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Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes

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Abstract

Organophosphate (OP) pesticides such as dimethoate and malathion intoxication has been shown to produce oxidative stress due to the generation of free radicals and alter the antioxidant defense system in erythrocytes. It is possible that vitamin E being present at the cell membrane site may prevent OP-induced oxidative damage. In the present study, rats were pretreated orally with vitamin E (250 mg/kg body wt, twice a week for 6 weeks) prior to oral administration of a single low dose of dimethoate and/or malathion (0.01% LD_{50}). The result showed that treatment with OP increased lipid peroxidation (LPO) in erythrocytes, however, vitamin E pretreated rats administered OP's showed decreased LPO in erythrocytes. The increase in the activities of superoxide dismutase (SOD) and catalase (CAT) and total-SH content in erythrocytes from dimethoate and/or malathion treated rats as compared to control appears to be a response towards increased oxidative stress. Vitamin E pretreated animals administered OP's showed a lowering in these parameters as compared to OP treated rats which indicates that vitamin E provide protection against OP-induced oxidative stress. The glutathione-S-transferase (GST) activity in erythrocytes was inhibited in OP intoxicated rats which partially recovered in vitamin E pretreated animals administered OP's. Inhibition in erythrocyte and serum acetylcholinesterase (AChE) activity was not relieved in vitamin E pretreated rats administered OP's probably due to the competitive nature of enzyme inhibition by OP's. The results show that vitamin E may amelierate OP-induced oxidative stress by decreasing LPO and altering antioxidant defense system in erthrocytes.

Keywords: Lipid peroxidation; Superoxide dismutase; Catalase; Glutathione-S-transferase; Acetylcholinesterase; Organophosphate pesticides

1. Introduction

Pesticides are occasionally used indiscriminately in large amounts causing environmental pollution and therefore, are a cause of concern. Residual amounts of organochlorines (OC) and organophosphate (OP) pesticides have been detected in the soil, water bodies, vegetables, grains and other foods products [1]. OP are known to cause inhibition of acetylcholinesterase (AChE) activity in the target tissues [2] which accumulates acetylcholine and prevents the smooth transmission of nerve functions leading to convulsions and death. However, low intakes of OP's through food and water may not show clear symptoms of OP intoxication such as convulsions but it may show mild inhibition of AChE activity in erythrocytes and tissues. Recent studies indicate that dimethoate intoxication produce oxidative stress by the generation of free radicals and induce hepatic LPO in chicken [3] and in mice [4]. As some of the OP's may be present in blood of exposed humans and animals, it may produce oxidative stress in erythrocytes. The antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) as well as total-SH content in erythrocytes, however, may neutralize the oxidative stress. Vitamin E, a constituent of plasma membrane, is an effective antioxidant and as it is present at the site of free radical generation, it may neutralize the toxic effects of reactive oxygen species (ROS). The rats were thus, pretreated with vitamin E, prior to dimethoate and/or malathion intoxication in order to show whether vitamin E prevents OP-induced oxidative stress in erythrocytes.

2. Materials and methods

Male Wistar rats weighing 150–180 g were housed in polypropylene cages under standard conditions with free access to drinking water and basal diet. Rats were selected due to their easy availability as an experimental animal. The

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animals were regularly weighed. The animals were randomly divided into eight groups each comprising of six animals. The rats were administered orally 0.2 ml. dimethoate and/or malathion (0.01% LD_{50}) as a single dose in 1 ml groundnut oil as vehicle. Groundnut oil was administered to control rats and its feeding showed no affect on the study. LD_{50} for dimethoate and malathion are 300 mg/kg body wt and 1350 mg/kg body wt respectively [5]. The dose of diamethoate and malathion administered to rats was 0.03 mg/kg body wt. and 0.13 mg/kg body wt. respectively. The doses were selected to provide low level of pesticide intoxication. Some of the animals were pretreated orally with vitamin E (α-tocopherol acetate) at a dose of 250 mg/kg body wt., twice a week, up to 6 weeks and were then orally administered dimethoate and/or malathion at the above mentioned doses. Basal diet was pellet feed and was not loaded with vitamin E. Vitamin E dose was administered to provide higher levels of vitamin E in the erythrocytes. The animals were killed by decapitation after day 3 of dimethoate and/or malathion treatment. Blood was collected by cardiac puncture in vials containing citrate (2%). The blood was centrifuged and the erythrocytes were washed twice with 0.1 M phosphate buffered saline (PBS, 1:9), pH 7.4. Erythrocyte lysate was prepared according to the method of McCord and Fridovich [6] for the assay of antioxidant enzymes.

2.1. Lipid peroxidation in erythrocytes

LPO in erythrocytes was estimated by the thiobarbituric acid (TBA) reaction with malonyldialdehyde (MDA), a product formed due to peroxidation of lipids by the method of Stocks and Dormandy [7]. LPO was expressed as in nmoles of MDA formed/hr/g Hb using a molar extinction coefficient of MDA as 1.56×10^5.

2.2. Antioxidant enzymes

SOD activity was determined by the ability of the enzyme to inhibit the autoxidation of pyrogallol by the method of Marklund and Marklund [8]. Catalase was assayed by the decomposition of hydrogen peroxide by the method of Aebi [9]. Glutathione-S-transferase activity was determined by the method of Habig et al. [10].

2.3. Total-SH content in erythrocytes

Total-SH content was assayed using Ellman's reagent by the method of Beutler et al. [11].

2.4. Acetylcholinesterase activity in erythrocytes and serum

Acetylcholinesterase (AChE) activity was assayed by the method of de la Huerga et al., as described by Varley [12].

Haemoglobin was estimated using Drabkin's reagent by the method of Dacie and Lewis [13]. The protein content was determined by the method of Lowry et al. [14]. The statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison procedure to calculate the significance. $P < 0.05$ was considered significant.

3. Results

3.1. Lipid peroxidation in erythrocytes

Vitamin E treated rats (group 2) showed erythrocyte LPO comparable to control (Table 1). Treatment with dimethoate and/or malathion (group 3, 5 and 7) showed increased LPO in erythrocytes, as compared to control. Vitamin E pretreated rats administered dimethoate and/or malathion (group 4, 6 and 8) showed significant decrease in erythrocyte LPO as compared to rats administered dimethoate and/or malathion without vitamin E pretreatment (group 3, 5 and 7).

3.2. Superoxide dismutase activity in erythrocytes

SOD activity in vitamin E treated rats (group 2) was comparable to control (Table 1). Treatment with dimethoate and/or malathion (group 3, 5 and 7) showed increased activity of SOD in erythrocytes as compared to control. Vitamin E pretreated rats administered dimethoate and/or malathion (group 4, 6 and 8) showed significant decrease in erythrocyte SOD activity as compared to group 3, 5 and 7 respectively.

3.3. Catalase activity in erythrocytes

Vitamin E treated rats (group 2) showed erythrocyte CAT activity comparable to control (Table 1). Treatment with dimethoate and/or malathion (group 3, 5 and 7) showed increased erythrocyte CAT activity as compared to control. Vitamin E pretreated rats administered dimethoate and/or malathion (group 4, 6 and 8) showed decrease in erythrocyte CAT activity as compared to group 3, 5 and 7 respectively.

3.4. Total-SH content in erythrocytes

Erythrocyte -SH content in vitamin E treated rats (group 2) was comparable to control (Table 2). Treatment with dimethoate or malathion (group 3, 5 and 7) showed increased -SH content in erythrocytes as compared to control. Vitamin E pretreated rats administered dimethoate and/or malathion (group 4, 6 and 8) showed decrease in erythrocyte -SH content as compared to group 3, 5 and 7 respectively.

3.5. Glutathione-S-transferase activity in erythrocytes

Vitamin E treated rats (group 2) showed erythrocyte GST activity comparable to control (Table 2). Treatment

Table 1
Lipid peroxidation and activities of superoxide dismutase and catalase in erythrocytes

S.No.	Group	LPO[a]	SOD[b]	CAT[c]
1	Control	691 ± 5	28.6 ± 2.6	482 ± 58
2	Vitamin E	708 ± 21 (NS)	39.7 ± 9.1 (NS)	543 ± 84 (NS)
3	Dimethoate	999 ± 80 ($P < 0.001$)	81.7 ± 3.3 ($P < 0.001$)	1062 ± 31 ($P < 0.001$)
4	Vitamin E + Dimethoate	776 ± 42*** ($P < 0.05$)	58.7 ± 6.3* (NS)	779 ± 97* ($P < 0.05$)
5	Malathion	872 ± 63 ($P < 0.001$)	79.0 ± 8.7 ($P < 0.001$)	1089 ± 89 ($P < 0.001$)
6	Vitamin E + Malathion	673 ± 36*** (NS)	46.7 ± 6.4* (NS)	816 ± 85* ($P < 0.05$)
7	Dimethoate + Malathion	819 ± 53 ($P < 0.001$)	50.8 ± 9.2 (NS)	680 ± 86 (NS)
8	Vitamin E + Dimethoate + Malathion	706 ± 39*** (NS)	26.9 ± 5.8* (NS)	412 ± 93* (NS)

[a] = nmoles of MDA formed/hr/g Hb.
[b] = Units/mg protein.
[c] = μmoles of H_2O_2 decomposed/min/mg protein.
Values are mean ± SD of 6 animals in each group.
P value in parenthesis show significance of groups 3, 5 and 7 as compared to control and groups 4, 6 and 8 as compared to group 2.
*** $P < 0.001$, * $P < 0.05$ when group 4, 6 and 8 was compared to group 3, 5 and 7 respectively.

with dimethoate and/or malathion (group 3, 5 and 7) markedly inhibited erythrocyte GST activity as compared to control. Vitamin E pretreated rats administered dimethoate (group 4) showed recovery in GST activity as compared to group 3, however GST activity in group 6 was comparable to group 5 while GST activity in group 8 was significantly less as compared to group 7.

3.6. Erythrocyte and serum acetylcholinesterase activity

Vitamin E treated rats (group 2) showed erythrocyte and serum AChE activity comparable to control (Table 2). AChE activity in erythrocytes and serum was inhibited in dimethoate and/or malathion treated rats (group 3 and 5) as compared to control. Vitamin E pretreated rats administered

Table 2
Total-SH content and activities of glutathione-S-transferase and acetylcholinesterase in rat blood

S.No.	Group	Total-SH content[a]	GST activity[b]	Erythrocytes AChE[c]	Serum AChE[c]
1	Control	6.9 ± 0.2	222 ± 21	75.4 ± 3.2	98.3 ± 3.6
2	Vitamin E	7.0 ± 0.4 (NS)	220 ± 18 (NS)	73.3 ± 5.7 (NS)	95.1 ± 8.8 (NS)
3	Dimethoate	10.1 ± 0.4 ($P < 0.001$)	ND	51.0 ± 1.1 ($P < 0.001$)	20.4 ± 7.5 ($P < 0.001$)
4	Vitamin E + Dimethoate	7.2 ± 0.9*** (NS)	101 ± 12*** ($P < 0.001$)	53.2 ± 3.4† ($P < 0.001$)	31.8 ± 6.0** ($P < 0.001$)
5	Malathion	8.7 ± 0.4 ($P < 0.001$)	73 ± 4 ($P < 0.001$)	30.2 ± 6.8 ($P < 0.001$)	40.3 ± 8.7 ($P < 0.001$)
6	Vitamin E + Malathion	6.9 ± 0.8** (NS)	75 ± 9† ($P < 0.001$)	26.9 ± 5.9† ($P < 0.001$)	51.2 ± 4.8* ($P < 0.001$)
7	Dimethoate + Malathion	7.7 ± 0.6 ($P < 0.05$)	147 ± 25 ($P < 0.001$)	34.7 ± 2.9 ($P < 0.001$)	38.6 ± 8.1 ($P < 0.001$)
8	Vitamin E + Dimethoate + Malathion	5.7 ± 0.6*** ($P < 0.001$)	102 ± 2*** ($P < 0.001$)	28.0 ± 3.4* ($P < 0.001$)	26.6 ± 5.7** ($P < 0.001$)

[a] = μmoles of DTNB conjugated/g Hb.
[b] = nmoles of CDNB conjugated/min/mg protein.
[c] = Units/ml.
Values are mean ± SD of 6 animals in each group.
P values in parenthesis show significance of groups 3, 5 and 7 as compared to control and groups 4, 6 and 8 as compared to group 2.
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, † not significant when group 4, 6 and 8 was compared to group 3, 5 and 7 respectively. ND = not detectable.

dimethoate or malathion (group 4 and 6) showed erythrocyte AChE activity comparable to group 3 and 5 while serum AChE activity showed mild recovery (group 4 and 6) as compared to group 3 and 5. Treatment with dimethoate and malathion together (group 7) also inhibited erythrocyte and serum AChE activity without any recovery in vitamin E pretreated rats administered both the pesticides (group 8).

4. Discussion

The results show that treatment with dimethoate and/or malathion produce LPO in erythrocytes. Vitamin E pretreated rats administered dimethoate and/or malathion however, showed decreased LPO in erythrocytes as compared to OP treated rats indicating that it may have beneficial role in lowering OP toxicity. There are no reports to show protective effect of antioxidants in OP toxicity, however, some of the studies have suggested the protective role of antioxidant in OC pesticides, such as endrin-induced hepatic LPO [15, 16]. Vitamin E allow free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids (PUFA), thus breaking the chain of free radical reactions, the resulting antioxidant radical being a relatively unreactive species [17]. The consequences of dietary vitamin E deficiency result in the peroxidation of subcellular fractions of various organs [18,19] whereas dietary vitamin E gave protection against increased LPO [20, 21]. Vitamin E depleted erythrocytes were shown to be more susceptible to LPO and lysis than those from animal on normal diet [22]. The result indicate that vitamin E pretreatment may decrease LPO and protect the erythrocytes from OP-induced oxidative stress.

SOD and CAT activities in erythrocytes were increased in dimethoate and/or malathion treated rats probably to dismutate superoxide anions ($O_2^{\cdot -}$) and to decompose H_2O_2. The increase in these enzymes was probably a response towards increased ROS generation in OP toxicity. Vitamin E pretreated rats administered dimethoate and/or malathion showed decrease in erythrocyte SOD and CAT activities, as vitamin E scavenges ROS and lowers oxidative stress.

The increased erythrocyte total -SH content in dimethoate and/or malathion treated rats may probably be due to decreased activity of GST in erythrocytes. The decline in erythrocyte GST activity may lower the conjugation of -SH groups thus causing toxic conditions in the erythrocytes. Rats pretreated with vitamin E, prior to OP intoxication, however, showed decrease in total-SH content due to low oxidative stress. Since liver is the major source of blood glutathione, hepatic GSH efflux in toxic conditions may increase blood glutathione level [23]. Presumably, the increase supply of GSH from the blood pool can enhance the ability of extrahepatic tissues to cope with a generalized increase in tissue oxidative stress [24].

GST detoxicates a variety of electrophlic compounds to less toxic forms by conjugation with -SH groups such as GSH. The marked inhibition in erythrocyte GST activity in dimethoate and/or malathion treated rats indicate insufficient conjugation of electrophiles and detoxication of these species. However, the recovery in erythrocyte GST activity in vitamin E pretreated rats administered dimethoate and/or malathion may lower oxidative stress due to metabolism of electrophiles.

AChE activity in erythrocytes and serum was inhibited in OP intoxicated rats, however, the activity was not relieved by vitamin E pretreatment probably due to the competitive nature of AChE inhibition by OP's. However, the recovery in erythrocyte AChE activity may depend upon the renewal of the erythrocytes and breakdown of the older red cells, while the recovery in serum AChE may depend upon the synthesis of the protein in the liver [25]. The results show that vitamin E decrease OP-induced oxidative stress in erythrocytes, however, it may not have a direct role in relieving the inhibition of circulating AChE activity which may recover with time.

In conclusion, dimethoate and/or malathion produced LPO in erythrocytes, however, pretreatment with vitamin E lowers LPO in intoxicated rats. Erythrocyte SOD and CAT activities and GSH content increased in pesticide intoxicated rats, however, these erythrocyte antioxidants decreased in vitamin E pretreated animals intoxicated with OP's. Erythrocyte GST activity was inhibited in dimethoate and/or malathion treated rats which recovered in vitamin E pretreated rats intoxicated with pesticide. Erythrocyte and serum AChE activity was inhibited in pesticide treated rats, however, the AChE inhibition was not relieved in vitamin E pretreated rats administered pesticides.

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