A Comparison between Neuromuscular Effects of Parathion and Paraoxon on Chick Biventer Cervicis Nerve-Muscle and the Reversal of their Effects by Pralidoxime

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Abstract
Background: It is generally believed that the anticholinesterase effect is induced by the organophosphate insecticide parathion only through its bioactive metabolite (i.e., paraoxon) that is created in the liver.

Objectives: This study aimed to evaluate the intrinsic anticholinesterase effect of parathion, compared to its metabolite.

Methods: This study has been conducted to prepare the isolated chick biventer cervicis nerve-muscle using the twitch tension recording method.

Results: According to the results, paraoxon (0.1 µM) induced a highly significant increase (more than 100%) in the twitch height, while higher concentrations (0.3 and 1 µM) induced partial or total contractures. Furthermore, parathion induced almost the same percentage of increase in the twitch height at 1 µM and partial or total contractures at 3 and 10 µM. It should be noted that pralidoxime (2-PAM), at 300 µM, reversed the effects of paraoxon and its parent (i.e., parathion).

Conclusion: These results demonstrate that both parathion and its metabolite inhibit the acetylcholinesterase enzyme which can be reactivated by pralidoxime, whereas parathion is about 10 times less potent, compared to its metabolite. Therefore, the intrinsic toxic effects of parathion, regardless of its metabolite, should be considered in future studies.

Keywords: Chick biventer cervicis nerve-muscle, Organophosphate, Paraoxon, Parathion, Pralidoxime

1. Background

Nerve agents are a group of organophosphorus compounds that have been used as military weapons due to their physicochemical properties and toxicity (1). These chemicals have been used in many cases in terrorist attacks and warfare, such as the Halabja chemical attack of the Iran-Iraq War in 1988 (2-4). In general, organophosphorus compounds are most widely used as insecticides and pesticides in agriculture. In addition to the war and terrorist poisonings caused by nerve agents, contamination by organophosphorus compounds is a serious health problem with more than three million poisonings and 200,000 deaths worldwide annually (5).

More than 200 types of organophosphates and 25 types of acetylcholinesterase inhibitor carbamates have been synthesized so far which are accompanied by an increasing number. The organophosphorus insecticides are highly diverse and vary in different properties, such as fat solubility, half-life, active metabolites, antidote bonding, and delayed neuropathy. The criterion for the classification of these compounds is based on the symptoms and severity of poisoning (6, 7). Currently, much evidence has evaluated the effects of organophosphorus compounds in animal models (8).

One of the most widely used organophosphorus compounds in animal and laboratory studies is parathion and its active metabolite (i.e., paraoxon) (9). Paraoxon, the same as other organophosphorus compounds, irreversibly inhibits the acetylcholinesterase enzyme and produces muscarinic and nicotinic symptoms. The administration of paraoxon in animals has also caused progressive myopathy (10).

It is believed that the anticholinesterase effects induce by the organophosphate insecticide parathion mainly through its bioactive metabolite (i.e., paraoxon) that is generated in the liver (11, 12). The direct effects of parathion against neuromuscular function (regardless of active metabolites), comparison of the potency of their neuromuscular effects, and possible effects of pralidoxime on unmetabolized parathion have not been investigated in the literature so far.

2. Objectives

This study aimed to investigate the effects of different concentrations of parathion and paraoxon on the function of skeletal muscle and reversal of these effects by pralidoxime.
3. Methods

3.1. Animals and Chemicals

This experimental study was conducted on the chick biventer cervicis (CBC) isolated from the chicken neck obtained from the laboratory animal center of Baqiyatallah University of Medical Sciences (BUMS), Tehran, Iran. The CBCs were then utilized for the electrophysiological investigation of neuromuscular transmission. The chicks used in this study were about 4-12 days old, weighed 37-47 g, and were fed and maintained under physiological conditions (with access to water and food ad libitum at 24-28°C). Both parathion and paraoxon were prepared from Sigma America (purity of more than 99%). Furthermore, the solvents and other reagents were purchased from Merck (Germany) or Sigma Chemical Company (Sigma-Aldrich, USA).

3.2. Experimental procedure

As the nerve is stimulated (electrical stimulation), this muscle will respond exactly like the rat diaphragm (Twitch). Moreover, when it is exposed to acetylcholine-like compounds, it produces a strong slow contraction that resembles the frog's abdominal smooth muscle response (13). For CBC nerve-muscle preparation, the CBC muscles and associated nerves were dissected from 4-12-day-old chicks killed by exposure to a lethal dose of CO₂. Totally, two preparations were mounted in 10 ml tissue baths containing modified Krebs-Henseleit solution maintained at 32°C, pH 7.3-7.4, and bubbled with 95% O₂+5% CO₂. The modified Krebs-Henseleit solution was composed of (mM): NaCl, 118.4; KH₂PO₄, 1.2; glucose, 11.1; NaHCO₃, 25; CaCl₂, 2.5; MgSO₄, 1.4 and KCl, 4.7 (14). As described by Khodaei et al. (2004), 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), carbachol (20-40 M), and KCl (20-40 mM) were recorded (in absence of nerve stimulation) before the addition of toxin and at the end of the experiment. The preparations were washed free of these drugs and allowed 20-30 min to stabilize before the application of toxins. In the absence of toxin, the twitch height or responses to exogenously applied acetylcholine, carbachol, or KCl did not change in the control experiment groups (up to at least 2 h). Twitches and contractures were recorded isometrically using Washington, Grass model 79 and Grass model 79B polygraphs, and SRI or Grass FT03 force transducers (16).

The nerve and muscle were placed at 0.1 µM paraoxon concentration solutions, and the impulses were recorded for 60 min. Pralidoxime 300 µM concentrations were also used in a separate set of experiments with paraoxon, and impulses were recorded for 60 min.

3.3. Ethical consideration

This study was carried out regarding the ethical recommendations of laboratory animal care of BUMS and was approved by the ethics committee and supported by the research deputy of BMSU.

3.4. Data analysis and presentation

The data were expressed as a percentage of impulse height, and one-way ANOVA was used to compare the results with those of the control group. All statistical tests were performed in SPSS software (version 20.0), and a p-value less than 0.05 was considered statistically significant. In total, six tissues were included in each test, and all data were expressed as mean±SE.

4. Results

Figure 1 demonstrates the effects of paraoxon on twitch height in response to electrical stimulation and the reversal of that effect by pralidoxime. Furthermore, paraoxon, at 0.1 µM, induced a significant increase (more than 100%) in the twitch height. The maximum contraction was obtained after 20 min of exposure. Moreover, higher concentrations of paraoxon (0.3 and 1 µM) induced partial or total contractures. Pralidoxime, at 300 µM, reversed (when it was used as post-treatment 20 min after the exposure to a toxin) the increasing effect of paraoxon in the twitch height.

Similarly, parathion, at 1 µM, induced almost the same percentage of increase in the twitch height (Figure 2), while higher concentrations (3, 10, and 30 µM) induced partial or total contractures. Pralidoxime, at 300 µM, reversed (when it was used as post-treatment 20 min after the exposure to a toxin) the increasing effect of paraoxon in the twitch height.

Figure 1. Time-dependent elevation of muscle impulses after electrical neuromuscular stimulation of chick biventer cervicis which presented as twitch height (% of control) after exposure to paraoxon (0.1 micro molar) or parathion (0.1 micro molar) and pralidoxime (300 micro molar) at 20 min post-exposure. Each point represents the mean±SE of the six experiments.
However, pralidoxime, at 300 µM, reversed the twitch height (when it was used as post-treatment 20 min after the administration of parathion).

5. Discussion

One of the toxic effects of nerve agents' warfare and organophosphorous insecticides is skeletal muscle weakness that often occurs in the ocular, neck, bulbar, proximal limb, and respiratory muscles (17, 18). Oximes can prevent or reverse these toxic effects on striated muscles (19). Several studies have been performed to evaluate the enzymatic effects of organophosphate pesticides; however, fewer functional studies have been performed in this regard so far (20-22).

Parathion is an organophosphate insecticide, and it was believed that it acted on the enzyme acetylcholinesterase indirectly (23). After exposure to humans and other organisms, parathion is quickly oxidized to paraoxon, and it is believed that most of the toxic effects of parathion come from this active metabolite (24). However, the functional potency of parathion in comparison to paraoxon was not defined. There are several studies on the different biological aspects of these compounds; however, our investigation was the first in its kind that studied and compared the neuromuscular effects of parathion and paraoxon on CBC nerve-muscle.

The results of current and recent studies show that muscle responses to the effects of different concentrations of paraoxon and reversal of these effects by pralidoxime effects are significant, repetitive, and reliable. Moreover, this method was used to investigate the cholinergic-nicotinic effects of different substances, and it is considered very useful for the study of striated muscle function (16, 25).

However, our preliminary results suggest that parathion itself also has an anticholinesterase activity which has not been reported in the literature. Despite the general belief, parathion, the same as its active metabolite paraoxon, induced a similar increase in response to electrical stimulation of the nerve of the preparation. The results of the present study suggest that parathion does not necessarily need to turn into its metabolite in order to be toxic. These findings were consistent with those observed by other researches in enzymatic studies (26). Reversal of these effects by pralidoxime indicates that observed effects of parathion are also due to the inhibition of acetylcholinesterase enzyme. Although parathion showed to be considerably less potent than paraoxon, it seemed to have some destructive effects on the tissue, which needs more investigations. It has been confirmed that the greatest effect of paraoxon anticholinesterase activity occurs in the striated muscles within 30 min after exposure, whereas myopathy develops within 24 h (27).

5.1. Limitations and suggestions

Due to the ability of a whole living organism to convert parathion to paraoxon, it is practically impossible to compare the potency of these two compounds with the activity of cholinesterase under in vivo conditions. Moreover, it is not possible to compare the chronic and sub-chronic toxicity of parathion with that of paraoxon. Therefore, it is suggested that future studies compare the biological activities of these two compounds and similar compounds under in vitro conditions.

6. Conclusion

These findings indicate that both parathion and its major metabolite (i.e., paraoxon) inhibits the acetylcholinesterase enzyme in striated muscle and leads to an increase in the twitch height which can be deactivated by pralidoxime although parathion is about 10ntimes weaker than paraoxon. Consequently, the intrinsic toxic effects of parathion, regardless of its metabolite, should be considered in future investigations.

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Footnotes

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

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

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