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Vitamin E prevents buthionine sulfoximine-induced biochemical disorders in the rat

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Abstract

Antioxidant therapy can improve the protection and metabolic activity of cells and tissues. In this study, the effect of vitamin E administration on buthionine sulfoximine (BSO)-induced glutathione (GSH) depletion in the rat lung and liver was investigated. Hepatic GSH was depleted by intraperitoneal administration of BSO (4 mmol kg^{-1}), twice a day, for 30 days to rats. We also investigated whether the lung and liver mitochondrial GSH contents were influenced by BSO administration and whether an extracellular supply of vitamin E could prevent the changes caused by BSO-mediated GSH depletion. Glutathione levels in lung and liver tissues were depleted by 47% and 60%, respectively. Depletion of hepatic and pulmonary GSH in turn causes decline in the levels of mitochondrial GSH, leading to impaired antioxidant defence function of mitochondria. Both the cytosolic and mitochondrial glutathione disulfides (GSSG) were altered during BSO treatment, and led to drastic increase in GSSG/GSH redox status. One of the experimental groups was given vitamin E (65 mg (kg diet)$^{-1}$) mixed with rat feed. The rats fed with vitamin E were found to have partially restored GSH levels in liver and lung, diminished levels of TBARS and minimized tissue damage. The current findings suggest that the impaired glutathione and glutathione-dependent enzyme status may be correlated with the elevated lipid peroxidation and mitochondrial membrane damage and that vitamin E therapy to the BSO-administered rats prevents the above changes. However, vitamin E did not have any effect on the activity of γ-glutamyl cysteine synthetase (γ-GCS).

Introduction

In recent years there has been a growing interest in establishing chemotherapy and radiotherapy against cancer in man (Kung et al 1990; Wattenberg 1993; Madesh et al 1998). However, neither type of therapy has been very successful in controlling cell proliferation due to the resistance developed by tumour cells to anti-tumour agents and radiation (Schneider et al 1995). It has been clearly demonstrated that an increased level of the non-protein thiol, reduced glutathione, is found in many resistant tumour cells (Schneider et al 1995; Wu & Kang 1998). Later studies on the effects of several anti-tumour agents on resistant and sensitive tumour cells were related to the cellular GSH levels (Mistry & Harrap 1991; Fracasso 2001). Thus, modulation of GSH concentration would be of great help in making tumour cells sensitive to chemotherapy and radiotherapy.

It has also been reported that GSH depletion decreases the rate of cell proliferation and inhibits cancer growth (Terradez et al 1993). Though studies by Hedley et al (1998) revealed the usefulness of buthionine sulfoximine (BSO) as a drug-resistance-reversing agent, toxic side effects in patients were encountered with BSO concentrations as low as 1 mmol. BSO is a potent and specific inhibitor of γ-glutamyl cysteine synthetase (γ-GCS) and it has been used in a number of biochemical and pharmacological studies as a specific agent for inhibiting GSH biosynthesis, thereby causing GSH depletion (Meister 1991; Wernerman & Hammarquist 1999; Rajasekaran et al 2002). However, the potential usefulness of BSO in chemotherapy and radiotherapy against transformed cells has also resulted in decreasing the GSH content in normal cells (Vanhoefer et al 1996). Further, depletion of antioxidants, decreased activity of

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antioxidant enzymes and lipid peroxidation have been noted in BSO-administered mice and rats (Meister 1991; Thanislass et al 1995; Rajasekaran et al 2002). Moreover, administration of BSO depletes GSH from tissues other than tumours. Lung tissue is found to be very sensitive to such GSH depletion, because it is exposed to the highest partial pressure of oxygen and air pollutants (Smith & Anderson 1992; Thanislass et al 1996).

Alpha-tocopherol, which is one of the most efficient antioxidants and a homologue of vitamin E in-vivo, plays a unique role in cellular defence against reactive oxygen species and other reactive intermediates (Seies et al 1992; Wang et al 2002; Petryna 2002; Gutierrez et al 2003; Leonetti et al 2003). This study was undertaken to understand the role of α-tocopherol in protecting cells from BSO-induced GSH depletion in the lung and liver of rats. Further, the study is focussed on the antioxidant effect of α-tocopherol on BSO-induced mitochondrial damage. This study will be of particular interest and importance in forming successful protocols for clinical trials with BSO administration to sensitize the resistant tumour cells towards chemotherapy and radiotherapy without any deleterious effects on normal cells caused by BSO-induced GSH depletion.

Materials and Methods

Male albino rats, 90–100 g, were purchased from FIPPAT (Chennai). Rats were divided into three groups. Group I served as control and received saline intraperitoneally, for 30 days; group II were administered BSO (4.0 mmol kg^{-1}, i.p.) (Meister, 1991) twice a day for 30 days; group III received BSO + α-tocopherol (65 mg (kg diet)$^{-1}$) (Maellaro et al 1990). All rats had free access to normal rat feed and water. The study was ethically approved by Ministry of Social Justices and Empowerment, Government of India.

At the end of the experimental period, the rats were killed by decapitation. Lung and liver tissues were excised immediately and kept in ice-cold saline. The tissues were homogenized in 0.01 M Tris HCl buffer, pH 7.2, to give a 10% homogenate. Tissue (1 g) was taken for mitochondrial isolation (Johnson & Lardy 1967). Tissue GSH was estimated by the method of Moron et al (1979) and lipid peroxidation by the method of Draper & Hadley (1990). The oxidized glutathione (GSSG) content was determined by the method of Tietze (1969) modified by Griffith (1980). Antioxidants (ascorbic acid (Omaye et al 1971), vitamin E (Desai 1984)) and antioxidant enzymes (superoxide dismutase (SOD; Misra & Fridovich 1972), catalase (Beers & Seizer 1952), glutathione peroxidase (GPX; Rotruck et al 1973), glutathione reductase (Staal et al 1969)) were assayed. The activity of γ-GCS (Mooz & Meister 1969) and glucose-6-phosphate dehydrogenase (G-6-PDH) (Zinkham 1978) was also determined. Protein and non-protein thiols, and total thiols, were measured by the method of Sedlack & Lindsay (1968). Lipids were extracted from mitochondrial fractions by the method of Folch et al (1957). Spectrophotometric estimation of cholesterol (Parekh & Jung 1970) and phospholipids (Rouser et al 1970) was also performed.

Statistical analysis

Data are expressed as mean ± s.d. for 6 individuals in each group. Analysis of variance followed by the Student's Newman–Keul multiple comparison test was used to determine whether there were significant differences among the groups. P values less than 0.05 were considered significant (Zar 1990).

Results

Figure 1 indicates the changes in body weights of control, BSO and BSO + vitamin E treated rats. There were no statistically significant ($P > 0.05$) changes observed during the course of BSO administration or vitamin E supplementation.

Tables 1 and 2 show the activity of γ-GCS and antioxidant enzymes and the levels of antioxidants and TBARS from lung and liver tissues, respectively. Administration of BSO at a dose of 4 mmol kg^{-1} twice a day, for a period of 30 days, significantly inhibited ($P < 0.001$) the activity of γ-GCS in both the tissues.

Inhibition of the activity of γ-GCS led to a significant ($P < 0.001$) decrease in glutathione levels, when compared with control. GSH levels in lung (Table 1) and liver (Table 2) were depleted by 47% and 60%, respectively. Altered levels of oxidized glutathione (GSSG) and drastic increase in GSSG/GSH status were also recorded in the lung and liver tissue of BSO-administered rats. The oxidation–reduction status of glutathione was found to be normalized upon vitamin E therapy (Tables 1 and 2).

The levels of total thiols, protein thiols and non-protein thiols were significantly ($P < 0.05$) decreased in BSO-induced GSH-depleted rat lung (Table 1) and liver (Table 2) when compared with control. Upon vitamin E therapy to BSO-treated rats, the levels of these parameters were normalized.

BSO treatment diminished the levels of other antioxidants, ascorbic acid ($P < 0.01$) and α-tocopherol ($P < 0.05$), in lung and liver (Table 1 and 2, respectively). The activity of antioxidant enzymes SOD ($P < 0.01$), catalase

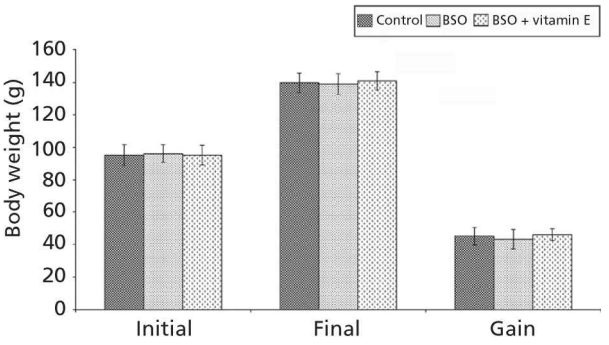

Figure 1 Changes in body weight of control, BSO-treated, and BSO + vitamin E-treated rats. Values are expressed as means ± s.d. for 6 rats in each group and the variations are not statistically significant ($P > 0.05$).

Table 1 Effect of vitamin E supplementation on pulmonary γ-GCS, antioxidants, thiol status and lipid peroxidation in BSO-treated rats.

Parameter	Control	BSO	BSO + Vitamin E
γ-GCS (μg phosphate lib/100 mg protein)	1225.50 ± 60.99^{a}	703.89 ± 42.42^{b}	765.2 ± 31.98^{b}
Reduced glutathione (μg (mg protein)$^{-1}$)	7.07 ± 0.99^{a}	3.72 ± 0.64^{b}	5.21 ± 0.91^{a}
Oxidised glutathione (GSSG, μg (mg protein)$^{-1}$)	0.72 ± 0.03^{b}	1.12 ± 0.08^{a}	0.62 ± 0.07^{b}
GSSG/GSH ratio	0.10 ± 0.01^{b}	0.30 ± 0.04^{a}	0.12 ± 0.02^{b}
Total thiols (μmol (mg protein)$^{-1}$)	30.12 ± 2.31^{a}	23.36 ± 2.01^{b}	29.74 ± 1.51^{a}
Protein thiols (μmol (mg protein)$^{-1}$)	15.01 ± 1.23^{a}	11.03 ± 1.01^{b}	14.26 ± 1.21^{a}
Non-protein thiols (μmol (mg protein)$^{-1}$)	14.86 ± 0.86^{a}	11.64 ± 1.08^{b}	15.59 ± 2.10^{a}
Ascorbic acid (μg (mg protein)$^{-1}$)	3.18 ± 0.38^{a}	2.93 ± 0.39^{b}	3.12 ± 0.75^{a}
α-Tocopherol (μg (mg protein)$^{-1}$)	3.57 ± 0.21^{b}	2.13 ± 1.20^{c}	6.21 ± 0.40^{a}
Superoxide dismutase (U (mg protein)$^{-1}$)	4.33 ± 0.33^{a}	2.48 ± 0.13^{b}	4.17 ± 0.14^{a}
Catalase (μmol H_2O_2 con/min/mg protein)	49.83 ± 7.33^{b}	24.01 ± 3.43^{b}	49.13 ± 2.71^{a}
Glutathione peroxidase (μg GSH con/min/mg protein)	44.85 ± 4.26^{a}	30.11 ± 11.05^{b}	40.36 ± 3.69^{a}
Glutathione reductase (μg $NADP^{+}$ formed/min/mg protein)	88.01 ± 22.8^{b}	148.1 ± 6.35^{a}	128.2 ± 28.07^{ab}
G-6-PDH (U min^{-1} (mg protein)$^{-1}$)	3.49 ± 0.08^{a}	2.40 ± 0.18^{b}	3.12 ± 0.41^{a}
TBARS (nmol MDA/100 mg protein)	143.01 ± 13.20^{b}	498.08 ± 13.53^{a}	154.60 ± 8.31^{b}

Values are means ± s.d. for 6 rats in each group. Within a line, values without a common letter are significantly different from the control group at $P < 0.05$ as determined by analysis of variance.

Table 2 Effect of vitamin E supplementation on hepatic γ-GCS, antioxidants, thiol status and lipid peroxidation in BSO-treated rats.

Parameter	Control	BSO	BSO + Vitamin E
γ-GCS (μg phosphate lib/100 mg protein)	3498.05 ± 112.06^{a}	2138.68 ± 32.38^{b}	2303.31 ± 201.62^{b}
Reduced glutathione (μg (mg protein)$^{-1}$)	9.92 ± 0.84^{a}	3.92 ± 0.28^{b}	5.72 ± 0.58^{a}
Oxidised glutathione (GSSG, μg (mg protein)$^{-1}$)	1.09 ± 0.10^{a}	0.78 ± 0.08^{b}	0.75 ± 0.06^{b}
GSSG/GSH ratio	0.11 ± 0.01^{b}	0.20 ± 0.03^{a}	0.13 ± 0.02^{b}
Total thiols (μmol (mg protein)$^{-1}$)	38.12 ± 3.21^{a}	28.36 ± 2.14^{b}	37.43 ± 2.86^{a}
Protein thiols (μmol (mg protein)$^{-1}$)	21.62 ± 1.83^{a}	15.41 ± 1.23^{b}	21.01 ± 1.74^{a}
Non-protein thiols (μmol (mg protein)$^{-1}$)	17.01 ± 1.42^{a}	13.41 ± 1.23^{b}	16.08 ± 1.49^{a}
Ascorbic acid (μg (mg protein)$^{-1}$)	2.97 ± 0.02^{a}	1.79 ± 0.28^{b}	2.83 ± 0.13^{a}
α-Tocopherol (μg (mg protein)$^{-1}$)	3.54 ± 0.29^{b}	1.77 ± 0.14^{c}	5.31 ± 0.21^{a}
Superoxide dismutase (U (mg protein)$^{-1}$)	1.48 ± 0.07^{a}	0.87 ± 0.07^{c}	0.91 ± 0.09^{b}
Catalase (μmol H_2O_2 con/min/mg protein)	45.61 ± 3.31^{a}	24.60 ± 2.72^{b}	44.80 ± 1.63^{a}
Glutathione peroxidase (μg GSH con/min/mg protein)	61.53 ± 2.33^{a}	28.63 ± 1.51^{b}	54.65 ± 2.98^{a}
Glutathione reductase (μg $NADP^{+}$ formed/min/mg protein)	177.57 ± 3.26^{c}	270.20 ± 2.37^{a}	205.46 ± 6.63^{b}
G-6-PDH (U min^{-1} (mg protein)$^{-1}$)	3.78 ± 0.83^{a}	1.68 ± 0.12^{b}	3.63 ± 0.73^{a}
TBARS (nmol MDA/100 mg protein)	120.85 ± 6.78^{c}	473.65 ± 7.45^{a}	145.32 ± 4.90^{b}

Values are means ± s.d. for 6 rats in each group. Within a line, values without a common letter are significantly different from the control group at $P < 0.05$ as determined by analysis of variance.

($P < 0.001$ in lung, $P < 0.05$ in liver) and GPX ($P < 0.01$) in both the tissues was inhibited significantly in BSO-treated rats, whereas the activity of glutathione reductase was increased ($P < 0.01$). The activity of all these enzymes was normalized in the vitamin-E-supplemented group.

The activity of G-6-PDH was decreased in both lung and liver tissue during BSO treatment and was partially normalized upon vitamin E supplementation to BSO-treated rats (Tables 1 and 2).

BSO treatment resulted in a 248% and 294% increase in the concentration of malondialdehyde (MDA) of lung and liver, respectively, when compared with controls. Vitamin E therapy in the BSO-treated rats reduced the MDA concentration to near normal level (Tables 1 and 2).

Table 3 depicts the antioxidant and lipid peroxidation profiles of lung and liver mitochondria of control, BSO-treated and vitamin-E-supplemented BSO-treated rats. A significant decrease in the GSH content of lung and liver mitochondria was observed in BSO-treated rats. Decreased activity of SOD ($P < 0.05$) and GPX ($P < 0.05$) or ($P < 0.001$) in lung and liver mitochondria was observed. The levels of TBARS were elevated ($P < 0.001$) in the lung

and liver mitochondria. The levels of these parameters were found to be normalized by vitamin E therapy to the BSO-treated rats (Table 3).

The cholesterol and phospholipid content of mitochondrial membranes isolated from rat lung and liver are presented in Table 4. A statistically significant increase ($P < 0.05$) in the cholesterol content of mitochondria from both tissues was observed, whereas the change in phospholipid was not statistically significant (Table 4). The cholesterol/phospholipid ratio was found to be altered significantly as a result of increased cholesterol concentration. Vitamin E supplementation aided in bringing back the concentration of all these parameters to near control levels.

Figures 2 and 3 show the histology of the lung and liver, respectively. Normal architecture of the bronchiole, with alveolar space, can be seen in the lung section of control rats (Figure 2A), whereas the lung section of BSO-treated rats (Figure 2B) shows bronchi with congestion, haemorrhage of alveolar space and dense mononuclear infiltration. Vitamin E supplementation prevented the damage caused during BSO administration (Figure 2C).

Normal architecture of the liver with central vein and cords of hepatocytes is observed in control rats (Figure 3A), whereas the liver section of BSO-administered rats (Figure 2B) shows hepatocytes with dysplastic changes like nuclear condensation, nuclear cytoplasmic alteration and crowding of hepatocytes. Supplementation with vitamin E resulted in minimal damage with occasional nuclear condensation and, rarely, diffused lymphocytic infiltration (Figure 3C).

Discussion

Vitamin E compounds, tocopherol and tocotrienols, are well recognized for their effective inhibition of lipid oxidation in foods and biological systems (Parker 1991). α-Tocopherol, the biologically most active form of vitamin E, has been the lipid-soluble antioxidant of major interest; its plasma and tissue levels can be modulated by diet and supplementation (Chow 1991; Seies et al 1992; van Dam et al 1999).

Results presented in this study clearly demonstrate that BSO inhibited the activity of γ-GCS significantly, which further led to extensive depletion of glutathione in lung and liver tissues (Tables 1 and 2). The extent of lipid peroxidation was evident from the observed increase in TBARS level from both the tissues of BSO-administered rats. These findings can be correlated with our previous reports (Thanislass et al 1995; Rajasekaran et al 2002).

While BSO inhibited the activity of γ-GCS, the level of GSH was partially restored in γ-tocopherol-supplemented

Table 3 Effect of vitamin E supplementation on lung and liver mitochondrial antioxidants and lipid peroxidation in BSO-treated rats.

Parameter	Control		BSO		BSO + Vitamin E	
	Lung	Liver	Lung	Liver	Lung	Liver
Reduced glutathione (μg (mg protein)$^{-1}$)	4.79 ± 0.52^{a}	5.93 ± 0.21^{a}	1.80 ± 0.55^{b}	3.13 ± 0.21^{b}	2.63 ± 0.48^{a}	4.07 ± 0.42^{a}
Oxidised glutathione (GSSG, μg (mg protein)$^{-1}$)	0.57 ± 0.04^{a}	0.78 ± 0.11^{a}	0.43 ± 0.02^{b}	0.72 ± 0.08^{a}	0.28 ± 0.03^{c}	0.49 ± 0.06^{b}
GSSG/GSH ratio	0.12 ± 0.02^{b}	0.13 ± 0.02^{b}	0.24 ± 0.03^{a}	0.23 ± 0.03^{a}	0.11 ± 0.01^{b}	0.12 ± 0.02^{b}
SOD (U (mg protein)$^{-1}$)	2.13 ± 0.11^{a}	1.49 ± 0.05^{a}	1.49 ± 0.06^{b}	1.02 ± 0.03^{b}	2.01 ± 0.08^{ab}	1.34 ± 0.08^{a}
GPX (μg GSH con/min/mg protein)	21.48 ± 1.37^{a}	26.38 ± 0.60^{a}	14.52 ± 0.54^{b}	13.48 ± 0.49^{b}	20.81 ± 1.02^{a}	20.53 ± 0.45^{a}
TBARS (nmol MDA/100 mg protein)	77.12 ± 2.21^{b}	75.45 ± 2.04^{b}	115.12 ± 3.20^{a}	123.62 ± 2.21^{a}	81.21 ± 3.82^{b}	86.72 ± 2.71^{b}

Values are means ± s.d. for 6 rats in each group. Within a line, values without a common letter are significantly different from the control group at $P < 0.05$ as determined by analysis of variance.

Table 4 Effect of vitamin E on mitochondrial membrane lipid profile of lung and liver of BSO-treated rats.

Parameter (mg/100 mg mitochondrial protein)	Control		BSO		BSO + Vitamin E	
	Lung	Liver	Lung	Liver	Lung	Liver
Cholesterol	2.26 ± 0.22^{b}	2.18 ± 0.17^{b}	3.36 ± 0.48^{a}	3.64 ± 0.41^{a}	2.38 ± 0.21^{b}	2.83 ± 0.28^{ab}
Phospholipids	31.72 ± 2.08^{a}	28.12 ± 2.31^{a}	29.87 ± 5.13^{a}	23.31 ± 2.12^{a}	30.63 ± 2.12^{a}	27.23 ± 1.78^{a}
Cholesterol/phospholipid	0.07 ± 0.02^{b}	0.10 ± 0.01^{b}	0.11 ± 0.01^{a}	0.16 ± 0.02^{a}	0.08 ± 0.01^{b}	0.11 ± 0.02^{b}

Values are means ± s.d. for 6 rats in each group. Within a line, values without a common letter are significantly different from the control group at $P < 0.05$ as determined by analysis of variance.

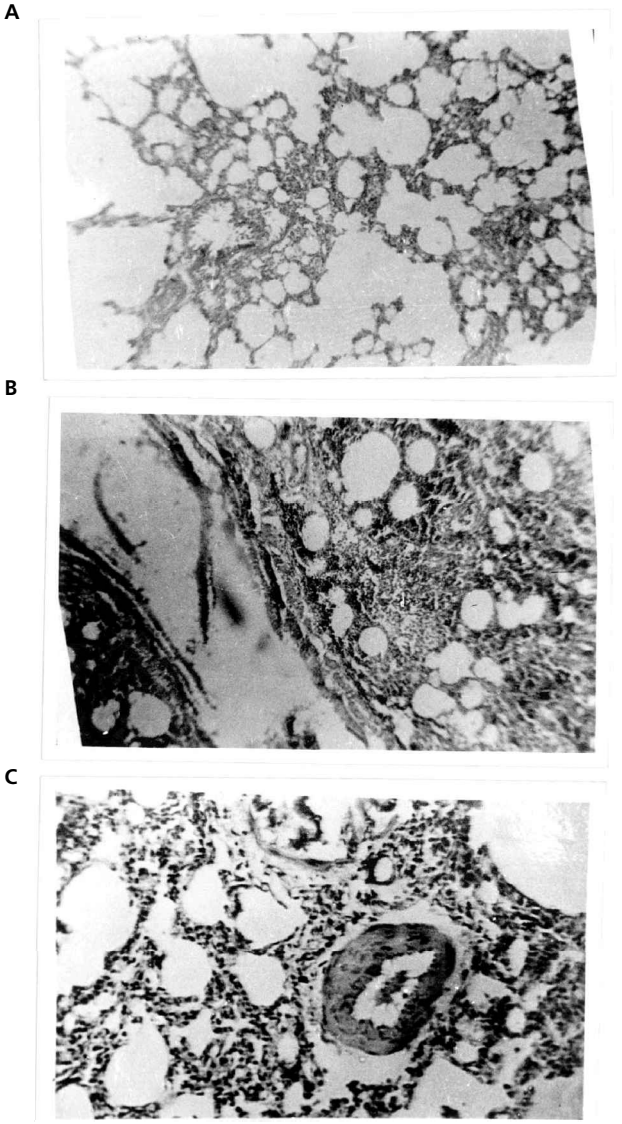

Figure 2 Histopathology of rat lung. Sections are stained with haematoxylin and eosin. A. Lung section of control rat showing normal architecture of bronchiole with surrounding alveolar space ($10 \times 10\times$). B. Lung section of BSO-treated rat showing bronchi with congestion, haemorrhage of alveolar space and dense mononuclear (lymphocyte) infiltration ($10 \times 10\times$). C. Lung section of BSO-treated rats supplemented with vitamin E showing the normal architecture of lung ($10 \times 10\times$).

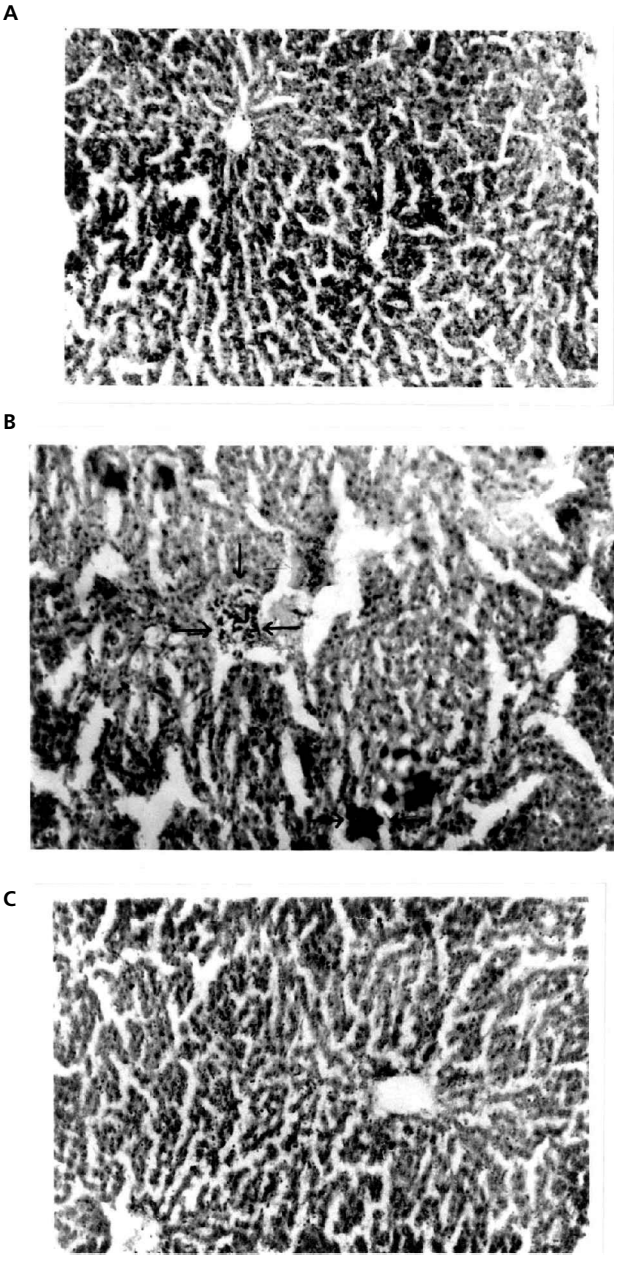

Figure 3 Histopathology of rat liver. Sections are stained with haematoxylin and eosin. A. Control liver section shows normal architecture of liver with central vein and cords of hepatocytes (around $10 \times 10\times$). B. Liver section of BSO-treated rats showing hepatocytes with dysplastic changes like nuclear condensation, nuclear cytoplasmic alteration and crowding of hepatocytes ($10 \times 10\times$). C. Liver section of vitamin E-supplemented BSO-treated rat showing hepatocytes with nuclear changes seen focally with occasional nuclear condensation and diffused lymphocytic infiltration (around $10 \times 10\times$).

rats. This indicates that vitamin E treatment spares the GSH function and helps in maintaining the antioxidant defence, thereby preventing lipid peroxidation (Wefers & Sies 1988). Oxidized glutathione (GSSG) levels in mitochondria and tissues were altered in BSO-administered rats, which resulted in a higher GSSG/GSH ratio. It has been reported that there is a direct relationship between m-DNA damage and the GSSG/GSH ratio. The oxidation of GSH is an index of oxidative stress, which occurs both in the cytosol and in the mitochondria (Esteve et al 1999).

Supplementation with vitamin E was found to induce the activity of glucose-6-phosphate dehydrogenase (G-6-PDH) and glutathione reductase in this study. Glutathione reductase is the enzyme that converts GSSG to GSH, with the help of NADPH, which is supplied by

G-6-PDH (Gaetani et al 1989). This may also be a reason for the increased level of GSH observed in vitamin E-supplemented rats. The sparing action of α-tocopherol on β-carotene was described in-vivo in man by Reaven et al (1993). During such action, tocopheroxy radicals are formed. For the regeneration of reduced tocopherol, vitamin C is required (Sharma & Buettner 1993). The levels of vitamin C, in turn, are dependent on GSH levels (Meister 1992). The decrease in BSO-induced GSH was associated with a corresponding increase in oxidised glutathione (GSSG), which resulted in decreased activity of GPX and increased activity of glutathione reductase as well.

It has been demonstrated that a dietary deficiency of vitamin E reduces the activity of hepatic catalase, GSH peroxidases and GSH reductase (Chow et al 1969; Muller 1990; Carr et al 2000), induces lipid peroxidation and cause neurological and cardiovascular disorders (Carr et al 2000), all of which can be reversed by dietary vitamin E supplementation. Intracellular depletion of GSH itself does not appear to be responsible for the large increase in the production of reactive oxygen species (ROS) and lipid peroxidation in general. In this study, chronic depletion of GSH by BSO had also resulted in significant decline in the levels of other major antioxidants – ascorbic acid and vitamin E. The observed decrease in activity of SOD, catalase and G-6-PDH in the lung and liver tissues of BSO-treated rats is probably due to an accumulation of ROS and severe oxidative stress condition created by BSO. It is also known that over-production of ROS is toxic to these enzymes (Escobar et al 1996; Gultekin et al 2000). Vitamin E supplementation, along with BSO, resulted in a drastic increase in the tissue level of vitamin E, which could have effectively scavenged the oxygen-derived free radicals as well as lipid peroxyl radicals. Thus the pronounced sparing action of vitamin E under GSH-depleted condition is evident and it is likely to minimize the accumulation of ROS. The tocopherols not only inhibit free-radical-induced lipid autoxidation, but they also inhibit the oxidation induced by singlet oxygen. Tocopherol reacts with singlet oxygen either by physical quenching or by chemical reactions (Kaisar et al 1990).

Previously, our group had reported that the lung and liver tissues of BSO-treated animals show haemorrhage, mononuclear cell infiltration and marked congestion, due to extensive glutathione-depletion-induced oxidative stress (Thanislass et al 1995; Rajasekaran et al 2002). Later findings demonstrated that the mitochondria do not have γ-GCS or GSH synthetase activity, indicating that mitochondrial GSH always depended on cytosolic GSH (Griffith & Meister 1985; Pascoe et al 1987; Thanislass et al 1996). In this situation, the decline in mitochondrial GSH is correlated with depletion of hepatic and pulmonary GSH. GSH is essential for the normal functioning of mitochondria and it provides a reservoir of reducing equivalents capable of preventing the effects of oxidants on sensitive thiols (Thanislass et al 1995). Mitochondrial GSH may be important in regulating inner membrane permeability by maintaining intra-mitochondrial thiols in the reduced state (Kosower & Kosower 1983).

Impaired antioxidant defence in liver and lung mitochondria resulted in oxidative stress, which is supported by the increased levels of TBARS in GSH-depleted mitochondria isolated from lung and liver of BSO-treated rats. Lipid peroxidation in mitochondria could lead to uncoupling of oxidative phosphorylation (Vladimirov et al 1980) and hence loss of membrane integrity and impairment of electron flow, affecting mitochondrial respiration, which in turn results in less ATP production (Shigenaga et al 1994). Cellular energy deficits caused by decline in mitochondrial function can impair normal cellular activity and compromise the cell's ability to adapt to physiological stress. In this investigation, we suggest that this oxidative damage and, in particular, oxidative damage to mitochondria, is prevented by vitamin E as is evident from the reduced MDA levels and normalized GSSG/GSH status in lung and liver mitochondria (Table 3). This is supported by recent reports (Naziroglu & Cay 2001).

Glutathione peroxidase (GPX), an enzymatic antioxidant, protects the plasma membrane and mitochondrial membrane from peroxidative damage (Thanislass et al 1996; Anandan et al 1999). Decreased GPX activity recorded in the BSO-administered rats might have contributed to altered mitochondrial functions.

It has also been revealed that mitochondria are more susceptible to lipid peroxidation because of their membranous structure. The fluidity of cellular membranes decreases with age (de la Asuncion et al 1996), a change that may be attributed, in part, to oxidation of plasma and mitochondrial membrane lipid components. Damage to mitochondria in the GSH-depleted condition is evidenced by the increased oxidation of NADH (Thanislass et al 1996). This investigation has shown a marked variation in the cholesterol concentration and hence significantly altered cholesterol/phospholipid ratio between normal and GSH-depleted lung and liver of rats. This suggests that alterations in the lipid composition seen in the lung and liver mitochondrial membrane of BSO-treated rats are due to severe depletion of thiols (Tables 1 and 2). This study is well correlated with our previous investigations in-vivo (Rajasekaran et al 2002) and also with the in-vitro studies performed by Madesh et al (1998). Altered cholesterol-to-phospholipid ratio (Table 4) suggests the possible alteration in membrane fluidity with a resultant alteration in membrane function. Vitamin E supplementation protected the deleterious effect of GSH depletion on lung and liver mitochondria, probably by its potential to fight against oxidants and also by partially maintaining the GSH levels in cytosol.

Several reports on enzyme inactivation by lipid peroxidation have shown that sulfhydryl enzymes are more susceptible to inactivation (Hermer & Story 1993). In our study, there was an enhanced lipid peroxidation that, in turn, decreased the protein sulfhydryl groups. It has been suggested that GSH acts as a buffer against the oxidation of protein thiols (Meister 1991). GSH also participates in scavenging free radicals directly and through GSH-dependent reactions (Meister 1991; Deneke 2000).

Some evidence is available linking free-radical reactions with a variety of pathological states, such as cancer, diabetes, atherosclerosis, etc. (Halliwell 1991; Haklor et al 1998). Generally, aerobic metabolism in the cells and tissues produces free radicals. It is widely accepted that

vitamin E behaves as a chain-breaking antioxidant for lipids in biological systems. The presence of a phenolic hydroxyl group in an active vitamin E compound plays a major role in free-radical scavenging function by transferring hydrogen from it (Chow 1991; Parker 1991; Gutierrez et al 2003). In this study, we observed increased lipid peroxidation in the lung and liver of rats that were exposed to BSO. This increase in lipid peroxidation may be due to BSO-induced GSH depletion. Similar observations have also been reported in vitamin-E-deficient animals (Chow 1991; Metin et al 2002).

It has been accepted that vitamin E is involved in the first line of defence against lipid peroxidation. It protects polyunsaturated fatty acids in cell membranes through its free-radical scavenging activity (Fatih et al 2001). In our study, increased levels of lipid peroxidation products recorded in BSO-administered conditions reverted back to normal levels in α-tocopherol-supplemented rats. Strong evidence from in-vivo and in-vitro studies suggests that vitamin E protects biological membranes against lipid peroxidation (Chow 1991; Sharma & Buettner 1993; Wang & Quinn 2002).

Regulation of intracellular lipid peroxidation is facilitated by catalase, superoxide dismutase, glutathione peroxidase, and by chain-breaking antioxidants like α-tocopherol, ascorbic acid, etc. They protect polyunsaturated fatty acids in membranes through their free-radical quenching activity. Decreased concentrations of all these substances were observed in BSO-administered rats, which reverted back to normal levels in α-tocopherol-supplemented rats. It is also evident from the current study, that vitamin E prevented the histopathological changes through its direct free-radical scavenging action and by enhancing GSH levels.

It is shown that depletion of intracellular and mitochondrial GSH resulted in cell injury and depletion of other antioxidants and enhanced the susceptibility of cells to further damage. Supplementation with α-tocopherol markedly elevated the α-tocopherol content in the liver and lung of BSO-treated rats, inhibited the associated lipid peroxidation and maintained intracellular GSH concentrations partially. Although levels of tissue and mitochondrial GSH were actually depleted in BSO-administered rats, the protective effect of vitamin E on BSO-treated rats was associated with a significant ($P < 0.05$) decrease in oxidised glutathione (GSSG) and concomitant restoration of cellular redox status (GSSG/GSH ratio) to control levels. The role of vitamin E in protecting against almost all the abnormalities resulting from BSO-mediated GSH depletion suggests that GSH and vitamin E have some similar targets in their actions against oxidative damage.

Conclusion

In conclusion, the above observations suggest that the impairment of oxidant–antioxidant balance, during BSO exposure, can be prevented by α-tocopherol. Hence, α-tocopherol supplementation therapy may consequently reduce lipid peroxidation, mitochondrial damage and cell death.

References

Anandan, R., Deepa Rekha, R., Devaki, T. (1999) Protective effect of picrorhiza kurroa on mitochondrial glutathione antioxidant system in D-galactosamine induced hepatitis in rats. *Curr. Sci.* **76**: 1543–1546

Beers, R. F., Seizer, I. W. (1952) A spectrophotometric method for measuring breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* **115**: 133–140

Carr, A. C., Zhu, B. Z., Frei, B. (2000) Potential antiatherogenic mechanisms of ascorbate (vitamin C) and α-tocopherol (vitamin E). *Circ. Res.* **87**: 349–354

Chow, C. K. (1991) Vitamin E and oxidative stress. *Free Radic. Biol. Med.* **11**: 215–232

Chow, C. K., Reddy, K., Tappel, A. I. (1969) Effect of dietary vitamin E on the activity of glutathione peroxidase *in vitro* and *in vivo* studies. *J. Clin. Invest.* **48**: 1957

de la Asuncion, J. G., Millan, A., Pla, R., Brusehini, L., Esteras, A., Pallardo, F. V., Sastre, J., Vina, J. (1996) Mitochondrial glutathione oxidation correlates with age-associated oxidative damage to mitochondrial DNA. *FASEB J.* **10**: 333–338

Deneke, S. M. (2000) Thiol-based antioxidants. *Curr. Top. Cell. Regul.* **36**: 151–180

Desai, I. D. (1984) Vitamin E analysis methods for animal tissues. *Methods Enzymol.* **105**: 138–147

Draper, H. H., Hadley, M. (1990) Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol.* **186**: 421–431

Escobar, J. A., Rubio, M. A., Lissi, E. A. (1996) SOD and catalase inactivation by singlet oxygen and peroxyl radicals. *Free Radic. Biol. Med.* **20**: 285–290

Esteve, J. M., Mompo, J., de la Asuncion, J. G., Sastre, J., Asensi, M., Boix, J., Vina, J. R., Vina, J., Pallardo, V. (1999) Oxidative damage to mitochondrial DNA and glutathione oxidation in apoptosis: studies *in vivo* and *in vitro*. *FASEB J.* **13**: 1055–1064

Fatih, G., Namik, D., Sulhattin, Y., Ibrahim, D. (2001) In vivo changes in antioxidant systems and protective role of melatonin and a combination of vitamin C and E on oxidative damage in erythrocytes induced by chlorpyrifos-ethyl in rats. *Arch. Toxiol.* **75**: 88–96

Folch, J., Less, M., Stanely, G. H. S. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509

Fracasso, P. M. (2001) Overcoming drug resistance in ovarian carcinoma. *Curr. Oncol. Rep.* **3**: 19–26

Gaetani, G. F., Galiano, S., Canepa, L., Ferraris, A. M., Kirkman, H. N. (1989) Catalase and glutathione peroxidase are equally active in detoxification of hydrogen peroxide in human erythrocytes. *Blood* **73**: 334–339

Griffith, O. W. (1980) Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal. Biochem.* **106**: 207–212

Griffith, O. W., Meister, A. (1985) Origin and turnover of mitochondrial glutathione. *Proc. Natl Acad. Sci. USA* **82**: 4668–4672

Gultekin, F., Ozturk, M., Akdogan, M. (2000) The effect of organophosphate insecticide chlorpyrifos-ethyl on lipid peroxidation and antioxidant enzymes (*in vitro*). *Arch. Toxicol.* **74**: 535–538

Gutierrez, M. E., Garcia, A. F., Africa de Madariaga, M., Sagrista, M. E., Mora, M. (2003) Interaction of tocopherols and phenolic compounds with men and their antioxidant activity in a liposomal model system. *Life Sci.* **72**: 2337–2360

Haklor, G., Sirikci, O., Ozer, N. K., Yalcin, A. S. (1998) Measurement of reactive oxygen species by chemiluminescence

in diet-induced atherosclerosis: protective roles of vitamin E and probucol on different radical species. *Int. J. Clin. Lab. Res.* **28**: 122–126

Halliwell, B. (1991) Reactive oxygen species in living system; source, biochemistry and role in human disease. *Am. J. Med.* **92** (Suppl. 3C): 14S–22S

Hedley, D. W., McCulloch, E. A., Minden, M. D., Chow, S., Curtis, J. (1998) Antileukemic action of buthionine sulfoximine: evidence for an intrinsic death mechanism based on oxidative stress. *Leukemia* **12**: 1545–1552

Hermer, L. M., Story, K. B. (1993) In vitro oxidative inactivation of glutathione S-transferase from a freeze tolerant reptile. *Mol. Cell. Biochem.* **124**: 149–158

Johnson, D., Lardy, H. (1967) Isolation of liver or kidney mitochondria. In: Estarbrook, R. W., Pullman, M. E. (eds) *Methods in enzymology*. Vol.10, Academic Press, New York, pp 94–96

Kaisar, S., DiMascio, P., Murphy, M. E., Sies, H. (1990) Physical and chemical scavenging of singlet molecular oxygen by the tocopherols. *Arch. Biochem. Biophys.* **277**: 101–108

Kosower, N. S., Kosower, E. M. (1983) In: Larsson, A., Orreniv, S. S., Holmgren, A., Mannervik, B. (eds) *Functions of glutathione: biochemical, physiological, toxicological and clinical aspects*. Raven, New York, pp 307–315

Kung, A. K., Zetterberg, A., Sherwood, S. W., Schimke, R. T. (1990) Cytotoxic effects of cell cycle phase specific agents: result of cell cycle perturbation. *Cancer Res.* **50**: 7307–7317

Leonetti, C., Biroccio, A., Gabellini, C., Scarsella, M., Maresca, V. F., Pace, A., Stoppacciaro, A., Zupi, G., Cognetti, F., Picardo, M. (2003) Alpha-tocopherol protects against cisplatin-induced toxicity without interfering with antitumor efficacy. *Int. J. Cancer* **104**: 243–250

Madesh, M., Benard, O., Balasubramanian, K. A. (1998) Glutathione modulates lipid composition of human colon derived HT-29 cells. *Int. J. Biochem. Cell. Biol.* **30**: 1345–1352

Maellaro, E., Alessandro, F., Bellow, C. B. D., Comporti, M. (1990) Lipid peroxidation and antioxidant systems in the liver injury produced by glutathione depleting agents. *Biochem. Pharmacol.* **39**: 1513–1521

Meister, A. (1991) Glutathione deficiency produced by inhibition of its synthesis and its reversal: application in research and therapy. *Pharmacol. Ther.* **51**: 155–194

Meister, A. (1992) On the antioxidant effects of ascorbic acid and glutathione. *Biochem. Pharmacol.* **44**: 1905–1915

Metin, G., Atukeren, P., Gumustas, M. K., Belce, A., Kayserilioglu, A. (2002) The effect of vitamin E treatment on oxidative stress generated in trained rats. *Tohoku. J. Exp. Med.* **198**: 47–53

Misra, H. P., Fridovich, I. (1972) The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **247**: 3170–3175

Mistry, P., Harrap, K. R. (1991) Historical aspects of glutathione and chemotherapy. *Pharmacol. Ther.* **49**: 125–132

Mooz, E. D., Meister, A. (1969) Glutathione biosynthesis. *Methods Enzymol.* **17B**: 483–500

Moron, M. S., Defierre, J. W., Mannervik, K. B. (1979) Levels of glutathione, glutathione reductase and glutathione-s-transferase activities in rat lung and liver. *Biochim. Biophys. Acta* **582**: 67–74

Muller, D. P. (1990) Antioxidant therapy in neurological disorders. *Adv. Exp. Med. Biol.* **264**: 475–484

Naziroglu, M., Cay, M. (2001) Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin. *Biol. Trace Elem. Res.* **79**: 149–159

Omaye, S. T., Turnball, J. D., Sauberlich, H. E. (1971) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. *Methods Enzymol.* **62**: 1–11

Parekh, A. C., Jung, G. H. (1970) Cholesterol determination with ferric chloride-uranyl acetate and sulphuric acid ferrous sulphate reagents. *Anal. Chem.* **42**: 1243

Parker, L. (1991) Protective role of vitamin E in biological system. *Am. J. Clin. Nutr.* **53**: 1050s–1055s

Pascoe, G. A., Fariss, M. W., Olafsdottir, K., Reed, D. J. (1987) A role of vitamin E in protection against cell injury. Maintenance of intracellular glutathione and biosynthesis. *Eur. J. Biochem.* **166**: 241–247

Petryna, L. H. (2002) The effect of various doses of gamma-radiation on the contents of vitamin E in rats. *Fiziol. Zh.* **48**: 51–55

Rajasekaran, N. S., Devaraj, H., Niranjali, S. D. (2002) The effect of glutathione monoester (GME) on glutathione (GSH) depleted rat liver. *J. Nutr. Biochem.* **13**: 302–306

Reaven, P. D., Khouw, A., Beltz, W. F., Parthasarathy, S., Witztum, J. L. (1993) Effect of dietary antioxidant combinations in humans: protection of LDL by vitamin E but not by β-carotene. *Arterioscler. Thromb.* **13**: 590–600

Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., Hafeman, D. G., Hoekstra, W. G. (1973) Selenium: biochemical role as a component of glutathione peroxidase. *Science* **179**: 588–590

Rouser, G., Fleischer, S., Yamamoto, A. (1970) Two dimensional thin layer chromatographic separation of polar lipids and determination of phospholipids by phosphorus analysis of spots. *Lipids* **5**: 738–743

Schneider, E., Yamazaki, H., Sinha, B. K., Cowan, K. H. (1995) Buthionine sulfoximine-mediated sensitisation of etopside resistant human breast cancer MCF7 cells over expressing the multidrug resistance associated protein involves increased drug accumulation. *Br. J. Cancer* **71**: 738–743

Sedlack, J., Lindsay, R. H. (1968) Estimation of total, protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Anal. Biochem.* **25**: 192–205

Seies, H., Stahl, W., Sundquist, A. R. (1992) Antioxidant functions of vitamins. Vitamin E and C, betacarotene and other carotenoids. *Ann NY Acad. Sci.* **669**: 7–20

Sharma, M., Buettner, G. R. (1993) Interaction of vitamin C and E. During the free radical stress in plasma. An ESR study. *Free Radic. Biol. Med.* **14**: 649–653

Shigenaga, M. K., Hagen, T. M., Ames, B. N. (1994) Oxidative damage and mitochondrial decay in aging. *Proc. Natl Acad. Sci. USA* **91**: 10771–10778

Smith, L. J., Anderson, J. (1992) Oxygen-induced lung damage. Relationship to lung mitochondrial glutathione levels. *Am. Rev. Respir. Dis.* **146**: 1452–1457

Staal, G. E. J., Visser, J., Veeger, C. (1969) Purification and properties of glutathione reductase of human erythrocytes. *Biochim. Biophys. Acta* **185**: 39–48

Terradez, P., Asensi, M., Lasso de la Vega, M. C., Puertes, I. R., Vina, J., Estrela, J. M. (1993) Depletion of tumor glutathione *in vivo* by buthionine sulfoximine: modulation by the rate of cellular proliferation and inhibition of cancer growth. *Biochim. J.* **292**: 477–483

Thanislass, J., Raveendran, M., Devaraj, H. (1995) BSO-induced GSH depletion – its effect on antioxidants, lipid peroxidations and calcium homeostasis in the lung. *Biochem. Pharmacol.* **50**: 224–229

Thanislass, J., Raveendran, N., Sivasithamparam, N., Devaraj, H. (1996) Effect of chronic glutathione deficiency on lung mitochondrial function. *Pulm. Pharmacol.* **9**: 119–122

Tietze, F. (1969) Enzymatic method for quantitative determination of nanogram amounts of total and oxidised glutathione:

applications to mammalian blood and other tissues. *Anal. Biochem.* **27**: 502–522

van Dam, P. S., Bravenboer, B., van Asbeck, B. S., Marx, J. J., Gispen, W. H. (1999) High rat food vitamin E content improves nerve function in streptozotocin-diabetic rats. *Eur. J. Pharmacol.* **376**: 217–222

Vanhoefer, U., Cao, S., Minderman, H., Toth, K., Skenderis, B. S., Slovak, M. L., Rustum, Y. M. (1996) D.L-buthionine (S.K). Sulfoximine potentiates the in vivo therapeutic efficacy of doxorubicin against multidrug resistant protein expressing tumors. *Clin. Cancer Res* **2**: 1961–1968

Vladimirov, Y. A., Olenev, V. I., Suslova, T. B., Cheremisina, Z. P. (1980) Lipid peroxidation in mitochondrial membrane. *Adv. Lipid Res.* **17**: 173–249

Wang, X., Quinn, P. J. (2002) The interaction of alpha-tocopherol with bilayers of 1-palmitc acid. *Biochim. Biophys. Acta* **1567**: 6–12

Wang, X., Falcone, T., Attaran, M., Goldberg, J. M., Agarwal, A., Sharma, R. K. (2002) Vitamin C and vitamin E supplementation reduce oxidative stress-induced embryo toxicity and improve the blastocyst development rate. *Fertil. Steril.* **78**: 1272–1277

Wattenberg, L. W. (1993) Prevention, therapy and basic science and the resolution of the cancer problem. *Cancer Res.* **53**: 5890–5896

Wefers, H., Sies, H. (1988) The protection by ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *Eur. J. Biochem.* **174**: 353–357

Wernerman, J., Hammarquist, F. (1999) Modulation of endogenous glutathione availability. *Curr. Opin. Clin. Nutr. Metab. Care* **2**: 487–492

Wu, H. Y., Kang, Y. J. (1998) Inhibition of buthionine sulfoximine-enhanced doxorubucin toxicity in metallothionin over expressing transgenic mouse heart. *J. Pharmacol. Exp. Ther.* **287**: 515–520

Zar, H. H. (1990) *Biostatistical analysis*. Prentice Hall Inc., USA

Zinkham, W. H. (1978) Defect of glutathione metabolism in erythrocytes from patients with a naphthalene induced hemolytic anaemia. *Bull. Johns Hopkins Hosp.* **102**: 169–175