spectrometry [7]. They broke down BChE partially by trypsin, a type of enzyme that can specifically cleave basic peptides bond on C terminal, to break down smaller pieces so that it can make analysis of protein mass spectrometry easier. Then they tested BChE with echothiophate, DFP, sarin, and VX for 4 days or more at room temperature,

which means most of the fragment of BChE has aged if the OPs are easy to age with BChE. The m/z peak should be smaller after aging process because the OPs lose alkyl groups. Mass spectroscopy for each hour that echothiophate was introduced to fragment of BChE sample. The graduate shift of peak on mass spectrometry can be clearly seen on Fig. 2. Dealkylation was happening and therefore OPs bind permanently by comparison of difference of two peaks. The difference between those 2 peaks is about 28 m/z , which corresponds to the loss of molecular mass of an ethyl group by hydrolysis of ester bond on echothiophate overtime. Therefore, the aging process is proven. The rate of aging process may differ from type of OPs. This also proven by experiment by showing the difference between the peak of mass spectroscopy of BChE fragment inhibited by each type of OPs for 4 days. The result of different types of OPs effects are listed in Fig. 3, Fig. 4 and Fig. 5.

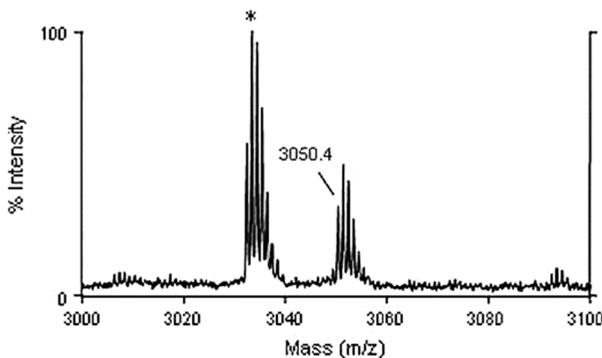


Fig. 3. The mass spectroscopy result for DFP-inhibited BChE after 4 days of aging [7].

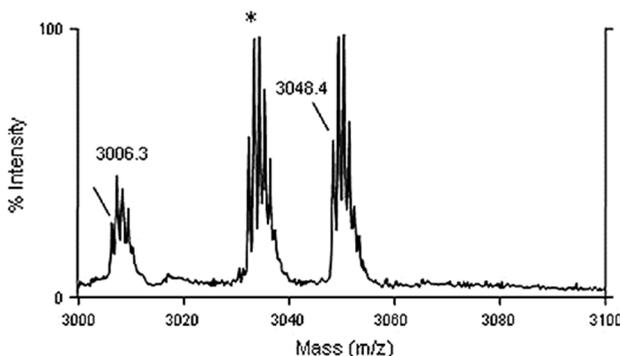


Fig. 4. The mass spectroscopy result for Sarin-inhibited BChE after 4 days of aging [7]

As the result of mass spectrometry shown above, we can clearly see that DFP had aged almost completely after 4 days since the peak of unaged BChE is insignificant. However, the mass spectrometry of sarin shown that there is still significant amount of unaged BChE while VX's one shown that BChE didn't aged at all, which has proven that aging effect of OPs depends on its types.

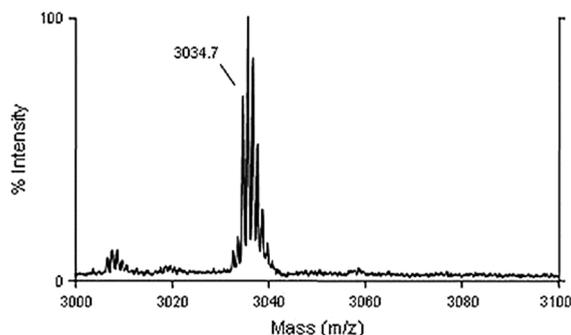


Fig. 5. The mass spectroscopy result for VX-inhibited BChE after 4 days of aging [7].

4 Solution of those problems

Since the process of aging is so fast that it happens only after several minutes from exposure, the intake of only 2-PAM will be inefficient to treat OPs poisoning in the most clinical cases. It needs drugs have interaction to relieve the negative effects. The first type of interactive drugs to help treatment of 2-PAM is called aging retardant. Retardant slows down the negative effects from OPs. It is the most widely used type of combination with 2-PAM. The most famous one is atropine, which commonly used with the treatment of 2-PAM such as Duo Dote® and it also played as main role in the treatment for massive patients after the infamous Tokyo sarin attack. Askew has conduct research in vivo on the toxicity and treatment of oximes against Ops [8]. Sarin was used as source of OPs and the combination effect of atropine and 2-PAM on mice and rats. The LD50 of sarin of mice and rats tested is listed below in Table 1.

Table 1. LD50 of sarin of mice and rats [8].

Species	No. of animal tested	LD50 (mg/kg)
mouse	80	0.214
rat	172	0.116

The individuals were given LD50 amounts of OPs and were divided into atropine only, PAM only and both PAM and atropine. The percentage increase of LD50 is shown in Fig. 6.

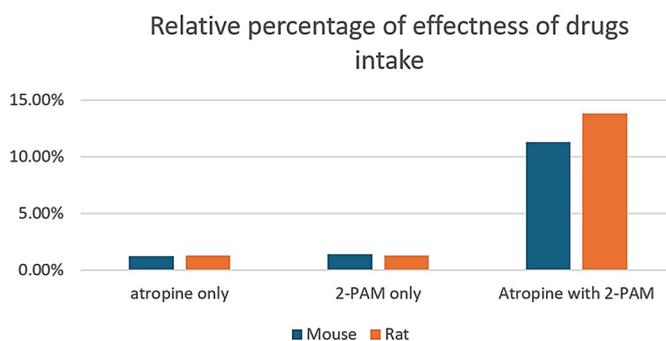


Fig. 6. The percentage increase of LD50 in mouse and rat [8].

Askew also performed the test on monkeys with DAM, also an oxime with similar mechanism with 2-PAM. The change of LD50 of sarin is shown in Fig. 7.

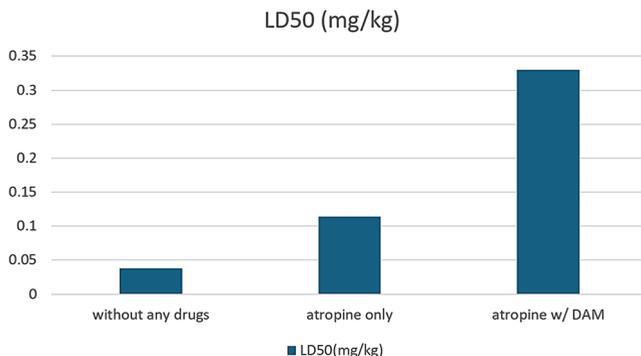


Fig. 7. The change of LD50 of sarin [8].

Therefore, what is the mechanism in action of atropine that it is so effective with 2-PAM? If you remember the mechanism of OPs poisoning, it is very easy to understand. OPs prevent the neurotransmitter, acetylcholine, from being broken down by acetylcholinesterase and therefore neurotransmitters overflow and bomb the acetylcholine receptor to cause acute toxicity. Atropine can relief those overflow by blocking acetylcholine receptor from the acetylcholine. And helps reduce the effect of aging of PAM when PAM is unable to bind with aged acetylcholinesterase. Another experiment done by Kose et al. *in vivo* with rats also suggested that treatment with pralidoxime and atropine can maximize the survivability [9].

Table 2. The level of cholinesterase [9].

Groups	Number of individual tested	Concentration of functional ChE (Unit/L)	Mortality rate (%)
Control	8	191.7 ± 47.7	0
Dichlorvos	15	77.4 ± 31.2	46.6
Atropine only	10	143.2 ± 79.9	0
PAM only	10	146.8 ± 90.4	0
Atropine and PAM	10	161.4 ± 84.1	0

As shown in Table 2, the treatment of atropine and PAM has highest recovering rate among the treatment with either PAM or atropine only. The recovering rate of cholinesterase based on the control group is 73.5% in comparison with atropine only being 57.6% and PAM only being 60.7%. This proven that the experiment that Askew is valid about the effectiveness of atropine and PAM combined.

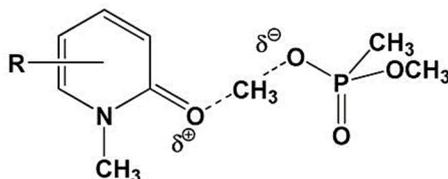


Fig. 8. Reaction mechanism of re-alkylation [10].

The second type of treatment that can work with oxime treatment is reverse the aging effect. Scientist found that aging process can be reversible by re-alkylation. One of the most well-known examples of re-alkylation drug is N-methyl-2-methoxypyridinium iodide. The mechanism is that Pyridium ion is used with similar mechanisms with 2-PAM to dock the anion part. Then the oxo group on the 2 position of pyridine acts as a good leaving group since the most electron in the molecule is mainly donated to the aromatic system of pyridine,

which means that the methyl group is electron poor group and can act as a nucleophile with dealkylated OPs [10]. The mechanism is shown in Fig. 8.

Therefore, the methyl group can be added to dealkylated OPs through nucleophilic substitution and therefore N-methyl-2-methoxypyridinium iodide can reactivate the reactivity between oxime and OPs.

5 Conclusion

As the clinical evidence shown, although oxime have been proven that it is somehow efficient, the effectiveness of oxime is still can be varied from inefficient to very efficient based on type of oxime, amount of intake, and type of OPs poisoning. It is important to take oxime treatment based on the clinical research. The most significant problem that oxime is ineffective of is aging process. Oxime are incapable of treating aged inhibited neural enzymes different OPs substance have different half-life to be dealkylated. To deal with aged enzymes, atropine is proven that it can relieve the symptoms of OPs poisoning and allow body to have more time to process along with oxime treatment oxime with synergy is proven to be the most effective drugs against OPs poisoning.

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