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The effect of glutathione monoester (GME) on glutathione (GSH) depleted rat liver

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Abstract

The effect of glutathione monoester (GME) on buthionine sulfoximine (BSO) mediated glutathione (GSH) depletion in rats was studied to understand the defensive role of intraperitoneally supplemented GSH. Administration of glutathione mono ester (GME) (at a dose of 5 mmole/kg body weight, twice a day for 30 days) significantly prevented the buthionine sulfoximine (at a dose of 4 mmole/kg body weight, twice a day for 30 days) induced alterations. This study suggests that glutathione mono ester is hepatoprotective and plays an important role in preventing lipid peroxidation, which leads to cytotoxic effects. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Glutathione; Buthionine sulfoximine; Glutathione monoester; Lipid peroxidation

1. Introduction

Intraperitoneal administration of BSO into adult rats leads to depletion of glutathione by inhibiting the activity of γ-glutamyl cysteine synthetase in normal cells and those of tumor [1]. As a selective agent for turning off GSH synthesis, BSO has been successfully employed to cause GSH depletion, which presumably, sensitizes cells to the toxic effects of several xenobiotics [2,3]. Depletion of cellular GSH has beneficial role on sensitizing tumor cells to chemo- and radio-therapy [3,4].

Increasing evidence of free radical production is reported for chronic deficiency of glutathione through repeated administration of BSO in vivo [5,6]. It has been recently reported that during prolonged BSO induced glutathione depletion, an altered antioxidant defense and enhanced lipid peroxidation occurred in cytosol [7]. Important studies made by Meister showed that GSH monoester protects the cells from toxicity [1]. Decades of intensive research have demonstrated that GSH is involved in numerous processes that are essential for normal biological function, but many problems that arise in association with decreased GSH concentrations are not remedied so far.

Glutathione monoesters have been found to be the ideal compound for increasing cellular GSH. However, we are focusing our studies towards the protective mechanism of GME. Eventhough several authors have reported the cellular uptake of GME, not much focus has been thrown towards the protective mechanism of GME after its uptake which is of our present interest.

2. Materials and methods

BSO was obtained from the Sigma Chemical Company, St. Louis, Missouri, USA. All other chemicals used were of analytical grade. GME was synthesized according to the method of Anderson and Meister.

Wistar Strain male albino rats weighing 80–100 g were obtained from the Fredrick Institute of Plant Protection and Toxicology, Padappai, Chennai, India and maintained in polyacrylic cages under hygienic conditions at normal room temperature (28–30°C). They were fed with commercial diet, which contained 5% fat, 21% protein, 55% nitrogen-free extract, 4% fiber (wt/wt) and adequate mineral and vitamin contents. The animals had access to food and water *ad libitum*. The animals were divided into three groups, each group consisted of six rats. Control animals were given physiological saline (0.5 ml), intraperitoneally, twice a day. Another group of rats were intraperitoneally injected with

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Table 1
Effect of BSO, BSO + GME on γ-glutamyl cysteine synthetase, antioxidants, antioxidant enzymes, MDA concentration, thiol status and protein content of rat liver

Parameters	Control	BSO	BSO + GME
γ-Glutamyl cysteine synthetase (μg phosphate liberated/mg protein)	2950.21 ± 103.42[a]	2658.01 ± 84.02[b]	2696.23 ± 76.48[b]
Antioxidants (mmol/kg wet tissue)			
Glutathione	7.08 ± 1.11[b]	2.13 ± 0.99[c]	9.15 ± 0.75[a]
Ascorbic acid	2.15 ± 0.06[a]	1.15 ± 0.10[b]	2.14 ± 0.06[a]
α-tocopherol	0.95 ± 0.10[a]	0.66 ± 0.05[b]	0.86 ± 0.08[a]
Antioxidant enzymes			
Glutathione peroxidase (μg of GSH consumed/min/mg protein)	48.93 ± 0.28[a]	19.43 ± 0.060[c]	32.23 ± 0.32[b]
Superoxide dismutase (units/min/mg protein)	1.87 ± 0.14[a]	1.38 ± 0.15[b]	1.59 ± 0.17[ab]
Catalase (μmol of H_2O_2 consumed/min/mg protein)	3.47 ± 0.33[a]	2.35 ± 0.01[b]	3.51 ± 0.54[a]
Lipid peroxidation products			
Malondialdehyde* (nmol MDA/mg protein)	123.01	226.90	130.53
Thiol status (μmol/mg protein)			
Total thiols	27.32 ± 2.81[a]	15.49 ± 1.38[b]	26.82 ± 2.63[a]
Protein thiols	19.81 ± 2.04[a]	12.08 ± 1.83[b]	18.26 ± 2.21[a]
Non protein thiols	7.51 ± 0.63[a]	3.38 ± 0.51[b]	8.56 ± 1.08[a]
Protein (g/kg wet tissue)	161.04 ± 6.82	155.84 ± 2.68	159.62 ± 4.36

Values are Mean ± SD for six animals 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 ANOVA.
* MDA levels are the average of triplicates analysed by HPLC.

BSO (0.5 ml, 4 mmol/kg body weight) twice a day. The dose selected for this study was based on Meister's report [1]. The third group of animals were intraperitoneally injected with BSO (0.5 ml, 4 mmol/kg body weight), twice a day. GSH monoester (5 mmol/kg body weight) injection was given 30–45 min after BSO administration. The dose given was based on the previous studies of Meister [1]. The treatment was continued for 30 days and the animals were fasted overnight and sacrificed by decapitation and the liver was dissected out immediately and washed with cold physiological saline. 500 mg of tissue was weighed and homogenized with 0.1 M Tris-HCl buffer (pH 7.0) at 4°C.

The non-enzymic antioxidants such as glutathione [8], ascorbic acid [9], α-tocopherol [10] and the activities of antioxidant enzymes-superoxide dismutase (SOD) [11], glutathione peroxidase (GPX) [12] and catalase [13] were measured. The activity of γ-glutamyl cysteine synthetase (γGCS) was analyzed according to the method of Mooz and Meister [14]. Lipid peroxidation products were determined using HPLC [15]. Briefly, the liver homogenate was centrifuged at 2000 rpm for 10 min, the supernatant was mixed with equal volume of acetonitrile to precipitate protein and this was centrifuged at 1000 rpm for 5 min 20 μl of filtered samples were injected by Hamilton 50 μl syringe in a C-18 HPLC column for detection of malondialdehyde, at the flow rate of 1 ml/min and UV absorbance at 270 nm. Thiol status of the liver was also measured [16]. Serum marker enzymes; lactate dehydrogenase [17], alanine transaminase [18], aspartate transaminase [18], γ-glutamyl transpeptidase [19] and alkaline phosphatase [18] were also measured to assess the tissue damage. Another 1 g of liver tissue was weighed accurately and lipid was extracted with chloroform:methanal (2:1, v/v) [20]. This was used to study the lipid profile; cholesterol [21], ester cholesterol [22], free cholesterol, free fatty acids [23] and triglycerides [24]. Phospholipid content was determined by the method of Rouser et al. (1970) [25] after digesting with perchloric acid [26]. The phosphorus content was multiplied by a factor of 25, which gave the amount of phospholipids.

3. Statistical Analysis

Data are expressed as mean ± SD. Analysis of variance (ANOVA) followed by the students 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.

4. Results

Regarding the body weight no significant changes were observed during the course of BSO administration. Table 1 indicates the activity of γ-GCS and the levels of antioxidants, lipid peroxides, thiols status and protein content of control, BSO, BSO + GME treated rat liver following treatment for a period of 30 days. Administration of BSO inhibits the activity of γ-GCS significantly ($P < 0.01$).

The levels of antioxidants; glutathione, ascorbic acid and α-tocopherol were significantly decreased in the BSO administered animals. Supplementation of GME showed near normal levels of the above antioxidants (Table 1). Activities of antioxidant enzymes such as catalase($P < 0.05$), glutathione peroxidase ($P < 0.01$) and superoxide dismutase ($P < 0.05$) were decreased significantly in BSO treated rats. An enhanced activity of SOD and catalase were recorded in the GME supplemented group and the activity of GPX was

Table 2
Effect of BSO and BSO + GME treatment on the levels of serum marker enzymes and lipid profile of rat liver

Parameters	Control	BSO	BSO + GME
Serum marker enzymes (μKat/L)			
Lactate dehydrogenase	3.08 ± 0.10^{b}	4.05 ± 0.13^{a}	3.19 ± 0.11^{b}
Aspartate transaminase	0.256 ± 0.02^{b}	0.266 ± 0.01^{a}	0.259 ± 0.04^{b}
Alanine transaminase	0.236 ± 0.006^{b}	0.264 ± 0.010^{a}	0.238 ± 0.007^{b}
Alkaline phosphatase	0.122 ± 0.019^{b}	0.174 ± 0.030^{a}	0.135 ± 0.019^{ab}
Gamma glutamyl transpeptidase	0.130 ± 0.015^{b}	0.159 ± 0.009^{a}	0.132 ± 0.015^{b}
Lipid profile (m mol/kg wet tissue)			
Cholesterol	8.76 ± 0.05^{b}	10.65 ± 0.10^{a}	8.27 ± 0.08^{c}
Free cholesterol	3.10 ± 0.08^{b}	4.06 ± 0.15^{a}	3.31 ± 0.23^{b}
Ester cholesterol	5.66 ± 0.26^{b}	6.59 ± 0.41^{a}	4.96 ± 0.39^{b}
Free fatty acids (mg/kg wet tissue)	610 ± 50^{b}	940 ± 40^{a}	490 ± 20^{c}
Triglycerides	2.53 ± 0.12^{b}	3.16 ± 0.11^{a}	2.55 ± 0.11^{b}
Phospholipids	703.28 ± 28.74^{a}	488.22 ± 9.68^{b}	723.94 ± 11.30^{a}
Cholesterol/Phospholipids	0.17	0.27	0.10

Values are Mean ± SD for six animals 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 ANOVA.

partially restored by GME when compared to control animals.

The concentration of malondialdehyde (MDA), the index of lipid peroxidation, was elevated (Table 1) in the glutathione depleted condition. GME therapy to the BSO treated animals reduced the MDA concentration to near normal value.

BSO administration also resulted in altered thiol status of liver. The levels of total thiols, non-protein thiols and protein thiols were significantly decreased ($P < 0.01$) in BSO-induced GSH-depleted rat liver when compared with control (Table 1). On GME therapy to BSO treated animals, the levels of these parameters were found to be normalized. The protein content of liver in the different groups did not show significant changes.

Table 2 depicts the effect of BSO and BSO + GME-treatment on the activities of biochemical markers of liver damage and lipid profile of rat liver. A significant increase ($P < 0.05$) in the activities of these enzymes was observed in the BSO-induced GSH depleted rats, which returned to near normal level in the GME supplemented group.

Total cholesterol, triglycerides, free cholesterol and ester cholesterol were significantly elevated ($P < 0.05$) on BSO administration. Levels of phospholipid were significantly decreased ($P < 0.01$) in the BSO treated animals when compared to the control group, whereas cholesterol/phospholipid ratio had increased. Supplementation of GME aided in bringing back these levels to near normal (Table 2).

5. Discussion

Therapeutic trials with BSO against drug resistant tumors cause damage through lipid peroxidation in normal cells too [27]. Altered levels of membrane phospholipids and cholesterol were observed in cancer patients. A few studies revealed that increased levels of cholesterol and phospholipids in women with breast cancer [28]. Earlier studies from our laboratory emphasized the depletion of intracellular GSH on BSO administration, which resulted in lipid peroxidation, thereby leading to subsequent alteration in protein thiols in rat lung [29]. In agreement with the above findings, the levels of total, protein and non-protein thiols were significantly decreased in the BSO-induced GSH-depleted rat liver. Due to the observed enhanced level of LPO, the liver is damaged in the BSO induced GSH depleted condition, which is evidenced by the increased levels of serum marker enzymes; lactate dehydrogenase (LDH), alkaline phosphatase (ALP), transaminases (ALT, AST) and γ-glutamyl transpeptidase (γ-GT).

Supplementation of glutathione brought back all the parameters, which were altered during glutathione depletion to near normal values, which confirms that the above changes are only due to the GSH depletion and not due to BSO. The activities of catalase, SOD and GPX were significantly decreased in GSH depleted condition due to severe oxidative stress and accumulation of H_2O_2. The decreased activity of GPX in our present study in the BSO administered group is due to the fact that BSO depletes the GSH which is the substrate for GPX. The observed decreased activity of GPX is thus due to glutathione depletion and could result in an accumulation of H_2O_2, which might have led to the diminished activities of catalase and SOD [30].

Increased levels of GSH and other thiols have been associated with increased tolerance to oxidant stress condition [31]. BSO induces lipid peroxidation, which can cause cross polymerization, polypeptide chain scission and chemical changes in individual aminoacids. Also, the lipid free radical species can oxidize protein sulfhydryl groups and enhance the formation of disulfide bridges. Thus, lipid peroxidation itself can cause a loss of protein thiols [32]. In the

present study, administration of BSO resulted in loss of protein thiols which shows the expression of toxicity and this is minimized by GME therapy which prevents the loss of thiol groups.

Ascorbic acid regenerates α-tocopherol from its tocopheroxyl radical, *in vivo* and *in vitro* [33]. In the present study, the decreased concentrations of α-tocopherol in BSO group was due to the significant decrease in ascorbic acid and phospholipid contents [34].

The reconversion of dehydroascorbate to ascorbate depends on the availability of reduced glutathione [35]. The depleted levels of glutathione consequent to BSO administration, may have led to the observed decrease in ascorbic acid content

It is well known that intake of ascorbic acid facilitates protection against alcohol induced hyperlipidemia in tissues [34]. Our results showed an increased level of ascorbic acid in GME supplemented animals. Regeneration of ascorbic acid might partially regulate lipid metabolism, which is evident in this study by decreased level of cholesterol recorded in the GME supplemented group. It has also been reported that the rate of cholesterol transformation to bile acids in the liver is directly related to the ascorbate concentration.

An altered membrane lipid composition was recorded in tumor cells due to decreased activity of superoxide dismutase, an antioxidant enzyme that protects cells against the damaging effects of superoxide radicals [36]. Phospholipid provides a major substrate for lipid peroxidation products in cells. Our results suggest that as increased lipid peroxidation occurs with BSO administration, there is a decrease in liver phospholipids with a corresponding increase in the cholesterol to phospholipid ratio. The change in the phospholipid content can be attributed to the enhanced LPO in the BSO administered group. The reason could be due to the abstraction of hydrogen atom from the PUFA in phospholipid by LPO. Dianzani has suggested that an increase in cholesterol is related to a loss of membrane fluidity [37]. In the present study, the altered c/p ratio might have an influence on the transport of glutathione.

The present data strongly indicate that the increase in cholesterol/phospholipid ratio and altered contents of other neutral lipids, triglycerides and free fatty acids disturbs the physical environment of the membrane bilayers. The results suggest that GME supplementation prevents the lipid peroxidation caused by BSO, with a decrease in the cholesterol to phospholipid ratio, Vitamin E and glutathione levels returning to their normal levels.

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