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The Effects of Fenthion on Lipid Peroxidation and Some Liver Enzymes: The Possible Protective Role of Vitamins E and C

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Abstract: The effects of fenthion on the serum activities of cholinesterase (ChE), enzymes concerning liver damage and lipid peroxidation (LPO), and the ameliorating effects of a combination of vitamins E and C against fenthion toxicity were investigated. The results of the in vivo experiment showed that fenthion caused a significant increase in LPO and the activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT) and lactate dehydrogenase (LDH), and a significant decrease in the activities of ChE and alanine aminotransferase (ALT). In addition, treatment with a combination of vitamins E and C led to a significant decrease in LPO and AST activity. In the in vitro experiment, the activity of ChE and ALT were inhibited by fenthion. From these results, it can be concluded that fenthion caused liver damage, and LPO may be one of the molecular mechanisms involved in fenthion-induced toxicity. Vitamins E and C can reduce LPO caused by fenthion.

Key Words: Fenthion, liver, lipid peroxidation, vitamin E, vitamin C

Introduction

Fenthion (O,O-dimethyl-O-(4-methylmercapto-3-methylphenyl)-phosphorothioate) is one of the most widely used organophosphate insecticides (OPIs) for agriculture and public health programmes. The widespread use of OPIs has long been shown to exert deleterious effects on living organisms (1). In general, OPIs are neurotoxic in nature by acting as inhibitors of neuronal cholinesterase (ChE) activity. However, some studies reported that OPIs caused liver damage and lipid peroxidation (LPO). In these studies, it has been suggested to be one of the molecular mechanisms involved in OPI-induced toxicity (2-4). Bagchi et al. (5) showed that the administration of fenthion results in the in vitro and in vivo induction of hepatic and brain LPO, production of chemiluminescence, increased DNA single strand breaks, and increased lactate dehydrogenase (LDH) leakage, suggesting that the reactive oxygen species and/or free radicals may be involved in the toxic manifestations of this insecticide. Few papers reported that a combination of vitamins E and C can reduce LPO caused by toxic substances (1,6). In these studies, vitamins had been administered before toxic substances. However, we could not find any investigations concerning the ameliorating effects of a combination of vitamins E and C against fenthion toxicity in rats. Therefore, the present research had the following objectives:

1. To investigate the effects of fenthion on serum activities of ChE, enzymes concerning liver damage and LPO in rats.

2. To investigate the ameliorating effects of a combination of vitamins E and C against fenthion toxicity in rats.

3. To investigate the effects of fenthion and a combination of vitamins E and C on the activities of ChE and other enzymes (in vitro).

Materials and Methods

Animals and treatment (in vivo experiment)

Twenty-one Wistar albino rats weighing between 200 and 230 g were divided into three experimental groups, each with seven rats, as follows: control group, fenthion treated group (Fenthion), and fenthion plus vitamin E plus vitamin C treated group (Fenthion+Vit). Fenthion and Fenthion+Vit groups were treated orally with a single dose of 54 mg/kg body wt. fenthion (54mg/kg = 0.25 of LD_{50}) (5) in 2 ml corn oil at 0 h. Only corn oil was given in the same way to the control rats. Vitamin E as α-tocopherol acetate (Evigen®, Aksu Farma) and vitamin C as sodium-L-ascorbate (Redoxon®, Roche) were injected at doses of 150 mg/kg body wt. (1 ml/kg body wt.) i.m. (1,6) and 200 mg/kg body wt. (2 ml/kg body wt.) i.p. (6-

8), respectively, 30 min after the treatment of fenthion in the Fenthion+Vit group. Equal amounts of physiologic saline instead of vitamins were given to the rats of the control and Fenthion groups. After all the rats received the above treatments they were fed ad libitum until midnight. The animals were fasted overnight for 12 h before the blood was collected. The rats were anaesthetized with ether and venous blood samples were collected by direct heart puncture at 24 h. Blood samples were centrifuged and serum was discarded.

In vitro experiment

A 10-ml venous blood sample was obtained from each of seven volunteers (four male, three female). Blood samples were centrifuged and serum discarded. The activities of serum enzymes were determined in each sample and these served as 0 h. Each sample was divided into four portions and each one served as experimental groups, as follows: control group, vitamin E plus vitamin C treated group (Vit), fenthion treated group (Fenthion) and fenthion plus vitamin E plus vitamin C treated group (Fenthion+Vit). Vitamin E and vitamin C were added at doses of 7.5 and 10 μg/ml, respectively, into Vit and Fenthion+Vit groups. Fenthion was added at doses of 0.7 mg/ml into Fenthion and Fenthion+Vit groups. In the control, Vit and Fenthion groups, physiologic saline was used instead of vitamins or fenthion. The activities of serum enzymes were determined in each sample at 24 h.

Biochemical parameters

An autoanalyser, Abbott Aeroset (IL, USA), was used to determine the serum activities of ChE, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and LDH.

Thiobarbituric acid reactive substances (TBARS), as a marker for LPO, were determined by the double heating method of Draper and Hadley (9). The principle of the method was a spectrophotometric measurement of the colour produced during the reaction to thiobarbituric acid (TBA) with malondialdehyde (MDA). For this purpose, 2.5 ml of 100 g/l trichloroacetic acid (TCA) solution was added to 0.5 ml serum in a centrifuge tube and placed in a boiling water bath for 15 min. After cooling in tap water, the mixture was centrifuged at 1000 g for 10 min, and 2 ml of the supernatant was added to 1 ml of 6.7 g/l TBA solution in a test-tube and placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a Shimadzu UV-1601 spectrophotometer (Japan) at 532 nm. The concentration of TBARS was calculated by the absorbance coefficient of MDA-TBA complex 1.56×10^5 cm^{-1} M^{-1} and expressed in nmol/ml.

Statistical evaluation

For statistical analyses, normality was firstly investigated, and it was shown that some values of the parameters did not fit the normal distribution. Therefore, as stated by Dawson-Saunders and Trapp (10), considering the small number of cases, the non-parametric Kruskal-Wallis test and Mann-Whitney U test were used to compare groups.

Results

The results of the in vivo experiment are shown in Table 1. TBARS were higher in the Fenthion group compared to the control group ($p < 0.01$) and decreased in the Fenthion+Vit group compared to the Fenthion group ($p < 0.01$). ChE activity was decreased in both the Fenthion and Fenthion+Vit groups compared to the control group ($p < 0.01$). The activities of AST, ALP, GGT and LDH were increased in both the Fenthion and Fenthion+Vit groups compared to the control group ($p < 0.01$). AST activity was decreased in the Fenthion+Vit group compared to the Fenthion group ($p < 0.01$). ALT activity was decreased in both the Fenthion and Fenthion+Vit groups compared to the control group ($p < 0.01$).

The results of the in vitro experiment are shown in Table 2. All enzyme activities remained unchanged in both control and Vit groups compared to 0 h. The activities of ChE and ALT were decreased in both the Fenthion and Fenthion+Vit groups compared to 0 h ($p < 0.01$, $p < 0.05$, respectively). There was no significant change in the activities of ChE and ALT between the Fenthion and Fenthion+Vit groups. The activities of AST, ALP, GGT and LDH remained unchanged in all groups.

Discussion

Toxicity from OPIs is primarily through the inhibition of ChE activity. Also in the present experiment, fenthion caused a significant decrease in ChE activity, and there was no significant effect of the combination of vitamins E and C on ChE activity in either the in vivo or in vitro

Table 1.   Activities of enzymes and levels of TBARS in Control, Fenthion and Fenthion+Vit groups (in vivo) (Values are mean ± SD for seven rats in each group).

	Control	Fenthion	Fenthion+Vit
TBARS (nmol MDA/ml)	2.307 ± 0.254	2.972 ± 0.16[a]	2.133 ± 0.268[b]
ChE (U/l)	2177.30 ± 196.4	48.00 ± 3.90[a]	48.50 ± 4.28[a]
ALT (U/l)	61.50 ± 6.05	51.67 ± 4.41[a]	51.33 ± 8.41[a]
AST (U/l)	84.16 ± 5.94	142.50 ± 9.35[a]	123.83 ± 4.96[a,b]
ALP (U/l)	65.00 ± 4.73	91.83 ± 9.81[a]	97.00± 15.63[a]
GGT (U/l)	0.93 ± 0.04	1.30 ± 0.18[a]	1.32 ± 0.20[a]
LDH (U/l)	807.50 ± 78.7	1296.33 ± 115.3[a]	1261.17 ± 35.4[a]

[a]: $p < 0.01$ (Fenthion or Fenthion+Vit groups compared with the control group)
[b]: $p < 0.01$ (Fenthion+Vit group compared with Fenthion group)

Table 2.   Activities of serum enzymes in 0 h, Control, Vit, Fenthion and Fenthion+Vit group (in vitro) (Values are mean ± SD for seven samples in each group).

	0 h	Control	Vit	Fenthion	Fenthion+Vit
ChE (U/l)	3297.4 ± 239.4	3303.6 ± 275.1	3221.9 ± 315.0	632.00 ± 56.94[a]	630.86 ± 44.28[a]
ALT (U/l)	45.29 ± 5.59	46.29 ± 6.50	44.57 ± 6.55	35.85 ± 7.22[b]	35.57 ± 6.45[b]
AST (U/l)	60.57 ± 9.07	62.29 ± 8.67	61.71 ± 8.46	63.86 ± 10.93	62.86 ± 9.84
ALP (U/l)	157.43 ± 14.62	164.57 ± 14.79	155.71 ± 11.76	162.57 ± 13.45	155.86 ± 11.82
GGT (U/l)	104.00 ± 16.26	107.57 ± 15.74	105.57 ± 14.27	110.86 ± 15.70	105.57 ± 13.99
LDH (U/l)	285.00 ± 24.64	265.29 ± 21.65	266.00 ± 21.02	265.14 ± 20.95	274.86 ± 17.67

[a]: $p < 0.01$, [b]: $p < 0.05$ (Fenthion or Fenthion+Vit groups compared with 0 h).
Note: There was no statistically significant difference in the control or Vit groups compared with 0 h, and in the Fenthion+Vit group compared with the Fenthion group.

experiments. However, recent findings indicate that toxic manifestations induced by OPIs may be associated with an enhanced production of reactive oxygen species (ROS) (1,5,11). Among ROS, superoxide anions, hydroxyl radicals and hydrogen peroxide enhance the oxidative process and induce lipid peroxidative damage in cell membranes. Hydroxyl radicals were previously proposed as initiators of LPO through an iron-catalysed Fenton reaction in membranes (12). The cell has several ways to alleviate the effects of oxidative stress, either by repairing the damage (damaged nucleotides and LPO by-products) or by directly diminishing the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants. Enzymatic and non-enzymatic antioxidants have also been shown to scavenge free radicals and ROS (1).

Non-enzymatic antioxidants such as vitamin E and vitamin C can also act to overcome oxidative stress, being a part of the total antioxidant system. Vitamin E is the most important lipophilic antioxidant and resides mainly in the cell membranes and thus helps to maintain membrane stability (13). Vitamin C is hydrophilic and is a very important free-radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damage (14). In addition to its antioxidant effects, vitamin C is involved in the regeneration of tocopherol from tocopheroxyl radicals in the membrane. Thus, vitamin E and vitamin C can have interactive effects (15).

Sheweita et al. (16) have reported that pretreatment of rats with vitamin E or vitamin C in single and repeated (12 consecutive days) doses prior to the administration of

CCl_4 reduced the induced level of TBARS caused by CCl_4. Gultekin et al. (1) have shown that pretreatment of rats with melatonin or a combination of vitamins E and C in repeated (6 consecutive days) doses prior to the administration of chlorpyrifos-ethyl reduced LPO caused by chlorpyrifos-ethyl. The results of the present experiment showed that fenthion caused a significant increase in LPO. In addition, treatment with a combination of vitamins E and C 30 min after the administration of fenthion led to a significant decrease in LPO.

Some studies reported that OPIs caused liver damage, in addition, LPO has been suggested as one of the molecular mechanisms involved in OPI-induced toxicity (2-4). Bagchi et al. (5) showed that administration of fenthion results in the in vitro and in vivo induction of hepatic LPO and increased LDH leakage. 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 their activities reflects active liver damage. Sheweita et al. (16) reported that single-dose pretreatment of rats with vitamin E or vitamin C could not restore the induction of AST or ALT activities caused by CCl_4 to the normal control level. However, repeated (12 consecutive days) dose pretreatment of rats with vitamin E restored the induction of AST and ALT activities caused by CCl4 to the normal control level. In our experiment, fenthion caused a significant increase in the activities of AST, ALP, GGT and LDH in rats. However, the treatment of fenthion-treated rats with the combination of vitamin E and C caused a significant decrease in the activity of AST. The activities of AST, ALP, GGT and LDH were not restored by the vitamins, although a restitution in LPO was observed. This may be due to the timing and dosing of vitamin E and C administration. Interestingly, ALT activity was decreased with fenthion treatment. Therefore, an in vitro experiment was performed to determine the direct effect of fenthion on the activities of ChE, ALT, AST, ALP, GGT and LDH in serum. In the present in vitro experiment, the activities of enzymes were not changed with the treatment of the vitamin E and C combination, but fenthion caused a significant direct inhibition in the activity of ALT. These results indicate that decreased ALT activity in rats may be due to the fact that ALT activity had been inhibited by fenthion, although increased ALT activity in fenthion toxicity is expected.

In conclusion, it is likely that fenthion caused liver damage. In addition, LPO may be one of the molecular mechanisms involved in fenthion-induced toxicity. Single-dose treatment with a combination of vitamins E and C 30 min after the administration of fenthion can reduce LPO caused by fenthion. We suggest that similar studies should be done on liver tissue due to the fact that the serum activities of enzymes concerning the liver are not completely specific.

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