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A Comparison of the Antidotal Effects of Three Oximes against an Organophosphate on the Chick Skeletal Muscle

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Abstract

Background: One of the most toxic effects of organophosphorus poisoning (OP) is the paralysis of skeletal muscles. The oximes are a group of available antidotes.

Objectives: This study investigated the effects of different concentrations of paraoxon on the function of skeletal muscle and reversal or prevention of these effects by three different oximes (i.e., pralidoxime, obidoxime, and HI-6).

Methods: This study was conducted based on the chicken biventer cervices (CBC) nerve-muscle preparation and the use of twitch tension recording technique. The twitches of the CBC were evoked by stimulating the motor nerve at 0.1 Hz with pulses of 0.2 msec duration and a voltage greater than that required to produce the maximum response. Moreover, twitches and contractures were recorded isotonically using Grass Biosystems.

Results: Paraoxon at 0.1 μM induced a significant increase (more than 100%) in the twitch amplitude, while higher concentrations (0.3 and 1μM) induced partial or total contracture. Therefore, paraoxon at a concentration of 0.1 μ M was used to examine the capability of oximes to prevent or reverse its effects. Pralidoxime, obidoxime, and HI-6 dose-dependently prevented (when it was used as pre-treatment, 20 min before or at the same time of administration of the toxin) and reversed (when it was used as post-treatment, 20 min after the administration of the toxin) the effect of paraoxon.

Conclusion: In conclusion, these results revealed that oximes were very useful in the prevention and reversal of the OP toxic effects on the skeletal muscle. Moreover, it was suggested that oximes were more effective when used as pre-treatment. Pralidoxime was more potent than obidoxime and HI-6. The HI-6, which is a newer oxime, was unexpectedly less effective than the other two.

Keywords: HI-6, Obidoxime, Organophosphorus pesticides, Paraoxon, Pralidoxime

1. Background

The use of organophosphorus (OPs) pesticides as insecticides or herbicides has been long prevalent in agricultural fields (1). Since many farmers are exposed to these pesticides, it is possible to absorb these agents through the skin which results in toxicity (2). Pollution of water and food resources with OPs is one of the environmental problems that has put many people at risk (3). Furthermore, the war and terrorist attacks with OPs and nerve agents is another serious health problem with numerous casualties around the world (4). Acetylcholinesterase plays a key role in the physiological function of the cholinergic and voluntary nervous system, the inhibition of which by OPs causes acute intoxication (5). One of the most toxic effects of OP poisoning has been the paralysis of skeletal muscles which can lead to respiratory muscle failure and death (6,7).

Paraoxon as a major active metabolite of parathion is one of the commonly used OP in experimental studies. It irreversibly inhibits the acetylcholinesterase enzyme and produces muscarinic and nicotine symptoms. The paraoxon exposure may also result in progressive myopathy (8). Oximes are chemical acetylcholinesterase reactivators that have been used clinically as the treatment of poisoning with OPs. They are the only antidotes that can reverse or prevent the toxic effects of OP insecticides and also nerve chemical warfare agents on the skeletal muscles. Although HI-6 has been considered the most effective compound in the H-groups of oximes, it has been reported to have some stability problems (9).

2. Objectives

A number of *in vitro* and *in vivo* studies as well as clinical reports have shown limited efficacy of conventional treatment with oximes in various OP pesticide toxicity scenarios (10). It seems that there is a necessity to choose a high efficacy oxime and conduct a study to make a comparison between them (11,12). This study aimed to investigate the effect of different concentrations of paraoxon on the function of skeletal muscle of chicken biventer cervices (CBC) and reversal or prevention of these effects by three leading oximes (i.e., pralidoxime, obidoxime, and HI-6).

3. Methods

This study was performed on the CBC isolated from the chicken neck. When the nerve is stimulated (electrical stimulation), this muscle will respond the same as the rat diaphragm (Twitch). When exposed to acetylcholine-like compounds, CBC produces a slow contraction that simulates the frog's abdominal smooth muscle response (13).

For nerve-muscle preparation, the CBC muscles and their associated nerves were dissected from 4-12 day old chicks killed by exposure to a lethal dose of CO_2. In total, two preparations were mounted in 10 ml tissue baths containing modified Krebs-Henseleit mixture maintained at 32°C, pH 7.3-7.4, and bubbled with 95% O_2+5% CO_2. The modified Krebs-Henseleit solution was composed of (mM): KH_2PO_4, 1.2; NaCl, 118.4; glucose, 11.1; $NaHCO_3$, 25; $MgSO_4$, 1.4; $CaCl_2$, 2.5 and KCl, 4.7 (14). In twitch tension recording, twitches were evoked by stimulating the motor nerve at 0.1 Hz with pulses 0.2 msec duration and a voltage greater than that required to produce the maximum response (15).

To detect any changes in postsynaptic sensitivity, responses to submaximal concentrations of acetylcholine (1-2 mM), potassium chloride (20-40 mM), and carbachol (20-40 M) were recorded (without nerve stimulation) prior to the addition of toxin and at the end of the experiment. The preparations were washed free of these compounds and allowed 20-30 min to stabilize before the application of paraoxon. In the absence of paraoxon, twitch height or responses to exogenously applied acetylcholine, carbachol or KCl did not change in control experiment groups (up to at least two hours). Twitches and contractures were recorded isometrically using Washington, Grass model 79 and Grass model 79B polygraphs, and SRI or Grass FT03 force transducers (15).

The nerve and muscle were exposed to different concentrations of paraoxon separately and the impulses were recorded for one hour. In separate sets of experiments, different concentrations of pralidoxime, obidoxime, and HI-6 (10, 30, 100, and 300 µM) were co-administered with paraoxon (0.1 µM), and impulses were recorded for an hour.

Paraoxon, pralidoxime, obidoxime, HI-6, and other reagents were prepared from Sigma America. The obtained data were presented as a percentage of impulse twitch height and compared to the paraoxon-only treated group (0.1 µM) using one-way ANOVA and Tukey's test, compared to the control group. Statistical tests were performed in SPSS software (Version 20.0), and a p-value less than 0.05 was considered statistically significant.

4. Results

4.1. Effects of different concentrations of paraoxon on twitch amplitude of CBC at different time intervals after exposure

Figure 1 presents the effects of different concentrations of paraoxon on twitch amplitude of CBC at different time intervals after exposure. Twitch height was increased in a time- and dose-dependent manner, and the concentration of 100 nM (0.1µM) of paraoxon after 20 min demonstrated the most elevation of muscle impulses after electrical neuromuscular stimulation of CBC. Therefore, paraoxon at a concentration of 0.1 µM was used to examine the capability of oximes to prevent or reverse its effects. The higher concentrations of paraoxon (0.3 and 1 µM) induced partial or total contractures.

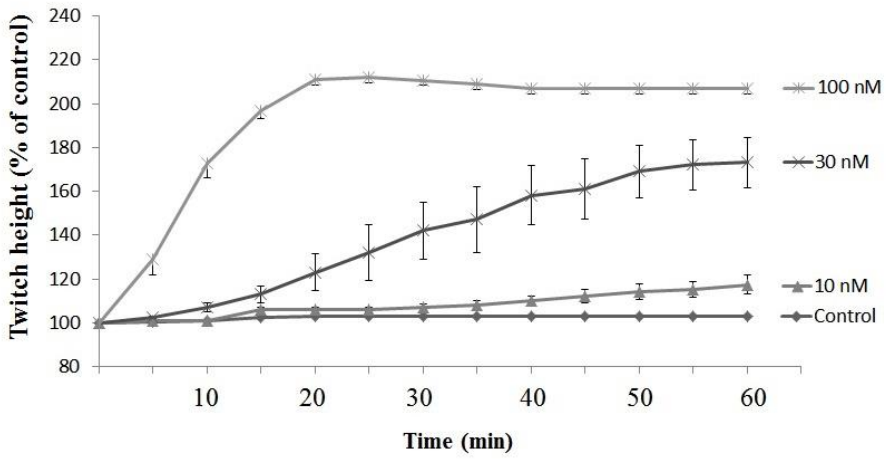

Figure 1. The time course effects of different concentrations of paraoxon on twitch height. Data obtained from the elevation of muscle impulses after electrical neuromuscular stimulation of chick biventer cervicis which are presented as mean±SE of twitch height (% of control) after exposure to paraoxon (10, 30, and 100 nM) (N=6)

4.2. Effects of different concentrations of three leading oximes (i.e., pralidoxime, obidoxime, and HI-6) on paraoxon-induced twitch height of CBC, before, at the same time, and after the administration of paraoxon

The effects of different concentrations (10, 30, 100, and 300 µM) of oximes (i.e., pralidoxime, obidoxime, and HI-6) were evaluated on paraoxon induced twitch height of CBC, 5 min before, at the same time, and 20 min after the administration of 0.1 µM paraoxon (Figure 2). As was expected, when used

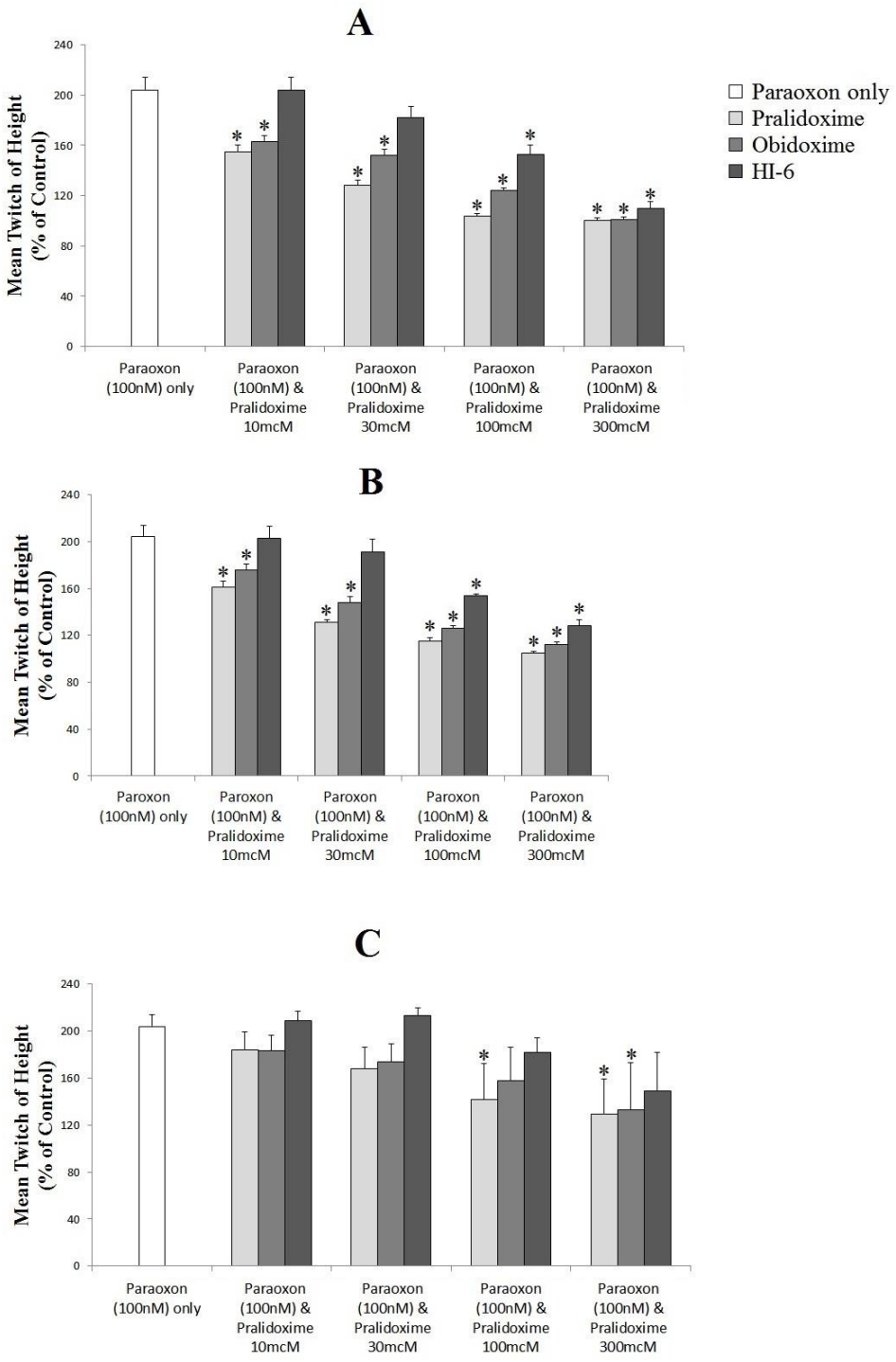

Figure 2. Effects of 10, 30, 100, and 300 µM of pralidoxime, obidoxime, and HI-6 5 min before (A), at the same time (B), and 20 min after the administration of paraoxon on muscle twitch height. Data obtained from the elevation of muscle impulses after electrical neuromuscular stimulation of chick biventer cervicis are presented as mean±SE of twitch height (% of control) with exposure to paraoxon (0.1 micromolar) (N=6)
* Significant differences at P<0.05, compared to paraoxon when administered alone.

before paraoxon administration, all three oximes at the concentrations of 100 and 300 µM, reduced the twitch height dose-dependently; moreover, pralidoxime, obidoxime, and HI-6 were more potent in descending order. It should be noted that pralidoxime and obidoxime (but not HI-6) were strong enough to have a similar effect at lower concentrations. These results were also replicated when oximes used concomitant to paraoxon.

When oximes were used 20 min after the administration of paraoxon, only pralidoxime at the concentrations of 100 µM and higher and obidoxime at a concentration of 300 µM were able to significantly reduce the twitch height. Overall, it seems that all three were more effective when used before or concomitant rather than after the administration of Paraoxon.

5. Discussion

The current treatment of OP poisoning involves the administration of a muscarinic antagonist, such as atropine and oximes, to reactivate acetylcholinesterase inhibited by OPs (10). None of the cholinesterase activators alone have the ideal performance for all OPs (16). Oximes have been widely used in OPs poisoning; however, their performance has not been satisfactory and their timing and route of administration remain controversial (17). The response of different OPs to oximes varies; accordingly, it is important to compare the oximes in terms of potency as well as the time of administration (18).

Paraoxon is one of the widely used OP pesticides that plays a considerable role in the chemical control of a wide range of pests in agriculture (19,20). Weakness and paralysis of striated muscles are the most important complications of OPs poisoning and nerve agents, such as paraoxon, and it has been shown that oximes have a great ability to prevent and even reverse these effects (21,22).

Most of the published studies have evaluated the effect of OP pesticides and their inhibitory effects on cholinesterase enzyme; however, fewer direct studies have been conducted on striated muscle function (23-25,10).

Pralidoxime, obidoxime, and HI-6 have been commonly used oximes in the management of OPs poisoning. The current study evaluated their comparative potency and efficacy by the time and dose of administration. Pralidoxime, obidoxime, and HI-6 may fully reverse the OPs effects on the skeletal muscle; however, their potency seemed to be different. Pre-treatment, simultaneous, and post-treatment administration with the oximes resulted in more effective inhibition of paraoxon effects in descending order. Finally, in terms of the potency of the three different oximes used, pralidoxime was more effective than obidoxime, and obidoxime in turn was more effective than HI-6. Although some earlier studies have shown that HI-6, obidoxime, and pralidoxime are more potent activators of the inhibited acetylcholinesterase in descending order, other recently conducted *in vivo* studies have reported that obidoxime is a more potent reactivator, compared to HI-6 in rat blood, diaphragm, and brain, which is consistent with the findings of the present study (26-28). It has been confirmed that only a high dose of oxime HI-6 is able to reactivate tabun inhibited-acetylcholinesterase (29).

6. Conclusion

In conclusion, our results suggest that this method can be used for the study of the neuromuscular effect of OPs and their antidotes. Moreover, pretreatment of oximes is more effective in reversing the toxic effects of Ops, and the oximes are effective in preventing and reversing the effect of OPs on the skeletal muscle. Furthermore, although HI-6 was expected to be more potent than the other two, it was less effective in this respect. Finally, the potency of these three oximes can be ordered as Pralidoxime>Obidoxime>HI-6.

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Footnotes

Authors' Contribution: Gholamreza Poorheidari developed the theoretical framework and performed the analytic calculations as well as numerical simulations. Mahdi Mashhadi Akbar Boojar contributed to the final version of the manuscript and supervised the project.

Conflict of Interests: The authors have no conflict of interest to declare.

Ethical Approval: This study was carried out regarding the ethical recommendations of laboratory animal care of Baqiyatallah University of Medical Sciences, Tehran, Iran. The study protocol was approved by the Ethics Committee of Research Deputy at Baqiyatallah University of Medical Sciences, Tehran, Iran.

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