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SHORT COMMUNICATION

Suppression of Plasma Estradiol and Progesterone Concentrations by Buthionine Sulfoximine in Female Rats*

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ABSTRACT. Glutathione (GSH) is an important factor involved in the resistance of tumor cells to anticancer agents. Buthionine sulfoximine (BSO), a specific inhibitor of GSH synthesis, effectively decreases cellular GSH concentrations both *in vitro* and *in vivo*. Depletion of GSH by BSO sensitizes a variety of cancer cells to chemotherapeutic agents. Therefore, BSO has been on clinical trial as an anticancer adjuvant. For this purpose, it is important to understand the effect of BSO treatment not only on the sensitivity of tumor cells to anticancer agents, but also on the metabolism and function of normal tissues. The present study was undertaken to determine the effect of BSO treatment on GSH concentrations in the blood, liver, and ovary, and changes in concentrations of ovarian hormones and other important components in plasma. Female Sprague–Dawley rats, 90 days of age, were treated with 2.0 mmol/kg BSO in saline by intraperitoneal injection, twice daily for 7 days. This treatment depressed GSH concentrations in the blood, liver, and ovary by 95, 75, and 85%, respectively. Several blood components were measured. These included red blood cells, hemoglobin, ceruloplasmin, hematocrit, mean corpuscular volume and hemoglobin concentration, alkaline phosphatase, urea nitrogrn, creatine and creatinine, glucose, cholesterol, triglycerides, triiodothyronine (T_3), thyroxine (T_4), and hormones including estradiol, progesterone, and prolactin. BSO treatment significantly ($P < 0.05$) elevated and lowered plasma concentrations of ceruloplasmin and urea nitrogen, respectively. More importantly, plasma concentrations of estradiol and progesterone were decreased markedly ($P < 0.05$) in the BSO-treated animals. The hormonal results suggest that investigations on the role of BSO-induced GSH depletion in the treatment of malignancies both with and without hormone dependence in women should be undertaken. BIOCHEM PHARMACOL 51;4:567–570, 1996.

KEY WORDS. buthionine sulfoximine; glutathione; estradiol; progesterone; ovary

GSH is a highly conserved non-protein thiol in mammalian cells. It functions in many cellular processes including amino acid transport, regulation of enzyme activity, maintenance of membrane structural integrity, synthesis of macromolecules, and cytoprotection against exogenous and endogenous toxicants [1]. GSH is synthesized intracellularly via two consequent enzyme reactions. The first and rate-limiting reaction is catalyzed by γ-glutamylcysteine synthetase and results in the formation of γ-glutamylcysteine from the constituent amino acids. The second reaction is catalyzed by GSH synthetase, which, by linking glycine to the dipeptide, produces the final product, GSH [1]. BSO, a selective inhibitor of γ-glutamylcysteine synthetase, has been used widely for studying GSH-dependent cellular functions [2]. It has been shown that depletion of GSH by BSO sensitizes many tumor cells, including breast cancers, to chemotherapeutic drugs [3, 4]. For this reason, BSO is currently on clinical trial for development as a cancer chemotherapeutic adjuvant [5]. BSO, however, does not decrease GSH concentrations only in the cancer cells *in vivo*. Changes in the GSH status in normal tissues may result in altered metabolism and/or malfunction. Metabolic and functional changes in normal tissues would lead to alterations in the blood concentration of hormones and other components. For example, if BSO is used for the treatment of breast cancers, it is important to know whether changes in GSH status affect concentrations of hormones and other vital components in plasma. In the present study, we treated female rats with BSO and examined the effect of this treatment on GSH

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† *Abbreviations:* BSO, L-buthionine-(S,R)-sulfoximine; GSH, glutathione; PCA, perchloric acid; T3, triiodothyronine; and T_4, thyroxine.

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concentrations in the blood, liver, and ovary. We then examined the effect of GSH depletion on concentrations of a variety of plasma components including ovarian hormones.

MATERIALS AND METHODS

Animals and Treatment

Female weanling Sprague-Dawley rats (34–42 g; Sasco, Lincoln, NE) were housed in quarters maintained at 22–24° with a 12-hr light/dark cycle. They were divided into two weight-matched groups (9 animals/group) having average weights of 39 g each and given free access to food and water. Diet was formulated as previously reported [6]. The primary ingredients were ground corn (73%), casein (16%), and corn oil (4%). Adequate amounts of vitamins and minerals were also provided by the diet. On day 70 of the experiment, rats (age 90 days) were injected intraperitoneally with either 0.9% saline or BSO in saline (L-buthionine-*S*,*R*-sulfoximine, Schweizerhall, Inc., Piscataway, NY), providing 2.0 mmol/kg body weight. Injections were given twice daily for 7 days. This dose regimen and the route of administration were based on previous studies [7]. After the last injection, the rats were fasted for 16 hr, weighed, and decapitated subsequent to anesthesia by sodium pentobarbital. These experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

Tissue Sample Preparation and Blood Analysis

The liver and ovaries were removed, placed in liquid nitrogen, and then stored at −80°C. Blood for cell counting and plasma assays was withdrawn from the inferior vena cava with a heparin-coated syringe. The number of red blood cells, the concentration of hemoglobin, hematocrit, mean corpuscular volume, and mean corpsuscular hemoglobin concentration were determined using a Coulter Counter (model S Plus 4, Hialeah, FL). Ceruloplasmin was determined according to Lehmann *et al.* [8]. Commercial kits from the Sigma Chemical Co. (St. Louis, MO) were used for the following measurements: alkaline phosphatase (Alkaline Phosphatase, Kit No. 104), urea nitrogen (Urea Nitrogen, Endpoint, Kit No. 640), glucose (Glucose, No. 510-6), cholesterol (Total Cholesterol, No. 352), and triglycerides (Triglyceride INT, No. 336-10). RIA methods were used for determining plasma hormones including estradiol, progesterone, and prolactin (Coat-A-Count kits: No. TK E21, No. TK PG1 and No. TK PR1, respectively; DPC, Los Angeles, CA), and T_3 and T_4 (Quanticoat T_3 and T_4; Kallestad Laboratories, Inc., Austin, TX).

Determination of Tissue and Blood GSH Concentrations

The HPLC assay described by Reed *et al.* [9] was employed with some modifications as described previously [10]. The tissues were homogenized with 10% PCA containing 1.0 mM bathophenanthrolinedisulfonic acid. The PCA supernatant was collected after centrifugation for GSH determination, and the precipitate was saved for protein assay by the method of Smith *et al.*[11].

Statistical Analysis

Data were analyzed by Student's *t*-test. All experiments were repeated two times, and data were presented from one representative experiment, as the mean ± SEM from 7–9 animals for each measurement. Differences between treatments were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

As shown in Table 1, GSH concentrations were decreased by BSO in blood, liver, and ovaries by 95, 75, and 85%, respectively. The effect of BSO treatment on concentrations of a variety of plasma components was determined. These included red blood cells, hemoglobin, ceruloplasmin, hematocrit, mean corpuscular volume and hemoglobin concentration, alkaline phosphatase, urea nitrogen, creatine and creatinine, glucose, cholesterol, triglycerides, T_3, T_4, and hormones including estradiol, progesterone, and prolactin. As shown in Table 2, among the plasma components measured the concentrations of estradiol and progesterone were decreased significantly ($P < 0.05$), being lowered by 37 and 64%, respectively. In addition, BSO treatment elevated plasma concentrations of ceruloplasmin by 20% and lowered urea nitrogen concentrations by 25%. This BSO treatment did not change the total body weight or weight gain (data not shown).

Under *in vitro* conditions, such as cell culture, BSO is applied directly to the tested system. The direct effect is depletion of cellular GSH. Because of the simplicity of the culture system, metabolic and functional changes can be ascribed to the GSH depletion. However, when BSO is used *in vivo*, its effect on GSH concentrations is not limited to a specific tissue. Therefore, two major problems are encountered in the application of BSO *in vivo*. The first is non-specific depletion of GSH in untested tissues, which may result in an overt side-effect confounding the direct effect of GSH depletion in the tested tissue. The second is the alteration of the overall metabolism and function resulting from the non-specific depletion of GSH in multiple tissues. For example, if the endocrine system is affected by GSH depletion, the concentrations

TABLE 1. Effects of BSO treatment on GSH concentrations in the blood, liver, and ovary of female rats

	GSH concentration (nmol/mg protein)	
	Non-BSO-treated	**BSO-treated**
Blood	10.2 ± 2.5	0.5 ± 0.1*
Liver	17.8 ± 2.6	4.5 ± 0.8*
Ovary	8.7 ± 2.4	1.1 ± 0.4*

Female Sprague-Dawley rats, 90 days of age, were treated with 2.0 mmol/kg BSO in saline by intraperitoneal injection, twice daily for 7 days. Values are means ± SEM from 9 animals for each measurement.

* Significantly different from non-BSO-treated ($P < 0.05$).

TABLE 2. Changes in concentrations of plasma components including sexual hormones in BSO-treated female rats

Plasma components	Non-BSO-treated	BSO-treated
Ceruloplasmin (U/L)	256.4 ± 9.3	306.0 ± 10.6*
Urea nitrogen (mg/100 mL)	17.9 ± 1.1	13.5 ± 0.7*
Estradiol (pg/mL)	41.0 ± 4.9	25.7 ± 2.3*
Progesterone (ng/mL)	37.8 ± 4.0	13.6 ± 0.5*

Female Sprague-Dawley rats, 90 days of age, were treated with 2.0 mmol/kg BSO in saline by intraperitoneal injection, twice daily for 7 days. Values are means ± SEM from 7–9 animals for each measurement.

* Significantly different from non-BSO-treated ($P < 0.05$).

of important regulatory secretagogues in plasma may be altered. This, in turn, would affect the metabolism and function of other tissues. Thus, it is important to know whether GSH depletion affects these vital components in plasma to understand the *in vivo* effects of BSO-induced GSH depletion.

In the present study, we examined the effect of BSO treatment on GSH concentrations in blood, liver, and ovary. BSO markedly decreased GSH concentrations in all these tissues. We then determined the effect of BSO on plasma concentrations of hormones secreted from the ovary and other important vital components, including those released from the liver. Estradiol and progesterone were decreased significantly in plasma. The results suggest that depletion of GSH in the ovary may affect the endocrine function of this organ. The decrease in plasma concentrations or urea nitrogen suggests that GSH depletion in the liver may affect the urea cycle, which needs to be investigated further.

Numerous studies have shown that GSH concentrations are elevated in many tumor cells including breast cancers, and this elevation is related to acquired resistance to anticancer agents [3, 4]. Because drug resistance is a major impediment in clinical treatment of cancers, various approaches using BSO as an adjuvant to cancer therapy have been under development. Experiments *in vitro* have established the potential of BSO as a cancer therapeutic sensitizer. A clinical trial is currently ongoing [5]. However, it is important to fully understand the effect of BSO-induced GSH depletion not only on tumor cells but also on normal tissues in order to develop it as a drug for clinical application in cancer therapy.

The results obtained in the present study demonstrate that the effect of BSO on the ovary function has to be considered in the application of this agent in clinical trials for cancer therapy, particularly in the treatment of breast cancers in women. A third of the breast cancers are estrogen dependent and respond to endocrine therapy. The use of agonistic analogues of luteinizing hormone releasing hormone (LHRH) is an established therapy for hormone-dependent metastatic premenopausal breast cancer [12]. The mechanism of action of LHRH in this disease is the suppression of ovarian estrogen production. Under this disease condition, the application of BSO may be beneficial. However, for the treatment of hormone-independent breast cancers in women, systemic chemotherapy causes estrogen deficiency [13]. Estrogen-replacement therapy has been applied and shown to prevent osteoporosis and reduce fracture risk in postmenopausal women [14]. Studies also show a marked reduction in cardiovascular disease by the estrogen-replacement therapy [15]. Therefore, the application of BSO under this disease condition may be problematic due to the estrogen deficiency.

In this study, depletion of GSH in the ovary and depression of estrogen and progesterone in plasma were observed. However, the relationship between the decreased production of estrogen and progesterone and the depletion of GSH was not established. It is possible that GSH plays a role in production and/or secretion of ovarian hormones. Other possibilities, however, also exist. Previous studies have shown that BSO inhibits cellular amino acid transport and causes cytostasis, which are independent of GSH depletion [16]. Clinical trial of BSO showed that it enhances melphalan-induced leukopenia and thrombocytopenia in humans [5]. These side-effects have not been demonstrated to result from GSH depletion. Whether the hormonal effects of BSO and those other effects observed in the present and previous studies correlate with cellular GSH depletion requires further investigation.

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