Cell. Mol. Biol. 57 (1): 70-79
Published on February 12, 2011; DOI 10.1170/T904

BIOCHEMICAL AND MORPHOLOGICAL PERTURBATIONS IN RAT ERYTHROCYTES EXPOSED TO ETHION: PROTECTIVE EFFECT OF VITAMIN E

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Abstract

Erythrocyte membranes are an excellent model system to study interaction of pro-oxidants with membranes. The aim of the present study is to examine the effect of vitamin E on ethion-induced biochemical and morphological alterations in erythrocytes. Ethion was administered to the rats orally at a daily dose of 2.7 mg/kg body weight for a period of 7, 14, 21 and 28 days. The results from the present study show that administration of ethion resulted in oxidative damage to erythrocyte membranes as evident by increased lipid peroxidation and decreased phospholipid content. This was accompanied by decrease in membrane cholesterol levels. In addition, ethion exposure inhibited the activities of membrane bound enzymes; Na^+ K^+ ATPase and Mg^{2+}ATPase. Scanning electron micrographs of erythrocytes from animals exposed to ethion revealed morphological changes. Supplementation of vitamin E (50 mg/kg body weight) to ethion exposed animals ameliorated the ethion-induced oxidative stress, restored membrane lipid composition and activity of membrane bound enzymes along with erythrocyte shape. The results clearly demonstrate that ethion-induced damage involves increase in oxidative stress that results in alterations in erythrocyte membrane structure and function. Furthermore, supplementation with vitamin E reversed ethion induced alterations suggesting its beneficial role in individuals exposed to ethion.

Key words: Erythrocytes, Ethion, Lipids, Membrane, Oxidative stress, Vitamin E.

Article information
Received on December 17, 2010
Accepted on January 5, 2011

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Abbreviations: **Ops**: Organophosphates; **AChE**: Acetylcholinesterase; **LD_{50}**: lethal dose; **MDA**: Malondialdehyde; **LPO**: Lipid Peroxidation; **SEM**: Scanning Electron Microscope; **PUFA**: Polyunsaturated Fatty Acids.

INTRODUCTION

Ethion (O, O, O′, O′-tetraethyl S,′ S - methylene-bis (phosphorodithioate) is a widely used organophosphate insecticide, which has been identified as a contaminant in many components of the global ecosystem (18). Approximately 3000-4000 metric tones of ethion are used annually in India and makes it a major public health hazard especially in rural India (23). Exposure to ethion is associated with high morbidity and mortality (15). Ethion exposure has been observed to have direct toxic effects on liver, kidney, intestine and brain (34). Acute ethion exposure primarily affects the nervous system through inhibition of acetylcholinesterase (35). In addition, ethion is documented to be genotoxic (8). DNA damage has recently been shown in agricultural workers exposed to organophosphate pesticides (3,26). The biochemical mechanisms involved in toxicity following chronic low dose exposure to ethion

are not fully understood as it does not involve cholinergic mechanisms. Oxidative stress caused by organophosphates is proposed to be a major non-cholingeric mechanism linking pesticide exposure to its health effects (48). However, studies demonstrating the role of oxidative stress as a mechanism involved in ethion toxicity following chronic exposure are limited.

Erythrocytes are a convenient model to understand oxidative damage to membranes from various pro-oxidants as they are particularly sensitive to oxidative stress (31). This is because erythrocyte membranes contain high amounts of polyunsaturated fatty acids (PUFA), have higher concentration of oxygen and heme (37). A correlation has been observed between inhibition of acetylcholinesterase and ethion toxicity suggesting that it is a biomarker of ethion exposure (34). We have earlier reported that *in vitro* ethion exposure causes concentration and time dependent membrane damage in terms of hemoloysis and K^+ leakage from erythrocytes (45) along with inhibition of antioxidant enzymes (44).

Organophosphate intoxication can result in derangement of antioxidant defense resulting in adverse effects in various tissues (29). Hence, treatments aimed at increasing antioxidant defense mechanisms might be effective in preventing OP toxicity (43). Of the antioxidants tested vitamin E holds most promise against chemical-induced toxicity. Vitamin E is a lipid soluble antioxidant that protects biological membranes from oxidative damage following exposure to toxicants or in a disease condition (33,36). In addition, vitamin E is critical to erythrocyte function as vitamin E depleted erythrocytes have been shown to be highly susceptible to oxidative stress and lysis than those with normal vitamin E levels (12). Some investigators have reported that administration of vitamin E may be useful in controlling the toxic effect of insecticides and other chemicals (24,40). We have recently reported that chronic ethion exposure induces oxidative stress in liver accompanied by histological alterations which were reversed on vitamin E supplementation (7). Therefore, the present study has been designed with an aim to investigate the role of oxidative stress and alterations in lipid composition as a mechanism involved in ethion-induced damage and to evaluate the protective role of vitamin E as a protective agent against ethion induced toxicity using erythrocyte membranes as a model system.

MATERIALS AND METHODS

Chemicals

Ethion (technical grade) was a gift from Rallies, India. Vitamin E (α-tocopheryl acetate, trade name Evion) was purchased from the Merck Pharmaceuticals (Mumbai, India). All other chemicals were purchased from Sigma Chemical Company, St. Louis, MO, USA and Sisco Research Laboratory (SRL), Mumbai, India.

Animals and treatment

Male rats (Wistar strain) weighing 160–180 g were purchased from the Central Animal House of Panjab University, Chandigarh. All the animals were housed in clean polypropylene cages and were fed standard pellet diet (Ashirwad Industries, Kharar, India) and had free access to water. All the experiments were performed according to guidelines for use and care of laboratory animals and were approved by the ethical committee of the University.

The animals were randomly divided into four groups, each comprising of six animals.

Control: Animals were administered vehicle (corn oil).

Vitamin E-treated: Animals were administered vitamin E (50 mg/kg body weight/day).

Ethion-treated: Animals were administered ethion (2.7 mg/kg of body weight /day) dissolved in corn oil

Ethion + vitamin E-treated: Animals were administered ethion along with vitamin E.

The dose of ethion used in the study was $1/10^{th}$ oral LD_{50} for rats and was optimized in our laboratory (47). The rationale for administering Vitamin E at a dose of 50mg/kg was based on the doses reported in the literature (2). Blood samples were collected at regular intervals (7, 14, 21 and 28th day) and used for biochemical and scanning electron microscopy studies.

Preparation of erythrocytes

Animals were fasted overnight and blood was collected from the tail of the rats under mild ether anesthesia. Erythrocytes were prepared according to the method of Lohr and Waller (28). 0.5 ml of blood was taken into a test tube containing 0.5 ml sodium citrate solution (3.8% w/v) and centrifuged at 1000g for 10 min. The cells were washed twice with 5 ml physiological saline and the sediments were suspended in 1 ml of physiological saline.

Preparation of erythrocyte membranes

Erythrocyte membranes were prepared by the method of Blostein (10). The blood samples containing anticoagulant were centrifuged at 1000 g for 10 min at 4^oC. The pellet containing cells were washed thrice with ice cold solution of saline; buffy coat was removed at the end of each centrifugation. The cells were then hemolysed by adding 10 volumes of distilled water to one volume of packed cells (osmotic lysis). After centrifugation at 20,000 x g, the post-hemolytic residue was washed twice with 1.0 mM Tris-HCl containing 1 mM Tris-EDTA (pH 7.4), once with 10 mM Tris-EDTA (pH 7.4) and then three times with 2 mM Tris-HCl (pH 7.4). The preparation obtained was membrane residue and was half of the original packed cell volume of the washed cells. The erythrocyte membranes were stored at -80^0C for further studies.

Biochemical Assays

Lipid peroxidation

Peroxidation of erythrocyte membranes was determined by the method of Wills (52). As an index of lipid peroxidation, the amount of malonaldehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as nmoles MDA/mg protein using molar extinction coefficient of MDA thiobarbituric chromophore ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Erythrocyte Membrane Lipid Composition

Total lipids were measured by the method of Fringes and Dunn (19). Phospholipid content of the erythrocyte membranes was estimated by the method of Bartlett (5) as modified by Marinetti (32). Cholesterol was estimated according to the method of Zlatkis et al. (56)

Acetylcholinesterase (AChE)

Erythrocyte AChE activity was analyzed by the method of Ellman et al. (17) using acetylthiocholine as substrate. The rate of hydrolysis of acetylthiocholine was followed by increase in absorbance at 412 nm. AChE activity was calculated using molar extinction coefficient of 5-mercapto-2-nitrobenzoate ($13.6 \times 10^3\ M^{-1}\ cm^{-1}$). Results were expressed as nmoles acetylthiocholine hydrolyzed/min/mg protein.

Mg^{2+}ATPase and Na^+ K^+ ATPase

Mg^{2+}ATPase and Na^+ K^+ ATPase enzymes activities were assayed in the erythrocyte membranes according to the method of Quigley and Gotterer (38).

Protein Estimation

Protein content in erythrocyte membrane was quantified by the method of Lowry *et al.* (30) using bovine serum albumin as a standard.

Scanning electron microscopy (SEM)

Blood samples were fixed in 2.5% glutaraldehyde prepared in 0.2M phosphate buffer (pH 7.2). After 2 hrs of fixation, cells were centrifuged at 1000-1500 rpm. The fixative was discarded and pellet was re-suspended in the phosphate buffer. This process was repeated 2-3 times, and the supernatant was discarded every time. The pellet was suspended in distilled water, centrifuged and reconstituted in distilled water. Finally, the pellet was suspended in a minimum amount of distilled water. A drop of sample was smeared on the metallic SEM stub which was loaded with a conductive silver tape on its top. These stubs were then coated with gold to a thickness of 100Å using sputter i on coater with gold source for 5 min. These specimens were finally observed under scanning electron microscope (JSM-6100, Jeol, Tokyo, Japan) at the Regional Sophisticated Instrumentation Centre of the University.

Statistical analysis

All values were expressed as mean ± standard deviation of six observations. Data were analyzed using one way analysis of variance (ANOVA) followed by Bonferroni's post-hoc test for multiple pair wise comparisons between the various treated groups. Values with $P<0.05$ were considered as statistically significant.

RESULTS

Effect on acetylcholinesterase activity

Fig. 1 shows the effect of administration of ethion on AChE activity in erythrocyte membranes. A significant decrease in AChE activity was observed after ethion exposure for various time intervals. The decrease was 26.74 % after 7 days, 32.74 % after 14 days, 37.12 % after 21 days and 41.7 % after 28 days treatment as compared to control animals. Co-administration of vitamin E along with ethion resulted in a significant increase in the AChE activity and the increase ranged from 16 to 18 % as compared with the animals treated with ethion alone.

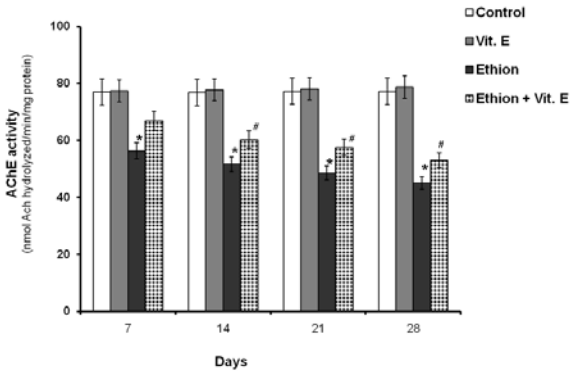

Figure 1. Effect of vitamin E on ethion-induced alterations in acetylcholinesterase activity from erythrocyte membranes. Values are mean ± S.D. of 6 animals/group.Data was analyzed by one-way ANOVA followed by Bonferroni's post hoc test.*Significantly different from control; #significantly different from Ethion treated group ($p<0.05$)

Effect on lipid peroxidation in erythrocyte membranes

Effect of *in vivo* administration of ethion on LPO in erythrocyte membranes is shown in Table 1. Vitamin E treated rats showed LPO levels comparable to the controls at all the time intervals of treatment. Treatment with ethion significantly increased MDA levels in erythrocyte membranes as compared to controls at all the intervals studied. Lipid peroxidation increased significantly with the duration of the treatment of ethion (79.41 % after 7 days, 144.93 % after 14 days, 240.58 % after 21 days and 358.82 % after 28 days). A significant decrease in the LPO was observed when animals were treated with vitamin E along with ethion (10.66 % after 7 days, 14.80 % after 14 days, 14.04 % after 21 days and 17.31 % after 28 days) as

compared to ethion treated rats. Increase in lipid peroxidation in the membrane suggests induction of oxidative stress by ethion.

Effect on membrane lipid composition

Table 2 shows that ethion administration decreased total lipids in erythrocyte membranes at all the time points studied. The change was found to be significant after 7 days treatment (11.39%) and the effect was found higher after 14 days (18.03 %), 21 days (29.01 %) and 28 days (34.49 %) of ethion administration. Administration of vitamin E along with ethion had beneficial effect on day 28 day as compared with the ethion treated group. The findings show that vitamin E alleviates the effect of ethion.

In vivo administration of ethion to rats also resulted in significant decrease in phospholipid content in erythrocyte membranes. The decrease was by 11.78 % after 7 days, 21.94 % after 14 days, 34.89 % after 21 days and 50.31 % after 28 days. Administration of vitamin E along with ethion resulted in ameliorating the effect of ethion on phospholipid content. The effect was not significant at day 7 (6.5 %), but significant after 14 (12.45 %), 21 (21.53 %) and 28 (73.13 %) days of ethion exposure. Co-administration of vitamin E with ethion resulted in restoring the phospholipid content of the erythrocyte membranes as compared with groups treated with ethion alone for 14, 21 and 28 days respectively.

Table 1. Effect of vitamin E on ethion-induced alterations in lipid peroxidation in erythrocyte membranes

	Lipid Peroxidation (nmoles MDA/mg protein)			
	Days			
	7	14	21	28
Control	0.68 ± 0.02	0.69 ± 0.07	0.69 ± 0.05	0.68 ± 0.04
Vitamin E	0.63 ± 0.04	0.60 ± 0.03	0.52 ± 0.03	0.49 ± 0.09
Ethion	1.22 ± 0.06*	1.69 ± 0.03*	2.34 ± 0.09*	3.12 ± 0.41*
Ethion+ Vitamin E	1.09 ± 0.06#	1.44 ± 0.07#	2.02 ± 0.10#	2.58 ± 0.11#

Values are mean ± S.D. of 6 animals/group.
Data was analyzed by one-way ANOVA followed by Bonferroni's post hoc test.
*Significantly different from control; #significantly different from Ethion treated group ($p<0.05$).

Treatment with ethion decreased cholesterol content in erythrocyte membranes as compared to control at all time intervals studied. The effect of ethion administration was more pronounced with increasing duration of ethion exposure; at 14 day (21.2 %), 21 day (35.3 %) and 28 day (47.03 %). The administration of vitamin E along with ethion resulted in improving the cholesterol content. Vitamin E administration increased cholesterol levels by 4.85 % after 7 days, 20.69 % after 14 days, 26.45 % after 21 days and 44.90 % after 28 days. However, the change was significant post 21 and 28 days of exposure.

Effect on Membrane Bound Enzyme Activities

Table 3 shows the effect of ethion administration on the activities of membrane bound enzymes; Na^+, K^+ ATPase and Mg^{2+}ATPase. Vitamin E treated rats had Na^+ K^+ ATPase and Mg^{2+}ATPase activities comparable to their corresponding controls. Na^+, K^+ ATPase and Mg^{2+}ATPase activities were inhibited in ethion treated groups as compared to control groups at all time interval of treatment. Na^+, K^+ ATPase and Mg^{2+}ATPase activities were found to be inhibited by 16.4 and 14.63 % after 7 days, 26.41 and 24.05 % after 14 days, 35.41 and 48.59 % after 21 days and 55.51 and 61.12 % after 28 days treatment as compared to controls. Inhibition of Na^+, K^+ ATPase activity was found to be significant after 7, 14, 21 and 28 days of ethion administration, whereas, the effect on Mg^{2+}ATPase was significant after 14, 21 and 28 days. However, co-administration of vitamin E along with ethion showed mild

Table 2. Effect of vitamin E on ethion-induced alterations in lipid composition of erythrocyte membranes

	Days			
	7	14	21	28
		Total Lipids (mg/mg protein)		
Control	10.18±0.22	10.26±0.28	10.34±0.58	10.41±0.14
Vitamin E	10.35±0.73	10.47±0.54	10.55±0.86	10.61±0.59
Ethion	9.02±0.77*	8.41±0.34*	7.34±0.75*	6.82±0.55*
Ethion+ Vitamin E	9.31±0.68	9.06±0.49#	8.39±0.21#	8.33±0.38#
		Phospholipids (mg/mg protein)		
Control	3.14 ± 0.12	3.19 ± 0.15	3.21 ±0.18	3.22 ± 0.2
Vitamin E	3.3 ± 0.1	3.42 ± 0.09	3.49 ±0.11	3.57 ±0.09
Ethion	2.77 ± 0.06*	2.49 ± 0.11*	2.09 ±0.11*	1.59 ±0.11*
Ethion+ Vitamin E	2.95 ± 0.17#	2.8 ±0.12#	2.54 ±0.14#	2.77 ±0.26#
		Cholesterol (mg/mg protein)		
Control	1.81±0.13	1.84±0.11	1.87±0.17	1.85±0.09
Vitamin E	1.86±0.16	1.89±0.14	1.92±0.14	1.95±0.07
Ethion	1.65±0.13	1.45±0.07*	1.21±0.09*	0.98±0.07*
Ethion+ Vitamin E	1.73±0.07	1.75±0.12#	1.53±0.08#	1.42±0.07#

Values are mean ± S.D. of 6 animals/group.
Data was analyzed by one-way ANOVA followed by Bonferroni's post hoc test.
*Significantly different from control; #significantly different from ethion treated group ($p<0.05$).

Table 3. Effect of vitamin E on ethion-induced alterations on activity of enzymes in erythrocyte membranes

	Days			
	7	14	21	28
	Na^+ K^+ ATPase (nmol Pi hydrolyzed/min/mg protein)			
Control	11.31±0.89	11.55±0.93	11.88±0.66	11.98±0.10
Vitamin E	14.18±0.71	15.34±0.73*	16.27±0.81	18.00±0.99
Ethion	9.45 ±0.46*	8.50±0.44*	7.67 ±.054*	5.33 ± 0.74*
Ethion+ Vitamin E	10.59±0.96#	9.56±0.43#	8.17 ±0.63*#$	6.10 ±0.60*#
	Mg^{2+}ATPase (nmol Pi hydrolyzed/min/mg protein)			
Control	15.58±1.10	15.47±1.61	15.93±1.01	15.69±1.58
Vitamin E	16.82±0.72	17.23±0.77	17.89±1.36	18.35±0.64
Ethion	13.3±0.46	8.50±1.08*	8.19±1.56*	6.10 ± 0.74*
Ethion+ Vitamin E	11.28±0.96#	13.39±2.28#	11.69±1.11#	9.35±1.46#

Values are mean ± S.D. of 6 animals/group.
Data was analyzed by one-way ANOVA followed by Bonferroni's post hoc test.
*Significantly different from control; #significantly different from ethion treated group ($p<0.05$).

recovery in erythrocyte Na^+, K^+ ATPase ranging from 12 to 15 % but had higher recovery in Mg^{2+}ATPase activity after 21 and 28 days of treatment.

Morphological changes in erythrocytes

Scanning electron microscopy of erythrocytes revealed that administration of ethion resulted in prominent morphological changes in rat erythrocytes. It is evident from the electron micrograph that the erythrocytes of the control group were perfect discocytes, that is, typical biconcave disks. Distortions of normal discocytes to different pathological forms were observed following ethion administration (Fig. 2). Most of the erythrocytes were spherocytes. Appearance of irregular margins, central and peripheral protuberances were prominently observed in the ethion exposed animals. Erythrocytes of ethion and vitamin E treated group showed improvement in erythrocyte topography as compared with ethion treated group indicating normalization of the morphology.

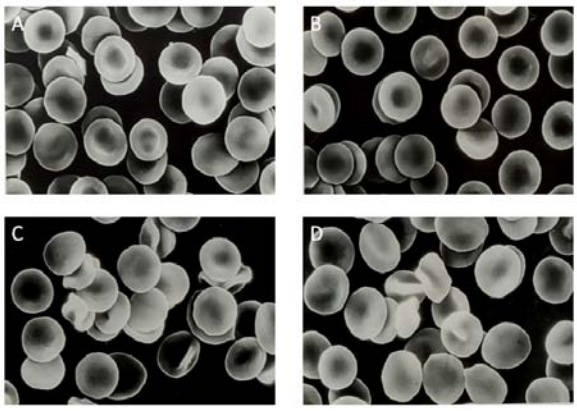

Figure 2. Scanning electron micrographs of erythrocytes from rats at day 28 at 2500x, 10 kV. A, Control; B, Vitamin E-treated; C, Ethion treated; D, Ethion+Vitamin treated.

DISCUSSION

Organophosphate compounds exert their toxic effects mainly by inhibition of AChE activity at the synapse and neuromuscular

junction. In the present study, we observed that *in vivo* administration of ethion inhibited the erythrocyte membrane AChE activity at all the treatment periods studied. Vitamin E administration improved the ethion inhibited AChE activity to a limited extent. Erythrocyte AChE is inhibited by various organophosphates (13). There is no report available in literature regarding the effect of ethion administration on erythrocyte membrane AChE. However, we have earlier reported that ethion inhibited erythrocyte membrane AChE *in vitro* (44). In another study, AChE was found to be inhibited in plasma and red blood cells of goats after intravenous injection of ethion at high dose (34). The decreased activity of AChE in erythrocyte membranes observed in the present study might be due to phosphorylation of serine at the active site of enzyme. Our results are contrary to the findings of John et al. (22) who reported inhibition in erythrocyte AChE activity by organophosphate pesticides was not relieved in vitamin E pretreated rats. However, Yu et al. (54) observed vitamin E ameliorated OP inhibited AChE activity in retina. These results suggest that decrease in AChE activity might be partially contributed by peroxidation of erythrocyte membrane lipids by ethion and protection by vitamin E can be attributed to its antioxidant action.

Oxidative stress in biological systems originates as a result of imbalance between the generation of oxidizing species and cellular antioxidant defenses (25). Various studies have suggested that LPO acts as one of the molecular mechanisms involved in OP induced toxicity (48). MDA is an indicator of LPO, and its level increases in tissues when they are exposed to oxidative stress. The increased MDA levels observed following exposure to ethion suggests accentuation of oxidative stress by ethion. In a recent study by Rastogi et al. (39) increased oxidative stress with depleted antioxidant status was observed in blood samples of agricultural workers exposed to organophosphorus insecticides during spraying. Ethion might accentuate oxidative stress by directly increasing the production of reactive oxygen species or decreasing the antioxidant ability of the cell. The beneficial effect of vitamin E on ethion-induced oxidative stress suggests it potential role in ethion toxicity. Beneficial effect of vitamin E on organophosphate-induced lipid peroxidation in erythrocytes have been reported by us earlier (7, 20) and by other investigators (16, 22). Mechanism of antioxidant action of the vitamin E is due to its ability to scavenge the lipid peroxyl and other free radicals and, hence it is able to prevent peroxidation of membrane lipids. Vitamin E is believed to be membrane stabilizer due to its lipophilic nature (51). In addition, Vitamin E has been shown to exert its antioxidant action by protecting glutathione-dependent enzymes (50).

Peroxidation of polyunsaturated fatty acids in membrane lipids has been suspected to be a major mechanism of oxidant injury leading to membrane dysfunction and subsequently to alterations in cellular functions (21). A loss of membrane lipids following ethion exposure appears to be due to peroxidation of phospholipids along with reduction in cholesterol levels. Our results are in accordance with the previous studies demonstrating alterations in erythrocyte membrane lipid composition as a result of decrease in total lipids, phospholipids and cholesterol after the organophosphate pesticide administration (14). The decrease in phospholipid content might be attributed to loss of phospholipids as a result of lipid peroxidation (53). Administration of vitamin E was found to reverse ethion-induced alterations in lipid composition thereby indicating that oxidative stress is involved in perturbations in lipid composition. Vitamin E has been shown to protect against oxidative damage to lipids particularly PUFA-rich biomembranes (49). Normal erythrocyte function is completely dependent on intact erythrocyte membranes. The erythrocyte membranes serves as a variable barrier to oxygen transport, the changes in its composition can induce cellular hypoxia in the tissue bed. Furthermore, as the size, shape, and diffusion capacity of a red blood cell depends on the structure of its membrane, alterations in membrane structure could lead to a decrease in tissue oxygenation (11).

Lipid peroxidation and perturbed lipid composition are known to disturb structural integrity of the membrane that might in turn affect the activity of membrane bound enzymes (41). Ethion-induced alterations in membrane lipid composition resulted in decreased activities of membrane bound enzymes; Na^+ K^+ ATPase and Mg^{2+} ATPase. The effect was more pronounced with the duration of treatment. OP compounds are known to affect the activities of

membrane bound ATPases (1). OPs have been shown to induce inhibitory effects on the Na^+, K^+-ATPase activity in erythrocytes (45) and brain (6). Further, it has been reported that OPs inhibit Na^+, K^+-ATPase in a noncompetitive manner by excluding the enzyme protein from its normal lipid milieu (9). Zhang et al. (55) have observed decrease in erythrocyte membrane fluidity following organophosphate exposure. A recent study revealed that osmotic fragility as a potential biomarker of oxidative membrane damage in pesticide-induced oxidative membrane damage to erythrocytes (42). Vitamin E had a beneficial effect of activity of membrane bound enzyme further supporting the fact that decrease in activity is due to alterations in membrane lipid milieu.

The alterations in membrane structure and function suggest that ethion exposure might result in morphological changes in erythrocytes, which is critical to its function. Alterations in lipid composition affect the membrane permeability which further leads to the alterations in the cell shape with adverse hematological consequences. SEM studies on erythrocytes revealed that administration of ethion resulted in prominent morphological changes in rat erythrocytes. Distortions of normal discocytes to different pathological forms have been reported. Most of the erythrocytes became spherocytes. Prominent observations such as appearance of irregular margins, central and peripheral protuberances were observed. Administration of vitamin E along with ethion resulted in normalization of the morphology to some extent. The alterations in the shape of normal erythrocytes (discocytes) following ethion exposure which primarily appears to be through oxidative stress mediated alteration in membrane structure. A large number of studies have suggested abnormalities in erythrocyte shape following OP exposure (46). Changes in lipid composition of the membrane are the key reason for such deformities in the shape of erythrocytes in response to various chemical treatments (27). Vitamin E administration reversed ethion-induced alterations in erythrocyte shape. Azzi (4) have suggested that vitamin E is effective as an antioxidant in membrane environment and it also stabilizes the membranes because of interactions with unsaturated fatty acids.

In conclusion, our findings demonstrate that chronic ethion exposure induces oxidative damage to erythrocyte membranes resulting in alterations in membrane structure (lipid composition) and function (membrane bound enzymes; Na+ K+ ATPase and Mg2+ ATPase activities) ultimately affecting erythrocyte shape. These results suggest that alterations in membrane structure and function contribute to ethion toxicity. Further, Vitamin E supplementation might be effective in protecting erythrocytes and other cells against the oxidative damage induced by ethion suggesting its beneficial role in ethion poisoning.

Acknowledgements – The authors thank the University Grants Commission, New Delhi for Rajiv Gandhi National Fellowship to GKB.

Other articles in this theme issue include references (57-72).

REFERENCES

1. Aggarwal, M., Naraharisetti, S.B., Sarkar, S.N., Rao, G.S., Degen, G.H. and Malik, J.K., Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: a biochemical study. Arch. Environ. Contam. Toxicol. 2009, 56: 139-148.
2. Atarashi, K., Ishiyama, A., Takagi, M., Minami, M., Kimura, K., Goto, A., Omata, M., Vitamin E ameliorates the renal injury of Dahl salt-sensitive rats. Am. J. Hyperten. 1997, 10: 116S-119S
3. Atherton, K.M., Williams, F.M., Egea González, F.J., Glass, R., Rushton, S., Blain, P.G. and Mutch, E., DNA damage in horticultural farmers: a pilot study showing an association with organophosphate pesticide exposure. Biomarkers 2009, 14: 443-451.
4. Azzi, A., Molecular mechanism of alpha-tocopherol action. Free Radic. Biol. Med. 2007, 43:16-21.
5. Bartlett, G.R., Phospholipid assay in column chromatography. J. Biol. Chem. 1959, 234: 466-468.
6. Basha, P.M. and Nayeemunnisa., Effect of methyl parathion on Na^+/K^+ and Mg^{2+}-adenosine triphosphatase activity in developing central nervous system in rats. Indian J Exp. Biol. 1993, 31: 785-789.
7. Bhatti, G.K., Kiran, R. and Sandhir, R., Modulation of ethion-induced hepatotoxicity and oxidative stress by vitamin E supplementation in male wistar rats. Pestic. Biochem. Physiol. 2010, 98: 26-32
8. Bhunya, S.P. and Jena, G.B., Evaluation of genotoxicity of a technical grade organophosphate insecticide, Tafethion (ethion), in chicks. In Vivo. 1994, 8: 1087-109.
9. Blasiak, J., Allosteric inhibition of (Na^+/K^+)-ATPase by parathion and methyl parathion. Pestic. Biochem. Physiol. 1996, 54: 40-47.
10. Blostein, R., Relationships between erythrocyte membrane phosphorylation and adenosine triphosphate hydrolysis. J Biol. Chem. 1968, 243: 1957-1965.
11. Buchwald, H., Menchaca, H. J., Michalek, V. N., Rohde, T. D., Hunninghake, D. B. and O'Dea, T. J., Plasma cholesterol: an influencing factor in red blood cell oxygen release and cellular oxygen availability. J. Am. Coll. Surg. 2000, 191: 490–497.

12. Chow, C.K., Oxidative damage in the red cells of vitamin E-deficient rats. Free Radic. Res. Commun. 1992, 16, 247-258.
13. Costa, L.G., Current issues in organophosphate toxicology. Clin. Chim. Acta. 2006, 366: 1-13.
14. Datta, C., Gupta, J. and Sengupta, D., Interaction of organophosphorus insecticides phosphamidon & malathion on lipid profile and acetylcholinesterase activity in human erythrocyte membrane. Indian J. Med. Res. 1994, 100: 87-89.
15. Dewan, A., Patel, A.B., Pal, R.R., Jani, U.J., Singel, V.C. and Panchal, M.D., Mass ethion poisoning with high mortality. Clin. Toxicol .(Phila) 2008, 46: 85-88.
16. Durak, D., Uzun, F.G., Kalender, S., Ogutcu, A., Uzunhisarcikli, M. and Kalender, Y., Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E *in vitro*. Environ. Toxicol. 2009, 24: 235-242.
17. Ellman, G.L., Courtney, K.D., Andres, V., Jr. and Feather-Stone, R.M., A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol.1961, 7: 88-95.
18. Foster, L.J.R., Kwan, B.H. and Vancov, T., Microbial degradation of the organophosphate pesticide, Ethion. FEMS Microbiol. Lett. 2004, 240: 49–53.
19. Fringes, C.S. and Dunn, R.T., A colorimetric method of determination of total serum lipids based on the sulpho-phosphovanillin reaction. Am. J. Clin. Pathol. 1970, 53: 89-91.
20. Garg, D.P., Kiran, R., Bansal, A.K., Malhotra, A. and Dhawan, D.K., Role of vitamin E in mitigating methomyl induced acute toxicity in blood of male wistar rats. Drug Chem. Toxicol. 2008, 31: 487-499.
21. Halliwell, B. and Gutteridge, J.M., Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. Arch. Biochem. Biophys. 1986, 246: 501-514.
22. John, S., Kale, M., Rathore, N. and Bhatnagar, D., Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem. 2001, 12: 500-504.
23. Kalam, A. and Mukerjee A.K., Influences of hexacohazole, carbofuran and ethion on soil microflora and dehydrogenase activities in soil and intact cell. Indian J. Exp. Biol. 2001, 39: 90-94.
24. Kalender, S., Ogutcu, A., Uzunhisarcikli, M., Açikgoz, F., Durak, D., Ulusoy, Y. and Kalender, Y., Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicol. 2005, 21: 197-206.
25. Kappus, H., Oxidative stress in chemical toxicity. Arch. Toxicol. 1987, 60: 144-149.
26. Kisby, G.E., Muniz, J.F., Scherer, J., Lasarev, M.R., Koshy, M., Kow, Y.W. and McCauley, L., Oxidative stress and DNA damage in agricultural workers. J Agromed. 2009, 14: 206-214.
27. Kumar, V.V., Lipid molecular shapes and membrane architecture. Indian J. Biochem. Biophys. 1993, 30: 135-138.
28. Lohr, G.W. and Waller, H.D., Glucose-6-Phosphate Dehydrogenase, In: Methods in Enzymatic Analysis Bergmeyer, H.U. (Ed.), Academic Press, New York, 1974, pp. 636-643.
29. López, O., Hernández, A.F., Rodrigo, L., Gil, F., Pena, G., Serrano, J.L., Parrón, T., Villanueva, E. and Pla, A., Changes in antioxidant enzymes in humans with long-term exposure to pesticides. Toxicol. Lett. 2007, 171: 146-153.
30. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with Folin phenol reagent. J. Biol. Chem. 1951, 193: 265–275.
31. Mansour, S.A., Mossa, A.T. and Heikal, T.M., Effects of methomyl on lipid peroxidation and antioxidant enzymes in rat erythrocytes: in vitro studies. Toxicol. Ind. Health 2009, 25: 557-563.
32. Marinetti, G.V., Chromatographic separation, identification and analysis of phospholipids. J. Lipid Res.1962, 3: 1-20.
33. Melin, A.M., Corbonneau, M.A., Thomas, M.J., Perromat, A. and Clerc, M., Dietary vitamins A and E affect differently, lipid peroxidation in rat heart and testis. J. Clin. Biochem. Nutr. 1995, 18: 19–34.
34. Mosha, R.D. and Gyrd-Hansen, N., Toxicity of ethion in goats. Vet. Hum. Toxicol. 1990, 32: 6-8.
35. Nath, B.S. and Kumar, R.P.S., Toxic Impact of Organophosphorus Insecticides on Acetylcholinesterase Activity in the Silkworm,Bombyx moriL. Ecotoxicol. Environ. Saf. 1999, 42: 157-162.
36. Niki, E., Yamamoto, Y., Komuro, E. and Sato, K., Membrane damage due to lipid oxidation. Am. J. Clin. Nutr. 1991, 53: 20lS-205S.
37. Nikolaidis, M.G., Jamurtas, A.Z., Blood as a reactive species generator and redox status regulator during exercise, Arch. Biochem. Biophys. 2009, 490: 77-84.
38. Quigley, J.P., Gottere, G.S., Distribution of Na^+K^+-stimulated ATPase activity in rat intestinal mucosa. Biochem. Biophys. Acta 1969, 173: 456-468.
39. Rastogi, S.K., Satyanarayan, P.V., Ravishankar, D. and Tripathi, S., A study on oxidative stress and antioxidant status of agricultural workers exposed to organophosphorus insecticides during spraying. Indian J. Occup. Environ. Med. 2009, 13: 131-134.
40. Rendón-Ramirez, A., Cerbón-Solórzano, J., Maldonado-Vega, M., Quintanar-Escorza, M.A. and Calderón-Salinas, J.V., Vitamin-E reduces the oxidative damage on delta-aminolevulinic dehydratase induced by lead intoxication in rat erythrocytes. Toxicol. In Vitro. 2007, 21: 1121-1126.
41. Sandhir, R., Julka, D. and Gill, K.D., Lipoperoxidative damage on lead exposure in rat brain and its implications on membrane bound enzymes. Pharmacol. Toxicol. 1994, 74: 66-71.
42. Sharma, B., Rai, D.K., Rai, P.K., Rizvi, S.I. and Watal, G., Determination of erythrocyte fragility as a marker of pesticide-induced membrane oxidative damage. Methods Mol. Biol. 2010, 594: 23-28.
43. Shiva Shankar Reddy, C.S., Subramanyam, M.V., Vani, R. and Asha Devi, S., In vitro models of oxidative stress in rat erythrocytes: effect of antioxidant supplements. Toxicol. In Vitro. 2007, 21: 1355-1364.
44. Singh, M., Sandhir, R. and Kiran, R., Erythrocyte antioxidant enzymes in toxicological evaluation of commonly used organophosphate pesticides. Indian J. Exp. Biol. 2006, 44: 580-583.
45. Singh, M., Sandhir, R. and Kiran, R., In vitro effects of organophosphate pesticides on rat erythrocytes. Indian J. Exp. Biol. 2004, 42: 292-296.
46. Singh, N.N. and Srivastava, A.K., Haematological parameters as bioindicators of insecticide exposure in teleosts. Ecotoxicol. 2010, 19: 838-854.
47. Smith, G.J., Acute oral rat LD50 for organophosphorus and carbamate pesticides. In: Toxicology and Pesticide Use in Relation to Wildlife, Organophosphorus and

Carbamate Compounds. CRC Press, New York, 1992, pp161
48. Soltaninejad, K. and Abdollahi, M., Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. Med. Sci. Monit. 2009, 15: 75-90.
49. Terrasa, A.M., Guajardo, M.H., Marra, C.A. and Zapata, G., Alpha-tocopherol protects against oxidative damage to lipids of the rod outer segments of the equine retina. Vet. J. 2009, 182: 463-468.
50. van Haaften, R.I., Haenen, G.R., Evelo, C.T. and Bast. A., Effect of vitamin E on glutathione-dependent enzymes. Drug Metab. Rev. 2003, 35: 215-253.
51. Wang, F., Wang, T., Lai, J., Li, M. and Zou, C.G., Vitamin E inhibits hemolysis induced by hemin as a membrane stabilizer. Biochem. Pharmacol. 2006, 71: 799-805.
52. Wills, E.D., Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 1966, 99: 667–676.
53. Wiswedel, I., Gardemann, A., Storch, A., Peter, D. and Schild, L., Degradation of phospholipids by oxidative stress--exceptional significance of cardiolipin. Free Radic. Res. 2010, 44: 135-145.
54. Yu, F., Wang, Z., Ju, B., Wang, Y., Wang, J. and Bai, D., Apoptotic effect of organophosphorus insecticide chlorpyrifos on mouse retina in vivo via oxidative stress and protection of combination of vitamins C and E. Exp. Toxicol. Pathol. 2008, 59: 415-423.
55. Zhang, X., Yao, W., Jia, B., Sun, D., Ka W., He D., Wang, X. and Wen, Z., Acute dichlorvos poisoning induces hemorheological abnormalities in rabbits via oxidative stress. Clin. Hemorheol. Microcirc. 2010, 44: 207-216.
56. Zlatkis, A., Zak, B. and Boyle, A.J., A new method for direct determination of serum cholesterol. J Lab. Clin. Med. 1953, 41: 468-490.
57. Tripathi S., Mahdi A. A., Hasan M., Mitra K. and Mahdi F., Protective potential of *Bacopa monniera (Brahmi)* extract on aluminum induced cerebellar toxicity and associated neuromuscular status in aged rats. *Cell. Mol. Biol.* 2011, **57 (1)**: 3-15.
58. Mishra A., Kumar S., Bhargava A., Sharma B. and Pandey A. K., Studies on *in vitro* antioxidant and antistaphylococcal activities of some important medicinal plants. *Cell. Mol. Biol.* 2011, **57 (1)**: 16-25.
59. Kumar A., Bhatnagar A., Gupta S., Khare S. and Suman, *sof* gene as a specific genetic marker for detection of *Streptococcus pyogenes* causing pharyngitis and rheumatic heart disease. *Cell. Mol. Biol.* 2011, **57 (1)**: 26-30.
60. Kumar Rai P., Kumar Rai D., Mehta S., Gupta R., Sharma B. and Watal G., Effect of *Trichosanthes dioica* on oxidative stress and CYP450 gene expression levels in experimentally induced diabetic rats. *Cell. Mol. Biol.* 2011, **57 (1)**: 31-39.
61. Kirby K.A., Singh K., Michailidis E., Marchand B., Kodama E.N., Ashida N., Mitsuya H., Parniak M.A., and Sarafianos S.G., The sugar ring conformation of 4'-ethynyl-2-fluoro-2'-deoxyadenosine and its recognition by the polymerase active site of hiv reverse transcriptase. *Cell. Mol. Biol.* 2011, **57 (1)**: 40-46.
62. Singh M.P., Pandey V.K., Srivastava A.K. and Viswakarma S.K., Biodegradation of Brassica haulms by white rot fungus *Pleurotus eryngii*. *Cell. Mol. Biol.* 2011, **57 (1)**: 47-55.
63. Baig M. S., Gangwar S. and Goyal N., Biochemical characterization of dipeptidylcarboxypeptidase of *Leishmania donovani*. *Cell. Mol. Biol.* 2011, **57 (1)**: 56-61.
64. Tripathi R., Gupta S., Rai S. and Mittal P. C., Effect of topical application of methylsulfonylmethane (msm), edta on pitting edema and oxidative stress in a double blind, placebo-controlled study. *Cell. Mol. Biol.* 2011, **57 (1)**: 62-69.
65. Chakravarty S. and Rizvi S. I., circadian modulation of Sodium-Potassium ATPase and Sodium - proton exchanger in human erythrocytes: *in vitro* effect of melatonin. *Cell. Mol. Biol.* 2011, **57 (1)**: 80-86.
66. Siddiqi N. J., Protective effect of Magnesium Chloride ON Sodium Fluoride induced alterations in various hydroxyproline fractions in rat lungs. *Cell. Mol. Biol.* 2011, **57 (1)**: 87-92.
67. Siddiqi N. J., Al-Omireeni E. A. and Alhomida A. S., Effect of different doses of Sodium Fluoride on various hydroxyproline fractions in rat serum. *Cell. Mol. Biol.* 2011, **57 (1)**: 93-99.
68. Rohilla M. S., Reddy P. V. J., Sharma S. and Tiwari P. K., *In vitro* induction of the ubiquitous 60 and 70Kd heat shock proteins by pesticides monocrotophos and endosulphan in *Musca domestica*: potential biomarkers of toxicity. *Cell. Mol. Biol.* 2011, **57 (1)**: 100-111.
69. Janardhan Reddy P. V. and Tiwari P. K., Genomic structure and sequence analysis of *Lucilia cuprina* HSP90 gene. *Cell. Mol. Biol.* 2011, **57 (1)**: 112-121.
70. Mishra N. and Tewari R. R., Cytotoxic and genotoxic effects of mercury in house fly *Musca domestica* (Diptera: Muscidae). *Cell. Mol. Biol.* 2011, **57 (1)**: 122-128.
71. Tripathi M., Agrawal U. R. and Tewari R.R., Seasonal genetic variation in house fly populations, *Musca domestica* (Diptera: Muscidae). *Cell. Mol. Biol.* 2011, **57 (1)**: 129-134.
72. Rai D. K., Sharma R. K., Rai P. K., Watal G. and Sharma B., Role of aqueous extract of *Cynodon dactylon* in prevention of carbofuran- induced oxidative stress and acetylcholinesterase inhibition in rat brain. *Cell. Mol. Biol.* 2011, **57 (1)**: 135-142.

