Asian Journal of Chemistry; Vol. 25, No. 5 (2013), 2589-2592

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.13487

Phosalone Toxicity on Liver and Pancreas: Role of Vitamins E and C

HILMI DEMIRIN[1,*], OSMAN GÖKALP[2], ERTUGRUL KAYA[3], BORA BÜYÜKVANLI[4], GÖKHAN CESUR[4], AYBARS ÖZKAN[5] and MURAT KAYA[5]

[1]Department of Biochemistry, School of Medicine, University of Duzce, 81620 Duzce, Turkey
[2]Department of Pharmacology, School of Medicine, University of Dicle, 21280 Diyarbakir, Turkey
[3]Department of Pharmacology, School of Medicine, University of Duzce, 81620 Duzce, Turkey
[4]Department of Physiology, School of Medicine, University of Suleyman Demirel, Isparta, Turkey
[5]Department of Pediatric Surgery, School of Medicine, University of Duzce, 81620 Duzce, Turkey

*Corresponding author: E-mail: hilmidemirin@hotmail.com

(*Received*: 21 January 2012; *Accepted*: 15 November 2012) AJC-12408

Phosalone (6-chloro-3-[diethoxyphosphinothioylsulfanylmethyl]-1,3-benzoxazol-2-one) is one of the most commonly used organophosphorus pesticides in the pest control of crops. Subchronic phosalone exposure was evaluated for its effects on the serum activities of some enzymes concerning hepatic and pancreatic damage including aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyltransferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), cholinesterase (ChE); and finally protective effects of combination of vitamins E and C in 24 wistar-albino rats. Experimental groups were as follows: control group (n = 8); a group treated with 120 mg/kg body weight phosalone (P group, n = 8); and a group treated with 120 mg/kg body weight phosalone + vitamin E + vitamin C (P+V group, n = 8). The P and P+V groups were treated orally with phosalone on 5 days a week for 4 weeks. The serum activities of the above mentioned enzymes were analyzed. In the samples, phosalone significantly increased the activities of ALT, LDH and decreased ChE ($p < 0.05$). However no significant change was detected for the remainder enzymes ($p > 0.05$). In the P+V group, ALT and LDH activities were significantly increased and ChE decreased ($p < 0.05$). It is concluded that subchronic phosalone causes rat liver damage to an extent, which is somewhat reflected on the liver enzymes. Furthermore, a combination of vitamins E and C can reduce the toxic effects of phosalone on liver tissue of rats.

Key Words: Phosalone, Toxicity, Liver, Pancreas, Enzymes.

INTRODUCTION

The extensive use of organophosphate pesticides has long been pointed out to exert detrimental effects on the living organisms[1]. Organophosphate pesticides competitively inhibit pseudocholinesterase and acetylcholinesterase (ChE), preventing hydrolysis and inactivation of acetylcholine. Acetylcholine accumulates at nerve junctions, causing malfunction of the sympathetic, parasympathetic and peripheral nervous systems. Clinical signs of cholinergic excess develop. Numerous complications are seen in organophosphate pesticides intoxication cases[2-5]. There are many kinds of organophosphorus pesticides, however phosalone was reported to lead to pancreatitis and liver damage amongst. Phosalone is one of the most widely used organophosphate pesticides in agriculture. Some of the toxic effects of phosalone are due to the inhibition of acetylcholinesterase, an enzyme needed for proper nervous system function. It can overstimulate the nervous system causing nausea, dizziness, confusion and at very high exposures (*e.g.*, accidents or major spills), respiratory paralysis and death[6].

Phosalone has been reported to cause acute pancreatitis in humans[7]. Based on histochemical examination of the acinar tissue, pancreatic tissue-fixed butyryl cholinesterase is the target enzyme of organophosphate toxicity was suggested. It has been reported that inhibition of pancreatic butyryl cholinesterase leads to cholinergic hyperstimulation of the acinar cell, resulting in acute pancreatitis[8]. Pancreatitis incidence rate was reported 12 % in the literature[3]. According to the 1996 data, it is estimated that 363,000 people have experienced organophosphate-induced acute pancreatitis yearly[9]. However, unconsciousness of the patient and complexity of the manifestation make it harder to diagnose this condition[2]. Some authors have reported[10-13] that organophosphate pesticides cause severe hepatic damage, as well, which is marked by certain hepatic enzymes. Exposure of hepatocytes accelerates cellular damage as well as the loss of cellular ATP[14].

Phosalone, as well as some other organophosphate insecticides, was claimed to exert deleterious effects on various tissues besides acetylcholinesterase inhibition *via* increasing reactive oxygen species and these effects were reported to

TABLE-1
ACTIVITIES OF HEPATIC AND PANCREATIC ENZYMES (VALUES ARE MEAN ± SD FOR 8 RATS IN EACH GROUP)

Parameter (U/L)	Experimental groups		
	Control	P	P+V
AST	204.50 ± 116.20	212.69 ± 107.27	206.37 ± 109.03
ALT	61.17 ± 5.74	110.87 ± 13.15[b]	61.02 ± 25.23[d]
GGT	1.74 ± 1.16	2.81 ± 3.83	2.2 ± 1.87
LDH	1514.50 ± 776.55	2125.04 ± 164.68[a]	572.11 ± 147.28[b,d]
ALP	294.66 ± 75.11	277.25 ± 102.95	226.12 ± 55.11
Amylase	1371.70 ± 115.69	1594.97 ± 105.99[a]	1420.94 ± 123.31[c]
Lipase	14,02 ± 1.93	17.13 ± 5.36	15.38 ± 2.45
ChE	537.41 ± 90.99	289.50 ± 78[b]	444.75 ± 81.14[d]

AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; ChE, cholinesterase. P, Phosalone group; P+V, phosalone + vitamin group
[a] $p < 0.05$, the P or the P+V groups compared with the control group; [b] $p < 0.01$, the P or the P+V groups compared with the control group
[c] $p < 0.05$, the P+V group compared with the P group; [d] $p < 0.01$, the P+V group compared with the P group.

recover to a certain extent with antioxidant administration[15]. The constructive impact of the antioxidants may be a proof for the proposed free oxygen radical formation mechanism for the damage of hepatic and pancreatic tissues. The present study focuses on determining the effects of phosalone and a possible recovery of antioxidants, vitamins E and C, for the activities of some of the hepatic and pancreatic enzymes classic for specific tissue damage.

EXPERIMENTAL

Animals and treatment: Twenty-four adult male Wistar albino rats weighing 220-275 g were used as animal subjects in the experiment. The rats were randomly divided into three experiment groups as follows: control group (n = 8); a group treated with phosalone (Zolone, Aventis, Istanbul, Turkey) 120 mg/kg body weight (P group, n = 8) and a group treated with 120 mg/kg (0, 25 LD_{50}) body weight phosalone + vitamin E + vitamin C (P+V group, n = 8). The phosalone group and P+V groups were treated orally with phosalone in corn oil as a vehicle (due to its lipophilic nature) for 5 days a week for 4 weeks. Control rats received only corn oil. Vitamin E, as α-tocopherol acetate (Evigen; Aksu Farma, Istanbul, Turkey) and vitamin C, as sodium-L-ascorbate (Redoxon; Roche, Basel, Switzerland), were injected intramuscularly at doses of 150 mg/kg body weight[16] and 200 mg/kg body weight intra peritoneally[16], respectively, 30 min after the administration of P to the P+V animals. Equivalent amounts of physiological saline were given instead of vitamins to rats of the control and P groups.

The rats were caged individually and fed *ad libitum* without water restriction. The animals were starved overnight for 12 h before the samples were drawn. After 4 weeks of administration, the rats were anesthetized with ketamine + xylazine and venous blood samples were collected by direct heart puncture. An autoanalyser, Abbott Aeroset (IL, USA) was used to determine the serum activities of acetylcholinesterase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ-glutamyl transferase (GGT), alkaline phosphatase (ALP), pancreatic amylase and lipase. The experiments reported here complied with the current laws and regulations of the Turkish republic on the care and handling of experimental animals.

Statistical evaluation: Data are presented as means ± standard deviation (SD). A computer program (SPSS® 9.0 for Windows®, SPSS Inc. Chicago, IL, USA) was used for statistical analyses. Oneway ANOVA test was used to compare the groups. A p value of < 0.05 was accepted as statistically significant.

RESULTS AND DISCUSSION

Clinical signs: Weird movements (n = 13) resembling chewing were seen most early after the administration in the P and P+V group. Slight tremors and salivation in some rats (n = 10) began to emerge in this group. Six rats were witnessed to have a lacrimation-like symptom after 6 h in P group. A decrease in rectal temperature was not monitored and only two rats in groups P and P+V showed exophthalmus at the end of the study. All the signs kept up along with the administration period.

Laboratory findings: In the hepatic profile, the activities of ALT and LDH displayed significant increases in the P group compared to the control ($p < 0.01$ and $p < 0.05$, respectively) and all these were found to be decreased in the P+V group compared to the P group ($p < 0.01$ and $p < 0.01$, respectively). The remainder hepatic enzyme activities, namely AST and GGT, showed non-significant increases, whereas ALP decreased non-significantly ($p > 0.05$) in P group. Amylase increased in P group significantly ($p < 0.05$) and vitamins provided a significant decrease compared to the P group ($p < 0.05$); however the expected increase in lipase in P group and the decrease with vitamin administration were both non-significant ($p > 0.05$). Phosalone caused a significant decrease in acetylcholinesterase activity in p group and addition of the vitamins provided a significant recovery ($p < 0.01$ and $p < 0.01$, respectively) compared to the P group. The results are shown in Table-1.

According to the given data (6) that LD_{50} of phosalone for the male rats was 120-170 g/kg, the minimum dose of 120 mg/kg was chosen in the present study since deaths are likely to be seen in organophosphate insecticide studies. Neurologic symptoms seen in some groups were in concordance with the literature, showing the acute motor and autonomic neurogenic effects[17].

Generally speaking, organophosphate insecticides have a negative reputation for neurotoxicity in nature by acting as inhibitors of neuronal acetylcholinesterase activity. Although

the parents organophosphate pesticides are weak acetylcholinesterase inhibitors, they can be activated by P450-mediated reactions to oxons, which are potent acetyl cholinesterase inhibitors. However, recent studies indicate that toxic manifestations induced by organophosphate insecticides may be associated with an enhanced production of reactive oxygen species[18]. This was somewhat justified by several studies in recent years, that lipid peroxidation increased with the administration of MD, fenthion, phosalone and diazinon[19]. In the present study, the activity of the enzyme acetylcholinesterase showed a significant decrease ($p < 0.01$) in P group as expected and seen in previous reports, moreover the vitamin combination of E and C, administered as antioxidants in doses used on the basis of recent studies in which the doses exerted antioxidant effect on various organophosphate insecticide damages, defended against the effect of phosalone on acetylcholinesterase ($p < 0.01$), nearly conserving the basic activity of the enzyme. In harmony with the previous studies, these vitamins prove their defensive and ameliorative effects against the neurotoxic capacity of the organophosphate insecticides. Pancreas is severely affected from some cholinesterase inhibitors like organophosphates[3,20]. After exposure to organophosphate derivatives, acute pancreatitis can occur in several days[21]. The damage in the pancreatic tissue is reflected as an increase in serum activities of amylase and lipase. The effect of various organophosphate insecticides on pancreatic tissue was observed histopathologically, namely fatty necrosis, cellular and glandular degeneration and congestion[21]. In the present study, a significant difference was detected between the control and phosalone given groups in amylase activities ($p < 0.05$). Though at a non-significant level ($p > 0.05$), this result was valid for lipase activities, substantiating the organophosphate insecticide damage in the pancreas of the rats. The vitamin group had lower activities than of P group. The activity of amylase showed close increase in the P+V group to the control, setting a significant difference between P and P+V groups. It was previously reported that the co-administration of vitamin-C and vitamin-E could prevent organophosphate insecticide-induced pancreatitis[18]. In the present study, phosalone exerted its toxic effect on amylase as expected ($p < 0.05$), however lipase activity did not decrease in expected range ($p > 0.05$) despite the dose was in lethal range for the rats. The compound was administered in corn oil, thus the lipid fraction may be consumed in enzymatic digestion by lipase, probably resulting in a moderate activity oscillation compared to the amylase. The vitamins functioned well in amylase as phosalone did; a somewhat parallel decrease was gained in lipase in a non-significant range ($p > 0.05$). The temperate preserving effect of the vitamins on lipase would probably expand if perchance the amplitude of the increasing effect of phosalone would have been greater on lipase. As in concordance with previous reports, the effect of phosalone, too, on pancreas was deleterious and a combination of vitamins E and C defended against this effect.

Some studies have reported that organophosphate pesticides also caused liver damage[10-13]. Diazinon disturbs the cytochrome P450 system in human liver[22,23]. LPO has been suggested as one of the molecular mechanisms involved in organophosphate insecticide-induced toxicity[10,19] Serum enzymes including ALT, AST, ALP, GGT and LDH are mainly used in the evaluation of hepatic damage. Although these enzymes are not completely specific, an increase in the activities reflects active liver damage. Numerous organophosphate pesticides were studied in monitoring the hepatic damage with these enzymes. Most leads to an increase in these activities depending on exposure time and dose (R diazinon). In the present study, a non-significant increase in AST, ALP and GGT was gained with phosalone administration ($p > 0.05$), whereas ALT and LDH increased significantly ($p < 0.01$ and $p < 0.05$, respectively). As an *aidé-memoire* the compound is lipophilic and thus lipophilic drugs that are converted in the liver to hydrophilic metabolites permit better control, because the lipophilic agent can be eliminated in this manner[24]. The speed of formation of hydrophilic metabolite determines the drug's length of stay in the body. Phosalone was reported to be oxidized in the rat liver to its oxon and both of these converted to the putative intermediate thiol, with the phosphorus portion of phosalone forming diethyldithiophosphoric acid and that of the oxon forming diethylthiophosphoric acid, all of which is hydrophilic and excreted in the urine. In transforming the compound to the above mentioned metabolites, the tissue may have released the enzyme ALT more than AST since the former is much more specific to the liver. ALP and GGT are more specific to the obstruction of little and extra hepatic bile ducts. The four week experimental design may have provided a shorter time course for constituting a process of hepatic fibrosis and serum ALP activity is suggested to increase non-significantly, thereof. GGT increase in the serum does not reflect cell damage, but an increased amount is related to the increased cell turnover due to the induction as in phenytoin and phenobarbital use, which is not likely in phosalone and metabolites[24]. The sound effect of the compound on LDH activity in contrast to the petty increase in other enzymes bewildered the team since LDH was nonspecific to the liver[25].

The vitamins were capable of defending the liver against phosalone, particularly in terms of the enzymes ALT and LDH compared to the control (both, $p < 0.01$). Others were decreased, however all were in non-significant ranges ($p > 0.05$). All in all, phosalone caused hepatic and pancreatic damages to an extent and the vitamins could have blocked the effect in a sense, that the damages are suggested to be restored in 0.5 h period after intoxication, especially in applications to the emergency services.

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