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Serum Paraoxonase and Arylesterase activity after acute chlorpyrifos poisoning in rat

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ABSTRACT

Introduction: Paraoxonase 1 (PON1) is a high-density lipoprotein-associated enzyme with both aryl esterase and lactonase activity, and it possesses significant antioxidant and anti-inflammatory properties. PON1 hydrolyzes the active metabolites of several organophosphorus (OP) insecticides, including parathion, diazinon, and chlorpyrifos. This widely studied enzyme is recognized for its protective role against organophosphate poisoning and vascular diseases, as well as its potential as a biomarker for conditions related to oxidative stress, inflammation, and liver disease. However, limited knowledge exists regarding PON1 activity status following acute organophosphate intoxication. The aim of the present study was to investigate changes in serum PON1 activity after acute chlorpyrifos poisoning and its relationship with acetylcholinesterase (AChE) activity.

Method: Rats were orally given a single dose of chlorpyrifos (160 mg/kg), and blood samples were collected before treatment, as well as at 6 and 96 hours post-treatment. We measured serum cholinesterase, paraoxonase, and arylesterase activity of PON1.

Results: Signs of OP poisoning, including miosis, salivation, tremors, fasciculation, and paralysis, were observed following intoxication, accompanied by a significant inhibition of AChE activity. All symptoms resolved after 48 hours, and AChE activity returned to baseline levels at 96 hours. In contrast, paraoxonase and arylesterase activities progressively increased after 6h and 96h treatment respectively.

Conclusion: The results of our study indicated that serum cholinesterase activity and paraoxonase activity negatively associated in OP poisoning. Based on these findings, monitoring paraoxonase activity after OP intoxication may serve as a valuable biomarker for assessing intoxication.

Keywords: Paraoxonase, Arylesterase, Chlorpyrifos, Organophosphate poisoning

INTRODUCTION

Organophosphate poisoning remains a pressing public health issue, particularly in agricultural settings where these compounds are extensively used as pesticides and herbicides. Organophosphates, which inhibit the enzyme acetylcholinesterase (AChE), lead to an accumulation of acetylcholine in the synaptic cleft, resulting in overstimulation of both the central and peripheral nervous systems. This condition manifests as a cholinergic toxidrome, characterized by symptoms ranging from mild gastrointestinal distress to severe respiratory failure and neurological impairment(1, 2). The World Health Organization estimates that approximately 3 million cases of organophosphate poisoning occur annually, with mortality rates varying between 2% to 25%

depending on the severity of exposure and the healthcare resources available to manage such cases. In developing countries, where access to emergency care may be limited, the risk of fatal outcomes is considerably heightened, underscoring the need for effective monitoring and treatment strategies(3).

Paraoxonase 1 (PON1) is a calcium-dependent enzyme primarily associated with high-density lipoprotein (HDL) in the bloodstream. It plays a crucial role in hydrolyzing organophosphate compounds, including pesticides and nerve agents, PON1 exhibits three main enzymatic activities: paraoxonase, arylesterase, and lactonase. Paraoxonase activity refers to its ability to hydrolyze paraoxon, the toxic metabolite of parathion, while arylesterase activity involves the hydrolysis of aryl esters. Lactonase activity pertains to its ability to break down lactones, which are cyclic esters formed from fatty acids. These activities are vital for detoxifying various substrates, including oxidized lipids and homocysteine thiolactone, thereby contributing to its protective role against oxidative stress and inflammation(4). PON1 plays critical roles in the metabolism of organophosphates and are essential for understanding individual susceptibility to organophosphate toxicity. PON1 exhibits hydrolytic activity against several organophosphate metabolites, including chlorpyrifos oxon. Variability in PON1 activity among individuals is influenced by genetic polymorphisms, notably the Q192R and L55M variants. These polymorphisms can significantly affect an individual's ability to detoxify organophosphates, with lower PON1 activity correlating with increased vulnerability to adverse health effects from exposure(5, 6).

PON1 is a widely studied enzyme based on its protective role against poisoning by organophosphate (OP) insecticides and in vascular disease, as well as its use as biomarker of diseases involving oxidative stress, inflammation and liver diseases(7, 8) but little is known about PON1 activity status after acute organophosphate intoxication. Monitoring PON1 and arylesterase activity can provide valuable insights into the biochemical response following acute organophosphate exposure. In particular, measuring enzyme activity at multiple time points post-exposure can reveal the dynamics of detoxification processes and help identify individuals at higher risk for severe poisoning outcomes.

In this study, we specifically investigate paraoxonase, arylesterase, and cholinesterase activities in plasma following acute chlorpyrifos poisoning at 4 and 96 hours post-intoxication. By analyzing these enzymatic activities over time, we aim to elucidate their roles in detoxification processes and assess their potential as biomarkers for exposure severity and clinical outcomes. This research not only contributes to our understanding of the biochemical mechanisms underlying organophosphate toxicity but also has implications for improving clinical interventions and public health strategies aimed at reducing the burden of organophosphate poisoning.

MATERIAL AND METHODS

Materials

Paraoxone was purchased from sigma(USA). Chlorpyrifos powder (Technical grade, 99%) were obtained from the Melli Shimi Keshavarz (Iran). 5-dithiobis-2-nitrobenzoic acid (DTNB), Acetylthiocholine, phenylacetate were prepared from Merck.

Animals

In this study six male wistar rats (200±20 g) , obtained from faculty of Medical Sciences, Tarbiat Modares University, were used. Animals were housed in clean plastic cages and fed a standard laboratory diet and water ad libitum, exposed to a 12 h light/dark cycle and maintained at 20-22 ° C temperature. All animal experiments were approved by the ethical committee of the Tarbiat Modares University.

Animal experiment

Rats were treated with single dose of chlorpyrifos(160mg/kg), which is about 80% of LD50 dose and continually monitored for any organophosphate poisoning symptoms such as lacrimation, tremor and paralysis. Blood samples were taken before, 6 and 96h after treatment from tail vein. The serum was separated by centrifuging at 3000g and kept in -80° C until analysis.

Measurement of serum cholinesterase activity

Cholinesteras activity were measured by the Ellman method (9) .In this method, cholinesteras hydrolyzed acetylthiocholine to produce thiocholine and acetate. The thiocholine reduces the Dithiobis-Nitrobenzoic Acid(DTNB) and liberates yellow color nitrobenzoate (TNB) anions, which absorbs at 405 nm. The increase in absorbance at 405 is proportional to enzyme activity. The reaction start immediately after addition of 5µl serum to Ellman mixture which consist of 0.1 M phosphate buffer (pH=8), acetylthiocholine and DTNB in a ratio of 150:2:5 and absorbance was monitored in 405 nm by plate reader(BIO TEK). The activity of enzyme was calculated by use of extinction coefficient of TNB anions.

Paraoxonase activity assay

The paraoxonase activity of PON1 enzyme was measured with paraoxon as a substrate as previously described(10). Briefly ,40 µl paraoxon(6mM) was added to 150 µl assay buffer (containing 0.132 M Tris–HCl pH 8.5 and 1.32 mM CaCl2) then immediately after addition of 10µl sample , absorbance was monitored for 5minute at 405 nM by plate reader . In this assay paraoxonase hydrolysis the paraoxone to produce p-nitrophenol and the paraoxonase activity was calculated by using the extinction coefficient of p-nitrophenol (18050M−1 cm−1) and units were expressed as micromoles of paraoxon hydrolysed per minute.

Arylesterase activity assay

Arylesterase activity of PON1 was measured with phenyl-acetate substrate as described earlier(10, 11). Briefly,5µl of sample was added to 195µl substrate solution containing 3.26 mM phenyl-acetate, 100mM Tris–HCl pH=8 and 2 mM CaCl2and then the rate of generation of phenol was determined at 270 nm at 37°C, using a continuously recording spectrophotometer. The molar extinction coefficient of 1310 M−1 cm−1 was used for calculations of enzyme activity and units were expressed as micromoles of phenyl-acetate hydrolyzed per minute.

Statistical analysis

All data are expressed as the Mean ± SEM. Differences were evaluated using Repeated measure one-way ANOVA followed by Tukey's Multiple Comparison Test for pairwise comparison of groups. $p < 0.05$ considered statistically significant. Statistical analysis was conducted using Graph-Pad Prism 7 software.

RESULTS

Serum cholinesterase activity

The results of cholinesterase activity presented in figure 1. Administration of chlorpyrifos significantly decrease the serum cholinesterase activity compared to baseline (before treatment) activity. Significant deference was not observed between 96h after treatment and baseline cholinesterase activity.

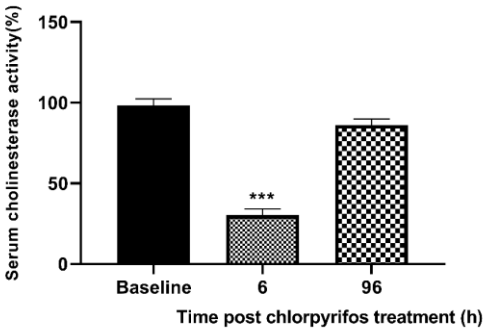

Figure 1. Serum cholinesterase activity before, 6 and 96 hours after chlorpyrifos (160mg/kg) treatment. Each bar graph represent the mean ± SEM (n=6), ***p<0.001 *vs* baseline

Serum paraoxonase activity

Significant increase in paraoxonase activity of PON1 enzyme was observed at 6 and 96h after treatment of chlotpyrifos compared to baseline activity. There was no significant difference between 6 and 96 h paraoxonase activity (figure2).

Serum arylesterase activity

The results of arylesterase activity measurement of PON1 enzyme in serum is shown in figure 3. The arylesterase activity was unchanged at 6h after treatment but significantly increased at 96h

after treatment in compared to baseline arylesterase activity.

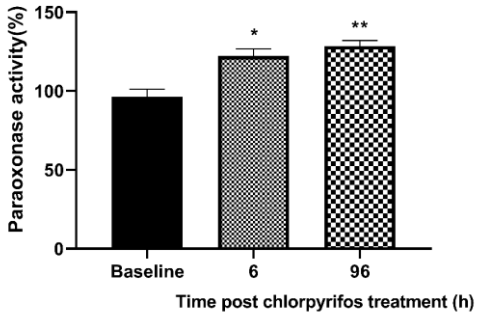

Figure 2. Serum paraoxonase activity before, 6 and 96 hours after chlorpyrifos (160mg/kg) treatment. Each bar graph represent the mean ± SEM (n=6), **p*<0.05, ***p*<0.01 *vs* baseline

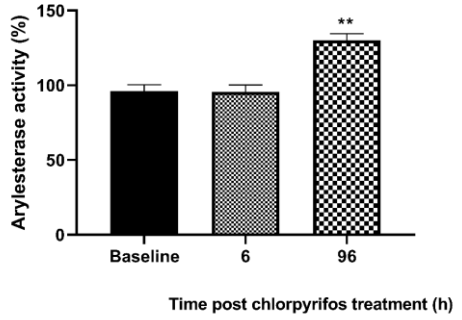

Figure 3. Serum arylesterase activity before, 6 and 96 hours after chlorpyrifos (160mg/kg) treatment. Each bar graph represent the mean ± SEM (n=6), ***p*<0. 01 *vs* baseline

DISCUSSION

The findings of this study provide valuable insights into the enzymatic responses following acute chlorpyrifos poisoning and highlight the dynamic nature of cholinesterase, paraoxonase, and arylesterase activities in the context of organophosphate intoxication.

The significant decrease in plasma cholinesterase activity observed at 6 hours post-intoxication aligns with established mechanisms of organophosphate toxicity, where the inhibition of acetylcholinesterase leads to an accumulation of acetylcholine at synaptic junctions. This accumulation is responsible for the characteristic symptoms of organophosphate poisoning, including cholinergic crisis, respiratory distress and neurological manifestations(12). Monitoring the animals symptoms confirm these findings. All animals indicated the OP poisoning signs such as miosis, salivation, tremor, fasciculation and paralysis. It is previously reported that these signs are observed at about 60-90% plasma cholinesterase activity inhibition(13). The recovery of cholinesterase activity by 96 hours suggests a potential regeneration capacity or compensatory mechanism within the body, allowing for a gradual restoration of enzymatic function. All OP intoxication signs resolved until 96 h after treatment that confirm the cholinesterase recovery. This finding underscores the importance of timely medical intervention, as early treatment may facilitate quicker recovery and mitigate the severity of poisoning symptoms and its consequences.

In contrast to cholinesterase, paraoxonase (PON1) activity showed a significant increase at both 6 and 96 hours after exposure, while arylesterase activity significantly increased only at 96 hours post-exposure. This elevation may indicate an adaptive response aimed at enhancing the detoxification of chlorpyrifos and its active metabolites. The role of PON1 in hydrolyzing toxic organophosphate compounds is well-documented(5), and its increased activity could suggest an upregulation in response to heightened levels of oxidative stress or inflammation following exposure. Recently, a population-based study revealed a nonlinear relationship between interleukin-6 (IL-6), a pro-inflammatory cytokine, and both paraoxonase and arylesterase activity of the PON1 enzyme. This study demonstrated that PON1 activity increased with rising IL-6 levels; however, at higher levels of IL-6, PON1 activity plateaued and then declined. (14). PON1 is an inducible enzyme with antioxidant and anti-inflammatory effects. (15). The upregulation of PON1 in cases of slight or moderate inflammation is a compensatory response to address inflammatory conditions.

In our study, the sustained elevation in PON1 activity suggests that individuals may benefit from enhanced detoxification capabilities, which could potentially reduce the risk of long-term neurotoxic effects associated with organophosphate exposure.

In conclusion, our study emphasizes the critical roles played by cholinesterase, paraoxonase, and arylesterase in responding to acute chlorpyrifos poisoning. The observed changes in enzyme activities not only contribute to our understanding of organophosphate metabolism but also underscore the potential for these enzymes to serve as biomarkers for exposure severity and therapeutic targets in managing organophosphate toxicity. Future research should focus on elucidating the mechanisms driving these enzymatic responses and exploring their implications for clinical practice and public health interventions aimed at reducing the impact of organophosphate poisoning.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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