

The Effects of Vitamin E and Selenium on Cypermethrin-Induced Oxidative Stress in Rats

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Abstract: The aim of this study was to investigate whether vitamin E (VitE) and selenium (Se) or VitE plus Se have any the protective and/or augmented effects on cypermethrin (CYP)-induced oxidative stress in liver, brain, kidney and blood in rats. Rats were treated orally with daily 50 mg/kg (~ 1/4 ED50) of CYP in corn oil for five days after administrations of VitE (100 mg/kg sc.), Se (0.1 mg/kg, sc.) and VitE plus Se for three days. Malondialdehyde (MDA) concentrations, glutathione peroxidase (GSH-Px) and catalase (CAT) activities were measured in tissues and blood samples. MDA concentrations in all tissues except plasma, and GSH-Px activities in liver and erythrocytes were significantly increased, but CAT activities in all tissues except erythrocytes were decreased in CYP treated group when compared with the control group. Treatment with VitE prior to CYP reduced sensitivity on CYP-induced oxidative stress. The use of Se was observed to increase both MDA concentrations and antioxidant systems such as GSH-Px and CAT activities. On the other hand, VitE plus Se caused complex alterations in the antioxidant system on the oxidative stress induced by CYP. The results suggest that CYP can induce oxidative stress and VitE can modify CYP metabolism and play a protective role against CYP-induced oxidative stress.

Key Words: Catalase, cypermethrin, glutathione peroxidase, lipid peroxidation, oxidative stress, selenium, vitamin E.

Ratlarda Sipermetrin Tarafından Oluşturulan Oksidatif Stres Üzerine Vitamin E ve Selenyumun Etkileri

Özet: Bu çalışmanın amacı ratlarda karaciğer, beyin, böbrek ve kanda sipermetrin tarafından oluşturulan oksidatif stres üzerine vitamin E, selenyum ve vitamin E ile birlikte selenyumun koruyucu ve/veya artırıcı etkilerinin olup olmadığını araştırmaktır. Ratlara 3 gün süreyle günlük olarak vitamin E (100 mg/kg), selenyum (0,1 mg/kg) ve vitamin E ile selenyumun birlikte verilmesinden sonra 5 gün süre ile mısır yağı içinde 50 mg/kg dozunda (~1/4 ED50) sipermetrin oral yolla uygulandı. Doku ve kan örneklerinde MDA düzeyi ile GSH-Px ve CAT aktiviteleri ölçüldü. Kontrol grubu ile karşılaştırıldığında tek başına sipermetrin uygulanan grupta MDA düzeylerinin plazma hariç tüm dokularda, GSH-Px aktivitelerinin ise karaciğer ve plazmada arttığı görüldü. Buna karşın CAT aktivitelerinin eritrosit hariç tüm dokularda azaldığı belirlendi. Sipermetrinden önce vitamin E uygulamasının oluşan oksidatif stresi azalttığı görüldü. Selenyumun ise hem MDA konsantrasyonlarında hem de GSH-Px ve CAT aktiviteleri gibi antioksidan sistemlerde artışlara neden olduğu gözlemlendi. Diğer yandan vitamin E ile birlikte selenyumun sipermetrin tarafından oluşturulan oksidatif stres üzerine antioksidan sistemde kompleks etkilere neden olduğu görüldü. Sonuç olarak sipermetrinin oksidatif stres oluşturabileceği ve vitamin E'nin sipermetrin metabolizmasını değiştirerek oksidatif strese karşı koruyucu bir rol oynayabileceği kanaatine varıldı.

Anahtar Sözcükler: Katalaz, sipermetrin, glutatyon peroksidaz, lipid peroksidasyon, oksidatif stress, selenyum, vitamin E.

Introduction

Synthetic pyrethroids have emerged as a new class of agricultural pesticides and have found wide use over organochlorine and organophosphate pesticides.

Pyrethroid pesticides show high toxicity to a wide range of insects, including resistant strains low toxicity to mammals and birds and rapid biodegradability (1-3). The pyrethroids are neurotoxins, and based on their structure

and on toxic signs observed in the rat, may be classified into two groups, namely type I and type II compounds. Cypermethrin (CYP) is an important photostable type II synthetic pyrethroid derivative of natural pyrethroids. CYP also exhibits good insecticide action and low toxicity in mammals (4,5). Pyrethroids are more hydrophobic than other classes of insecticides and this feature indicates that the site of action is biological membrane. The main target site is neuronal sodium channels and it increases sodium entry into the nerve cell and induces depolarization of the nerve membrane and blocks of nerve conduction at high concentrations. An additional mechanism for type II pyrethroids proposed inhibition of GABA receptors (6-8).

Abnormal production of free radicals leads to damage of some macromolecules including proteins, lipids and nucleic acids, and this is believed to be involved in the etiology of many chemicals and diseases (9-11). The induction of oxidative stress and alteration of antioxidant system by pyrethroids in animals and fishes have been reported. Investigators showed that lipid peroxidation in erythrocytes increased with pyrethroid treatment and antioxidant enzyme activities and MDA concentrations were altered after CYP exposure in fishes (2,12-14).

Under normal conditions, excessive formation of free radicals and concomitant damage at cellular and tissue concentrations is controlled by cellular defense systems. These preventive defense systems can be accomplished by enzymatic or non-enzymatic mechanisms including VitE, vitamin C and glutathione. The antioxidant enzymes such as GSH-Px and CAT may also have an important function in mitigating the toxic effects of reactive oxygen species. The preventive effect of VitE on CYP-induced elevation of thiobarbituric acid reactive substances (TBARS) in rat tissues observed in some studies is in agreement with its antioxidant activity (2,15-18). Giray et al. (19) reported that pretreatment of rats with VitE provided significant protection against the elevation of MDA concentrations in cerebral and hepatic tissues, induced by CYP. Additionally, some investigators suggested that the selective block of tetramethrin-modified sodium channels by VitE is one of the important mechanisms underlying VitE alleviation of paresthesia (20,21).

Selenium, in the form of GSH-Px, plays an important role in the protection of tissue from oxidative damage. Aside from being an integral part of GSH-Px, Se can antagonize the toxic effects of some chemical substances

(22-24). On the other hand, increases of dietary and tissue concentration of selenium, increases in plasma and hepatic GSH-Px activities occur, followed by ultimately hepatic lipid peroxidation (25-30).

The aim of the present study was to determine the protective and/or augmented effects of VitE and Se or VitE plus Se on CYP-induced oxidative stress.

Materials and Methods

Chemicals

Technical grade cypermethrin was kindly supplied by Novartis (Istanbul, Turkey) and had a purity of >90%. Vitamin E (DL- α -tocopherol) and Selenium (Sodium selenite) were obtained from Merck (Darmstadt, Germany). NADPH, Reduced glutathione, Glutathione reductase, Thiobarbituric acid, Fospho tungstic acide, n-Butanol, Hydrogen peroxide and other reagents were purchased from Sigma (St Louis, MO, USA).

Animals and Drug Administrations

Wistar rats weighing 180-250 g were provided from a local veterinary research institute. Rats were housed in plastic cages under standard conditions with free access to drinking water and basal diet. The animals were adapted to the laboratory conditions before use and were maintained in a room with controlled temperature (22 °C), relative humidity (60%) and 12-h light:12-h dark cycle. CYP (10 mg/ml) were suspended in corn oil and administered to animals by gavage at the doses of 50 mg/kg BW ($\sim 1/4$ ED₅₀). VitE (20mg/ml) were suspended in corn oil and administered to animals by sc at doses of 100 mg/kg BW. Selenium (0.02 mg/ml) was dissolved in distilled water and injected to animals at the dose of 0.1 mg/kg, sc. The doses used in this study were selected on the basis of the reports of previous studies (2,9,15,30). The rats were divided into five groups, each including six animals;

Group 1: (Control group): Rats received 1 ml corn oil orally for five days.

Group 2: This group received alone CYP orally for five days.

Group 3: This group received CYP orally for five days following treatment with VitE for three days.

Group 4: This group received CYP orally for five days following treatment with Se for three days.

Group 5: This group received CYP orally for five days following treatment with VitE plus Se for three days.

Sample Collection and Preparation of Tissue Homogenates

Animals in all groups were decapitated (between 9:00 and 11:00 am) 24 h after the last dose application. Blood samples were collected into tubes containing sodium oxalate (2%). The samples were centrifuged at 200 g for 5 min at +4 °C to separate their plasma and were kept at -20 °C until required. After separating the plasma, erythrocytes were washed three times with 0.9% NaCl solution to prepare erythrocyte hemolyzates. Brain, liver and kidney tissues were removed immediately and stored at -20 °C until the assay. The homogenization of tissues was carried out in Teflon-glass homogenizer with a buffer containing 1.5% KCl to obtain 1/10 (w/v) whole homogenate. Homogenates were centrifuged 30 min at 18,000 g at +4 °C to determine of GSH-Px and CAT activities.

Biochemical Assays

The concentrations of MDA were determined as preceding of lipid peroxidation. MDA concentrations of tissue homogenates were assayed according to a modified method of Ohkawa et al. (31) based on the reaction with thiobarbituric acid. Plasma MDA concentrations were measured by the modified method of Satoh (32). MDA concentrations in plasma and tissues were expressed as nmol/ml and nmol g tissue⁻¹, respectively. The activities of GSH-Px and CAT were measured as other indicators of oxidative stress. GSH-Px activities were determined by the procedure described by Beutler (33). The procedure of analysis performed was based on the oxidation of reduced glutathione peroxidase coupled to the disappearance of NADPH by glutathione reductase measured at 37 °C and 340 nm and were expressed as U g Hb⁻¹ in erythrocyte and U g protein⁻¹ in tissue. CAT activities were determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (34) and were expressed as k g Hb⁻¹ in erythrocytes and k g protein⁻¹ in tissues where k is the first-order rate constant. Protein concentrations were measured according to Lowry et al. (35). Hemoglobin concentrations were determined according to method of Drabkin (36).

Statistic Analysis

All results were expressed as mean ± SEM. Comparisons control group and Group 2 and between

Group 2 and remaining groups were evaluated by one-way ANOVA followed by Dunnet's test. The *P* values < 0.05 were considered significant.

Results

There was no mortality in any groups during the experiment. Slight nervousness and abnormal gait were noted in some rats treated with cypermethrin.

Treatment with alone CYP alone caused significant increases in MDA concentrations of liver, brain and kidney tissues but no change was detected in plasma when compared with the control group. A significant decrease was observed in MDA concentrations of liver, brain and kidney in rats treated with VitE prior to CYP but no significant changes were seen in plasma. When compared with the CYP treated group, an increase was observed in MDA concentrations of liver, brain and plasma, whereas a decrease was seen in the kidney Se treated group. While MDA concentrations decreased in liver, kidney, they showed slight increases in the brain tissue in VitE plus Se treated group (Table 1).

When compared with the control group, administration of CYP to rats caused increases in GSH-Px activities of liver and erythrocytes but decreases were coincided in brain and kidney. Although a decrease was determined in GSH-Px activities of liver and brain tissues in rats treated with VitE prior to CYP, a significant increase was found in kidney and erythrocyte when compared with CYP treated group. On the other hand, an increase was observed in GSH-Px activities of all tissues in rats treated with Se. In the group treated with VitE plus Se, increases in GSH-Px activities were determined in all tissues, except erythrocytes (Table 2).

Administration of CYP caused a decrease in CAT activities of tissues, except erythrocytes, when compared with the control group. While a significant decrease was observed in CAT activities of brain and erythrocytes, an increase was found in kidney in rats treated with VitE prior to CYP. Treatment with VitE did not affect liver CAT activity. When compared with the CYP treated group, an increase was observed in CAT activities of liver, kidney and erythrocytes, whereas a decrease was seen in brain in rats treated with Se. While CAT activities decreased in liver and brain, they showed an increase in the kidney, but no changes were observed in erythrocytes in VitE plus Se treated group (Table 3).

Table 1. Liver, brain, kidney tissues (nmol/g tissue) and plasma (nmol/ml) MDA concentrations.

	Liver	Brain	Kidney	Plasma
Control (Group 1)	1.53 ± 0.13	2.08 ± 0.01	1.75 ± 0.01	12.02 ± 0.31
CYP(Group 2)	2.50 ^a ± 0.17	2.74 ^a ± 0.39	2.12 ^a ± 0.01	11.45 ± 0.22
VitE + CYP (Group 3)	1.87 ^b ± 0.12	2.03 ^b ± 0.01	1.80 ^b ± 0.01	11.36 ± 0.19
Se + CYP (Group 4)	2.70 ± 0.11	3.11 ^b ± 0.12	1.83 ^b ± 0.01	13.63 ^b ± 0.22
(VitE + Se) + CYP (Group 5)	1.62 ^b ± 0.10	3.18 ^b ± 0.28	1.55 ^b ± 0.01	11.06 ± 0.12

Data are means ± S.E.M. of 6 rats for each group and concerning presented in same column. Statistical analysis was performed using a one-way analysis of variance followed by Dunnet's test.

CYP was administered at the dose of 50 mg/kg, orally in 1 ml corn oil for 5 days.

VitE was injected at the dose of 100 mg/kg s.c. in 1 ml corn oil.

Se was injected at the dose of 0.1 mg/kg s.c. in 1 ml distilled water.

^aP<0.05 (compared to controls).

^bP<0.05 (compared to the rats receiving only CYP).

Table 2. Liver, brain, kidney tissues (unit/g protein) and plasma (unit/g Hb) GSH-Px activities.

	Liver	Brain	Kidney	Erythrocyte
Control (Group 1)	2.40 ± 0.54	17.21 ± 3.02	8.56 ± 1.22	31.70 ± 2.72
CYP(Group 2)	2.63 ^a ± 0.12	8.96 ^a ± 1.23	4.21 ^a ± 0.77	41.60 ^a ± 2.95
VitE + CYP (Group 3)	2.34 ± 0.29	8.51 ± 0.37	8.93 ^b ± 0.77	55.10 ^b ± 5.42
Se + CYP (Group 4)	3.43 ^b ± 0.10	11.08 ^b ± 0.31	15.85 ^b ± 0.59	51.70 ^b ± 1.70
(VitE + Se) + CYP (Group 5)	3.60 ^b ± 0.27	15.00 ^b ± 2.39	11.95 ^b ± 0.22	36.20 ^b ± 3.03

Data are means ± S.E.M. of 6 rats for each group and concerning presented in same column.

Statistical analysis was performed using a one-way analysis of variance followed by Dunnet's test.

CYP was administered at the dose of 50 mg/kg, orally in 1 ml corn oil for 5 days.

VitE was injected at the dose of 100 mg/kg s.c. in 1 ml corn oil.

Se was injected at the dose of 0.1 mg/kg s.c. in 1 ml distilled water.

^aP<0.05 (compared to controls).

^bP<0.05 (compared to the rats receiving CYP alone).

Table 3. Liver, brain, kidney tissues (k/g protein) and plasma (k/g Hb) CAT activities.

	Liver	Brain	Kidney	Erythrocyte
Control (Group 1)	36.85 ± 3.55	1.15 ± 0.23	19.71 ± 1.77	42.28 ± 1.01
CYP (Group 2)	31.13 ^a ± 2.64	0.68 ^a ± 0.01	12.78 ^a ± 0.61	44.73 ^a ± 0.67
VitE + CYP (Group 3)	32.35 ± 1.30	0.09 ^b ± 0.01	19.95 ^b ± 1.23	21.16 ^b ± 2.87
Se + CYP (Group 4)	34.50 ^b ± 0.56	0.13 ^b ± 0.01	30.28 ^b ± 1.22	50.63 ^b ± 3.30
(VitE + Se) + CYP (Group 5)	24.46 ^b ± 1.18	0.10 ^b ± 0.01	18.50 ^b ± 0.40	43.10 ± 1.61

Data are means ± S.E.M. of 6 rats for each group and concerning presented in same column.

Statistical analysis was performed using a one-way analysis of variance followed by Dunnet's test.

CYP was administered at the dose of 50 mg/kg, orally in 1 ml corn oil for 5 days.

VitE was injected at the dose of 100 mg/kg s.c. in 1 ml corn oil.

Se was injected at the dose of 0.1 mg/kg s.c. in 1 ml distilled water.

^aP<0.05 (compared to controls).

^bP<0.05 (compared to the rats receiving only CYP).

Discussion

Lipid peroxidation may be due to oxidation of molecular oxygen to produce superoxide radicals. This reaction is also the source of H_2O_2 , which causes the production of MDA by initiating the peroxidation of unsaturated fatty acids in the membrane. Both H_2O_2 and $\cdot O_2^-$ produced highly reactive hydroxyl radical with Haber-Weiss reaction. The hydroxyl radical can initiate lipid peroxidation which is a free radical chain leading to loss of membrane structure and function (2,8).

Lipid peroxidation has been shown to increase in plasma and some tissues in CYP and other insecticides toxicities (1,12,13,15). The increase in the concentrations of MDA in the kidney and in the activities of GSH-Px in the liver suggested that CYP and its metabolites may be detoxified in these tissues (12,14). The lipophilic characteristics of CYP indicate that the site of action is sodium channels in the neuronal membrane (5-7) and it accumulates mostly in fat, skin, liver and kidney (4). In our study, the concentrations of MDA in the liver (63.3%), brain (31.8%) and kidney (21.1%) in alone CYP treated group were significantly higher than the control group. The increases in the concentrations of MDA are the indicator of CYP-induced lipid peroxidation. El-Tawil and Abdel-Rahman (3) and Giray et al. (19) observed that the liver can be accepted as source of GSH-Px, and therefore a higher activity was found in this organ compared to the other organs. Ünner et al. (14) reported that CYP caused an increase in GSH-Px activity while it caused a decrease in CAT activity in the liver and kidney of some fresh water fish species. Because GSH-Px is found mainly in cytosol and mitochondria, it is widely affected by xenobiotics (10,29). Investigations indicated that mammals have a good defense mechanism for lipid peroxidation because it can increase the hepatic CAT activity when needed. However, CAT is generally localized in peroxisomes and therefore its role in the other parts of the cell is limited. In particular, H_2O_2 at low concentration is destroyed by this enzyme (2,9,14). It is possible that pyrethroids can be transported through blood to the liver for metabolism and may produce cellular damage in erythrocytes (2,4,5,8). In the present study, CYP caused an increase in GSH-Px activity in liver and erythrocytes while it caused a decrease in CAT activity in all tissues, except erythrocytes. Our findings are in agreement with the observations of the above workers.

Studies carried out with antioxidants have shown that they inhibit free radical formation. VitE has also been used to prevent oxidative damage by interrupting the propagation of the oxidation of polyunsaturated fatty acids. Some investigators reported that administering VitE may be useful in controlling the hepatotoxic effects of insecticides and chemicals (9,15,16). Some investigators (17,19) showed the preventive effect of VitE against CYP-induced elevation of TBARS in rat tissues. In addition, Flannigan et al. (20) and Song and Narahashi (21) observed that VitE is able to reduce the cutaneous paresthesia caused by pyrethroids. In this study, VitE treatment decreased the MDA concentrations in liver, brain and kidney when compared with CYP treated group. In addition, liver and brain GSH-Px activities and CAT activities in brain and erythrocytes were decreased by VitE. A reasonable explanation for this may be that although VitE is not distributed uniformly in tissues, it can show a protective effect especially when administered prior to CYP. These observations might also indicate that VitE has prophylactic and therapeutic effects on CYP-induced oxidative stress.

Some investigators (11,26,28,30) suggested that Se toxicity is related to changes in intracellular concentrations of reduced glutathione. As dietary and tissue concentrations of Se increase, increases in plasma and hepatic GSH-Px activities occur, followed by increases in ratio of hepatic oxidized to reduce glutathione and ultimately hepatic lipid peroxidation. Zia and Islam (27) demonstrated that the content of TBARS was elevated dose-dependently in striatum but its concentration was depleted in the thalamus by sodium selenite. It was shown that when tissue concentration of Se was increased, GSH-Px activities also increase in plasma and liver. In addition, Avanzo et al. (18) determined decreases in GSH-Px activities in chicken fed diets deficient in Se and VitE, but increases in CAT activity. O'Grady et al. (28) observed that dietary Se supplementation did not affect GSH-Px activity in the presence or absence of VitE. In the present study, increases in MDA concentrations of liver, brain and plasma, GSH-Px activities in all tissues, CAT activities in liver, kidney and erythrocytes, were observed in rats treated with Se prior to CYP administration. Especially, alterations in GSH-Px activities are compatible with the reports of above workers and can be explained by effects of Se on cellular structures or its alternative effects on enzyme activities.

In conclusion, the concentrations of MDA in tissues were observed to increase significantly in rats treated with CYP. Treatment with VitE reduced sensitivity to oxidative stress. However, the use of selenium prior to CYP was observed to increase both MDA concentrations and antioxidant systems such as GSH-Px and CAT activities. On the other hand, VitE plus Se could cause complex alterations in the antioxidant system and

adversely could alleviate the oxidative stress induced by CYP.

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