Effect of Buthionine Sulfoximine Administration on Serum Glutathione Level in Pregnant and non-Pregnant Mice

Shiva Asadpour,[1] Jafar Soleimanirad,*[1] Leila Roshangar,[1] Hadi Karami[2]

1. Department of Histology, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran

Article information	Abstract
	Background: Buthionine sulfoximine is an agent that reduces intracellular glutathione and anti-oxidant enzymes and by this means is involved in pathology of some disease. Since low glutathione level may affect embryonic development, the aim of the present study is to investigate glutathione reduction in pregnant and non-pregnant mice. **Materials and Methods**: In the present study, the mice were divided into 4 groups (10 in each group). The groups included pregnant and non-pregnant, each one consisting of a control and an experimental subgroup. The experimental groups received 2 mMol/kg buthionine sulfoximine (in the pregnant group on the 10th day of pregnancy), then 12 hours after buthionine sulfoximine injection, the mice in all control and experimental groups were killed and the blood obtained from their heart, and the glutathione level were determined and compared with each other. **Results**: Glutathione level in experimental pregnant group was reduced significantly ($p<0.05$) in comparison with control pregnant group. In non-pregnant group, also the level of glutathione were reduced significantly ($p<0.05$) in comparison to control group. **Conclusion**: The results indicated that buthionine sulfoximine injection could reduce glutathione level both in the pregnant and non-pregnant mice. Also the effect of buthionine sulfoximine in the pregnant group was more extensive than that of non-pregnant group.

Introduction

Buthionine sulfoximine (BSO) is a compound that delays the synthesis of gamma-glutamyl cysteine synthetase enzyme (γ-GCS) and reduces glutathione (GSH) and antioxidant enzymes levels [1-3]. GSH is the major intracellular antioxidant and is abundant in humans and mammals and has an important role in biological processes; because its sulfhydryl group in cysteine is a strong nucleophile and acts as a first line defense against reactive oxygen species (ROS) and to neutralize free radicals [4-6]. When GSH and other endogenous antioxidants decrease, ROS and other oxidants accumulate in biological medium, then lead to oxidative stress followed by pathological effects such as cancer, AIDS and some infections, neurological and androgenic diseases which can lead to infertility [7-12]. For this reason, BSO is used as an anti-tumor drug in chemotherapy due to its ability to reduce GSH [13-15]. GSH is the main antioxidant in preventing the formation of new free radicals. This antioxidant transforms the different free radicals to molecules with less negative effects. Free radicals react with atoms or other molecules such as unsaturated fatty acids, proteins, nucleic acids, or lipopolysaccharide, thus abnormal compounds are formed. Meanwhile, the negative effects of lipid peroxides on somatic cells are inhibition of glycolysis pathway, erythrocyte breakdown, sulfhydryl oxidation, proteins and amino acid transformations, membrane destruction, disabling the membrane-bound enzymes, and DNA alterations [16, 17].

Since the effects of BSO on glutathione reduction during pregnancy were not specified, in this study we compared GSH reduction levels resulted from 2 mMol/kg BSO injection, in pregnant and non-pregnant mice, in order to obtain knowledge about the GSH decrease during pregnancy and prevent its adverse effects.

Materials and Methods

In this study, glutathione and total protein kits were obtained from Randox (Randox lab. Crumlin, UK), buthionine sulfoximine was obtained from Sigma (Sigma Aldrich, St. Louis, MO) and other chemicals were obtained from Merck. BSO was maintained in appropriate conditions (temperature below zero degrees Celsius) until the experiment time. In this research, glutathione was measured by the kit according to manufacturer's protocol.

For preparation of 2 mMol/kg BSO, 0.015 mg of BSO powder was dissolved in 32 cc normal saline, and then injected intraperitoneally according to animal weight. In this study, 40 Balb/C mice of both sexes, weighted averagely 20-25 gram were used. The mice were provided from the animal house of Tabriz Faculty of Pharmacy. They were kept in similar cages and place, under standard conditions with 22˚C and the same light cycles of 12

hours light and 12 hours of darkness. Food and water were provided as needed and on a daily basis.

For correct breeding of animals, the mice physiological and psychological factors must be considered. Thus, after preparation of the mice and prior to research, they were maintained in the animal house of the histology department for 2 weeks to adapt with the environment. Then half of the female mice were placed in a cage with the male mice for mating (two female mice with one male mouse). The next day, after seeing the vaginal plug (the first day of pregnancy), the mice were considered pregnant and were divided into two control and treatment groups (10 mice per group); the non-pregnant mice also were divided into two control and treatment groups (10 mice per group).

2 mMol/kg BSO was injected intraperitoneally to both non-pregnant and pregnant mice at the tenth day of gestation. After 12 hours, BSO receiving mice in both experimental and corresponding control groups were anesthetized with chloroform and blood was sampled from their heart.

The collected blood was slowly poured into the microtube and the plasma was immediately isolated from blood cells using microcentrifuge. GSH of the plasma was measured through cyclic enzymatic Tietz method [18, 19]. This method is based on enzymatic processes through which GSH is consecutively oxidized by glutathione reductase with DTNB (5, 5-dithiobis-2-Nitrobenzoic acid) and is reduced in the presence of NADH; the formation of TNB (2-Nitro-5-thiobenzoic acid) is determined with a spectrophotometer, and the quantification of GSH is performed using the standard curve. So that GSH reacted with DTNB and light absorbance changes were measured at 412 nm wavelength for 10 minutes with 1 minute intervals. Absorption curve changes plotted versus time and its slope was calculated. To prepare a standard curve, various concentrations (25, 50, 100, and 150 µMol) of reduced glutathione were used. All the above mentioned steps were repeated for standard solutions, and the absorption curve was plotted versus time and the slope was calculated. The final standard curve was plotted based on the slope of the concentrations. Then, total glutathione concentration in the unknown sample was compared with standard curve.

The data obtained from measurement of glutathione level of blood plasma were analyzed by Mann–Whitney *U* test with SPSS-15 statistical software and the data significance level was considered ($p<0.05$).

Results

The results obtained from GSH concentration measurement in pregnant mice is shown in graph [1]. As can be seen, in total, the mean level of GSH concentration in the pregnant control group and in the pregnant experimental group were 4.7±1.27 µMol/l and 2.44±1.25 µMol/l, respectively. The observed differences in the mean value of GSH concentration in the pregnant mice receiving BSO was reduced compared to the control group and this reduction was significant ($p=0.03$).

Measurement of GSH concentrations in non-pregnant mice is shown in graph [2]. As can be seen, in total, the mean level of GSH concentration in the non-pregnant control group and in the non-pregnant BSO receiving group were 3.90±0.80 µMol/l and 1.65±0.52 µMol/l, respectively. According to the test, the observed difference in the mean value of GSH concentration in non-pregnant mice of the experimental group was reduced compared to the control group and this reduction was significant ($p=0.04$).
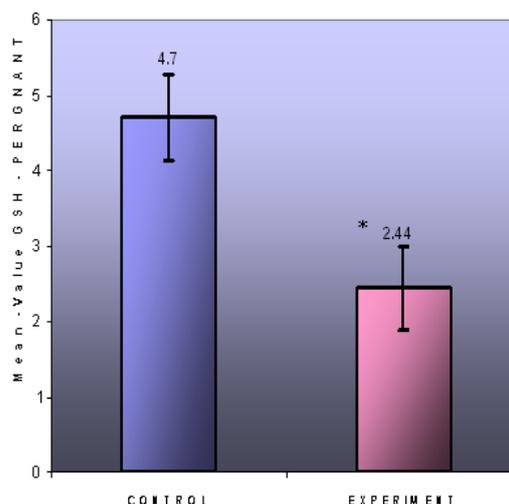

Figure 1. Comparison of GSH concentration in the pregnant samples of the experimental and control groups in terms of (µMol)
*The difference in GSH plasma levels between the experimental group compared to control group was significant ($p=0.03$)
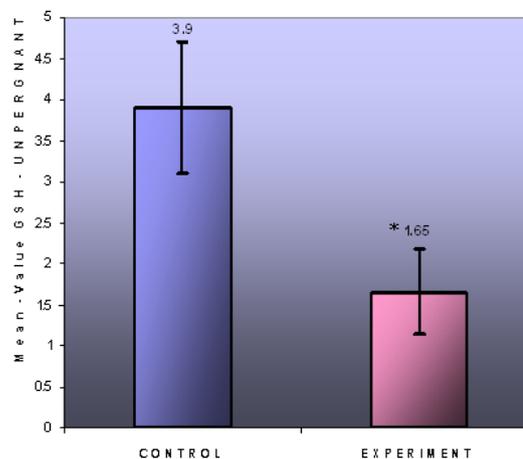

Figure 2. Comparison of GSH concentration in the non-pregnant samples of the experimental and control groups in terms of (µMol)
*The difference in GSH plasma levels between the experimental group compared to control group was significant ($p=0.04$)

Discussion

In the present study, intraperitoneal injection of 2 mMol/kg buthionine sulfoximine led to significant glutathione decrease in pregnant and non-pregnant mice. Consistent with the findings of the present study, Li et al. have shown that GSH levels decreased during treatment with BSO [20].

The mechanism of glutathione reduction by BSO can be justified with regard to the earlier findings as follows: BSO is a potent and specific inhibitor of gamma-glutamyl cysteine synthetase and when administered to animals or combined with tissue culture media, inhibits glutathione synthesis and reduces cellular glutathione levels [21]. The gamma-glutamyl cysteine synthetase enzyme catalyzes BSO phosphorylation through MgATP [22, 23].

The expression of API (transcription factors) is often associated with cell proliferation and differentiation. The activation of API (c-Jun, c-Fos, and NFKB) transcription factors depends on intracellular environment and it is observed that c-Fos and c-Jun mRNA levels increase in cells exposed to BSO. The API sites are in γ-GCS gene and this may be the answer to the mRNA expression of this gene. The intracellular GSH levels regulate the Fos and Jun genes. Increase in c-Fos and c-Jun genes expression leads to decreased GSH [24-27]. GSH synthesis is essential during development and is important for organogenesis [28, 29]. So that in the studies of Hales et al. it has been show that glutathione reduction with buthionine sulfoximine during pregnancy increases the number of dead fetuses and malformations [30]. Also the studies performed by Reliene et al. showed that GSH decrement during pregnancy increases the DNA deletions which lead to carcinoma [31].

The findings of the percent study indicate a 51% decrease in GSH caused by BSO in pregnant mice compared to 42% in non-pregnant mice. Thus, regarding to the toxic effects of BSO and since it can cause fetal anomalies we suggest that if BSO is required during pregnancy, its low doses can be used. The present study results showed that while the injection of BSO reduced glutathione level in pregnant and non-pregnant mice, the effect of reduced glutathione during pregnancy is longer than non-pregnancy period.

References

1. Zhao HW, Zhou D, Haddad GG. Antimicrobial peptides increase tolerance to oxidant stress in Drosophila melanogaster. J Biol Chem 2011; 286(8): 6211-8.

2. Salmen J, Skufca F, Matt A, et al. Role of glutathione in reproductive tract secretions on mouse preimplantation embryo development. Biol Reprod 2005; 73(2): 308-314.

3. Bełtowski J, Jamroz-Wisniewska A, Wojcicka G, et al. Renal antioxidant enzymes and glutathione redox status in leptin-induced hypertension. Mol Cell Biochem 2008; 319(1-2): 163-174.

4. Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. Fertil Steril 2003; 79(4): 829-843.

5. Irvine DS. Glutathione as a treatment for male infertility. Rev Reprod 1996; 1(1): 6-12.

6. Pastroe A, Federici G, Bertini E and Piemonte F. Analysis of glutathione: Implication in redox and detoxification. Clin Chim Acta 2003; 333(1): 19-39.

7. Wells PG, Bhuller Y, Chen CS, et al. Molecular and biochemical mechanisms in teratogenesis involving reactive oxygen species. Toxicol Appl Pharmacol 2005; 207(2 suppl): 354-66.

8. Hao H, Ying L, Zheo-Ning J and Guo-Qing L. Up-regulation of P-glycoprotein expression by glutathione depletion-induced oxidative stress in rat brain microvessel endothelial cells. J Neourochem 2006; 98(5): 1465-1473.

9. Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 1999; 222(3): 236-245.

10. Emanuele MA, Emanuele N. Alcohol and the male reproductive system. Alcohol Res Health 2001; 25(4): 282-287.

11. Ebisch IM, Peters WH, Thomas CM, et al. Homocysteine, glutathione and related thiols affect fertility parameters in the (sub) fertile couple. Hum Reprod 2006; 21(7): 1725-1733.

12. Maneesh M, Jayalekshmi H. Role of reactive oxygen species and antioxidants on pathophysiology of male reproduction. Indian J Clin Biochem 2006; 21(2): 80-89.

13. Ali-Osman F, Antoun G, Wang H, et al. Buthionine sulfoximine induction of gamma-L-glutamyl-L-cysteine synthetase gene expression, kinetics of glutathione depletion and resynthesis, and modulation of carmustine-induced DNA-DNA cross-linking and cytotoxicity in human glioma cells. Mol Pharmacol 1996; 49(6): 90-102.

14. Anderson CP, Keshelava N, Satake N, et al. Synergism of buthionine sulfoximine and melphalan against neuroblastoma cell lines derived after disease progression. Med Pediatr Oncol 2000; 35(6): 659-662.

15. Chew EH, Matthews CS, Zhang J, et al. Antitumor quinols: Role of glutathione in modulating quinol-induced apoptosis and identification of putative cellular protein targets. Biochem Biophys Res Commun 2006; 346(1): 242-251.

16. Davies KJ. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. IUBMB Life 2001; 50(4-5): 279-289.

17. Finkel T. Oxidant signals and oxidative stress. Curr Opin Cell Biol 2003; 15(2): 247-254.

18. Devine PJ, Sipes IG, Hoyer PB. Effect of 4-vinylcyclohexene diepoxide dosing in rats on GSH levels in liver and ovaries. Toxicol Sci 2001; 62(2): 315-320.

19. Eady JJ, Orta T, Dennis MF, et al. Glutathione determination by the Tietze enzymatic recycling assay and its relationship to cellular radiation response. Br J Cancer 1995; 72(5): 1089-109.

20. Li LY, Seddon AP, Meister A. Spermatogenic cell-somatic cell interactions are required for maintenance of spermatogenic cell glutathione. Biol Reprod 1989; 40(2): 317-31.

21. Belmonte MF, Stasolla C. Applications of DL-buthionine-[S,R]-sulfoximinedeplete cellular glutathione and improve

white spruce (Picea glauca) somatic embryo development. Plant Cell Rep 2007; 26(4): 517-23.

22. Ford JM, Yang JM, Hait WN. Effect of buthionine sulfoximine on toxicity of verapamil and doxorubicin to multidrug resistant cells and to mice. Cancer Res 1991; 51(1): 67-75.

23. Griffith OW. Mechanism of action, metabolism, and toxicity of buthioninesulfoximine and its higher homologs, potent inhibitors of glutathione synthesis. J Biol Chem. 1982; 257(22): 13704-12.

24. Salmen JJ, Skufca F, Matt A, et al. Role of glutathione in reproductive tract secretions on mouse preimplantation embryo development. Biol Reprod 2005; 73(2): 308-314.

25. Choe H, Hansen JM, Harris C. Spatial and temporal ontogenies of glutathione peroxidase and glutathione disulfide reductase during development of the prenatal rat. J Biochem Mol Toxicol 2001; 15(4): 197-206.

26. Obolenskaya MYu, Tschaikovskaya TL, Lebedeva LM, et al. Glutathione status of placenta from differently polluted regions of Ukraine. Eur J Obstet Gynecol Reprod Biol 1997; 71(1): 23-30.

27. Obolenska M. [Detoxicating function of the placenta of childbearing women from ecologically unfavorable regions of the Ukraine] [Ukraine]. Ukr Biokhim Zh 1998; 70(2): 89-97.

28. Shi ZZ, Osei-Frimpong J, Kala G, et al. Glutathione synthesis is essential for mouse development but not for cell growth in culture. Proc Natl Acad Sci USA 2000; 97(10): 5101-5106.

29. Yan J, Hales BF. Depletion of glutathione induces 4-hydroxynonenal protein adducts and hydroxyurea teratogenicity in the organogenesis stage mouse embryo. J Pharmacol Exp Ther 2006; 319(2): 613-621.

30. Hales BF, Brown H. The effect of in vivo glutathione depletion with buthionine sulfoximine on rat embryo development. Teratology 1991; 44(3): 251-257.

31. Reliene R, Schiestl R. Glutathione depletion by buthionine sulfoximine induces DNA deletions in mice. Carcinogenesis 2005; 27(2): 240-244.