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

Shiva Asadpour,1 Jafar Soleimanirad,2* Leila Roshangar,1 Hadi Karami2

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

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.

Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.

Introduction

Buthionine sulfoximine (BSO) is a compound that delays the synthesis of gamma-glutamyl cysteine synthetase enzyme ($\gamma$-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]. BSO 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).

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.

Acknowledgements

The authors of this research thank the research deputy of Tabriz University of Medical Sciences for funding of this research which is related to the thesis of Ms. Shiva Asadpour with code number 6/4-87/2.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

Tabriz University of Medical Sciences.

References

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.