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Taurine supplementation protects lens against glutathione depletion

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Abstract. – OBJECTIVE: Cataract which is defined as opacification of eye lens forms approximately 40% of total blindness causes all through the world. Age is the biggest risk factor for cataracts and oxidative stress is known to be one of the most important factors causing cataract formation. Age-related nuclear cataract (ARN) is associated with a loss of glutathione in the center of the lens. Taurine is an important antioxidant in lens tissue. Although, there is a high amount of taurine in lenses in early life, its concentration declines with age. In this study, we aimed to investigate the effects of supplemental taurine in lens tissues in an *in vivo* oxidative stress model which is induced by glutathione depletion to mimic ARN.

MATERIALS AND METHODS: Glutathione depletion was induced in rabbits subcutaneously with l-Buthionine –(S,R)-sulfoximine (BSO)– a glutathione inhibitor and the rabbits were treated with taurine. Total GSH, reduced GSH, GSH/GSSG ratio and MDA levels were measured.

RESULTS: BSO lowered the reduced GSH and total GSH levels and GSH/GSSG ratio. Taurine reversed these effects. On the other hand, BSO enhanced MDA level which is normalized by taurine.

CONCLUSIONS: These findings suggest that glutathione depletion with BSO may be a useful model to mimic ARN and dietary intake of taurine, may have an important role in decelerating the process of cataract formation.

Key Words:
Glutathione, Lens, BSO, Taurine, Cataract, Oxidative stress.

Introduction

Cataract, the opacification of the eye lens, is the leading cause of blindness worldwide, accounting for almost half of all blindness. It develops as a result of the progressive loss of transparency of the lens[1]. The lens has very high ATP levels and thus oxidative metabolism is very important in maintaining the lens in a transparent state[2]. Age is the biggest risk factor for cataract and oxidation is the hallmark of age-related nuclear (ARN) cataract. Loss of protein sulfhydryl groups and the oxidation of methionine residues are progressive and increase as the cataract worsens until >90% of cysteine and half the methionine residues are oxidized in the most advanced form[3]. UV light is an oxidative stress, and the eyes are more susceptible to UV damage with age. The levels of UV filters in our lens decrease linearly with age, at a rate of 12% per decade[4]. Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) plays a crucial role in protecting the mammalian tissues against oxidative damage[5,6]. Glutathione exists at high concentrations in cells, predominantly in reduced form (GSH), but small amounts of oxidized form (GSSG) are also detectible. The GSH/GSSG ratio is generally higher than 100:1 and is considered as an indicator of cellular redox status. In oxidative stress conditions, GSH concentration decreases and the associated increase in GSSG concentration results in an increased turnover of the GSH/GSSG cycle[7].

Glutathione is the essential and primary antioxidant in lens[1,8]. Lens contains a high concentration of reduced glutathione, which maintains the thiol groups in the reduced form[9]. Decreased GSH levels have been reported in human cataractous lenses[10-13]. This loss is thought to be due to oxidation of GSH, since the levels of oxidized glutathione rise significantly once cataract develops[3].

Treatment with buthionine sulfoximine (BSO), a selective inhibitor of the enzyme γ-glutamyl-L-cystein synthetase (γ-GCS) which catalyzes the formation of GSH, leads to decreased cellular GSH levels and its application can provide a useful experimental model of GSH deficiency[14]. This model depends on continuous depletion of GSH which is based on the inhibition of the enzyme

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γ-GCS by BSO which was previously shown in mice[15], rats[16] and rabbits[17-20].

Taurine (2-aminoethanesulfonic acid) is a free sulfur β-amino acid found in animal tissues[21]. Quantitative analysis of whole ocular tissue extracts of the rat eye revealed that taurine was the most abundant amino acid in the retina, vitreous, lens, cornea, iris, and ciliary body[22]. Yanshole et al[23] on quantitative analysis of whole ocular tissue extracts of the rat eye demonstrated a significant reduction in taurine levels with advancing age. The biosynthetic capacity of humans to produce taurine is limited in neonates (the effect amplified by prematurity) and also declines with aging and in some pathological conditions such as trauma and sepsis. In these situations, the diet is likely to be an important taurine source[24-26].

Previously, the beneficial effect of taurine is investigated in *in vitro* studies and diabetic cataracts[27-30]. However, it's *in vivo* effects in an *in vivo* glutathione depletion model were not investigated. Therefore, in this study we aimed to investigate the effects of taurine in an *in vivo* glutathione depletion model.

Materials and Methods

Amimal Study Design

Taurine (cat. # 86330), buthionine sulfoximine (cat. # B2515), bicinchoninic acid protein assay kit (BCA-1), were from Sigma-Aldrich, St. Louis, MO, USA. The animal experiments were carried out in accordance with guidelines described by the Ethics Committee of the Faculty of Pharmacy, Ege University that approved the study. A total of 20 rabbits were used in this study. White rabbits of either sex (2.5-3 kg) were divided into four groups, (1) control, (2) BSO, (3) BSO+Taurine, and (4) Taurine. The first group (n=5, control group) was given only the vehicle (0.9% NaCl, 0.8 ml kg-1 body weight day-1). The second group (n = 5, BSO group) received a single subcutaneous (s.c.) injection of BSO (75 mg kg-1 body weight day-1). The third group (n = 5, BSO+taurine group) received the same dose of BSO and taurine in drinking water (1.0%, w/v). The fourth group (n = 5, taurine group) received taurine in drinking water. Throughout the 2-week treatment period, all the rabbits were kept in separate cages and allowed free access to rabbit chow and tap water. At the end of the treatment period, the rabbits were sacrificed by means of an overdose of sodium pentobarbital (50 mg/kg) and lenses were excised and immediately frozen in liquid nitrogen. The samples were stored at –80°C until measurements were performed.

Determination of Glutathione Levels

The levels of reduced GSH and total GSH after dithiothreitol reduction in metaphosphoric acid-denaturated samples were measured by pre-column derivatization with orthopthaldialdehyde by HPLC with fluorescence detection. Reversed-phase chromatographic condition included a Macherey/Nagel Nucleosil MN C18 column (250/4.6 mm, 5 μm particle size), an isocratic separation with sodium acetate (50 mM)/acetonitrile (70:30) mixture at a flow rate of 0.7 ml/min, a column temperature at 30°C, and detector settings at Ex340/Em420. The level of reduced GSH was directly calculated from oxidized GSH graph and oxidized GSH from the equation [total GSH-reduced GSH/2][31] and was expressed as μmol/g wet tissue.

Determination of Malondialdehyde Levels

To assess lipid peroxidation, the concentration of malondialdehyde, dithiobarbituric acid adducts, were measured in lenses. Lens extracts were processed with dithiobarbituric acid and the levels of thiobarbituric acid reactive substances were determined on a HPLC system equipped with a xuorescence detector[32]. MDA levels were expressed as nanomoles per gram of wet tissue (nmol/g of wet tissue).

Statistical Analysis

All data are expressed as mean± S.E.M of the groups; n indicates the number of animals. Statistical analyses of data were performed using the software GraphPad Prism 5.01 (GraphPad Software, La Jolla, CA, USA). Group comparisons were carried out using the one-way analysis of variance and the Tukey post-hoc test. Difference was considered significant at p=0.05.

Results

Effects on Lens Opacification

In BSO treated rabbits, lens opacification was visible at the end of the treatment period, whereas BSO+taurine treated rabbits did not manifest a marked opacification. The lenses of the rabbits in control and taurine groups were clear. The representative photographs for each group are shown in Figure 1.

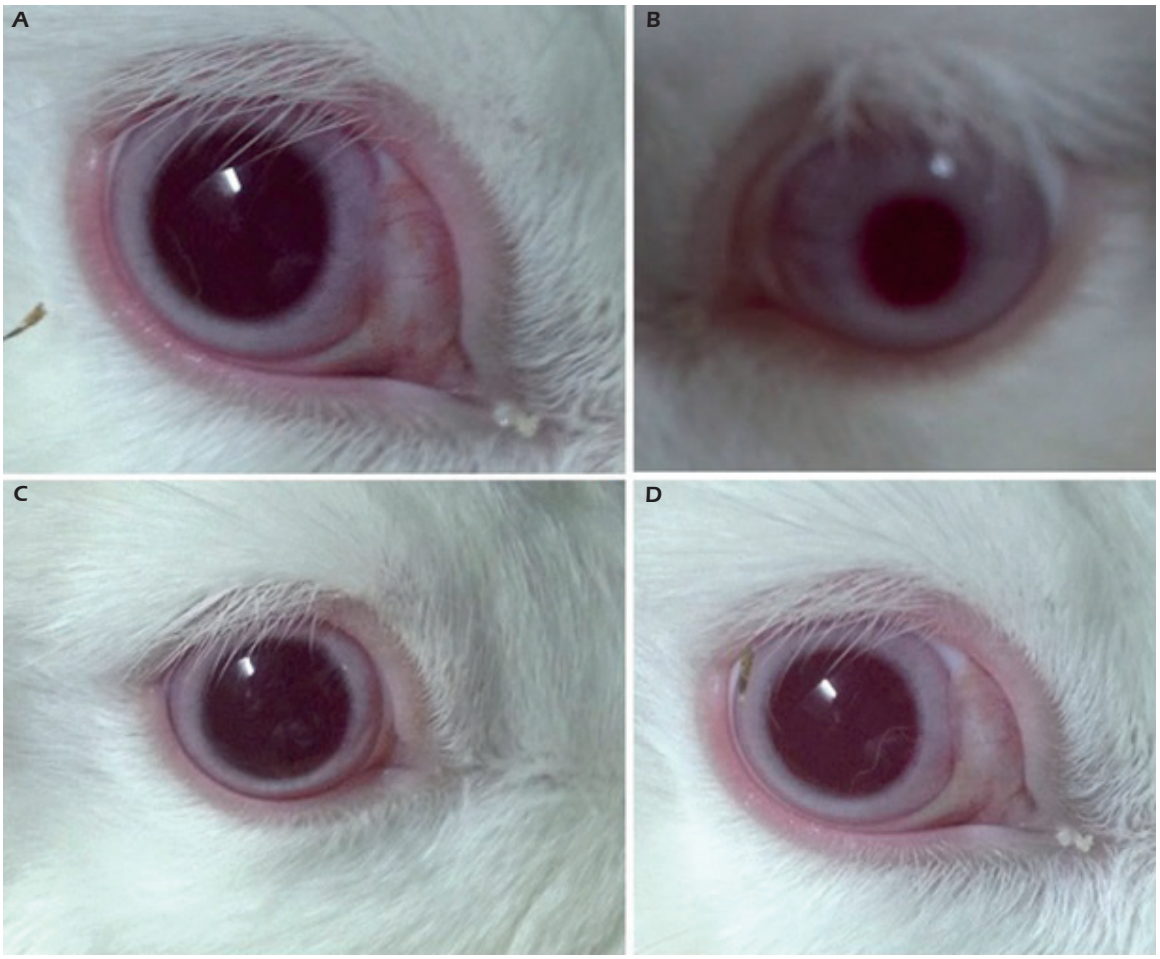

Figure 1. Representative images of lenses from (**A**) control animals, (**B**) animals treated with BSO, (**C**) animals treated with BSO+Taurine and (**D**) Taurine.

GSH Levels

Treatment with BSO significantly lowered the reduced glutathione (GSH) and total glutathione levels. When the animals were fed with taurine at the same time of BSO treatment both GSH and total glutathione levels were reversed back (Figures 2 and 3, respectively). Taurine alone had no effect on GSH levels when compared to control group (Figures 2 and 3, respectively). One of the important parameters in evaluation of glutathione capacity is the ratio between GSH and GSSG. BSO treatment lowered GSH/GSSG ratio as well and taurine reversed this effect (Figure 4).

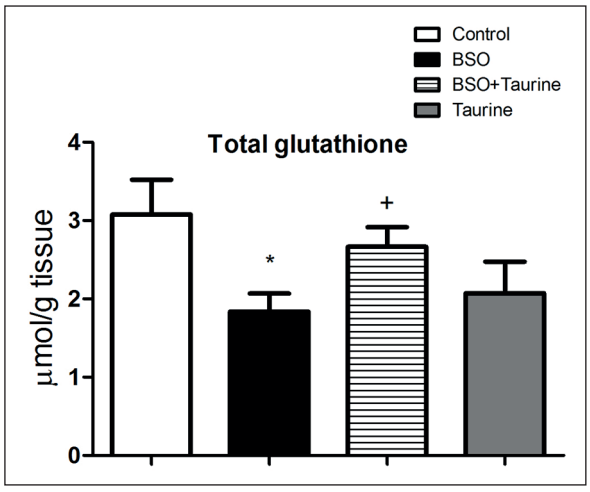

Figure 2. Levels of total glutathione in rabbit lenses of control animals and animals treated with BSO, BSO+Taurine or Taurine. Data are means ± SEM of values from five rabbits from each group. *p<0.05 Control *vs.* BSO +p<0,05 BSO *vs.* BSO+Taurine.

MDA Levels

BSO treatment enhanced MDA level. When the animals were fed with taurine at the same time of BSO treatment the MDA level was re-

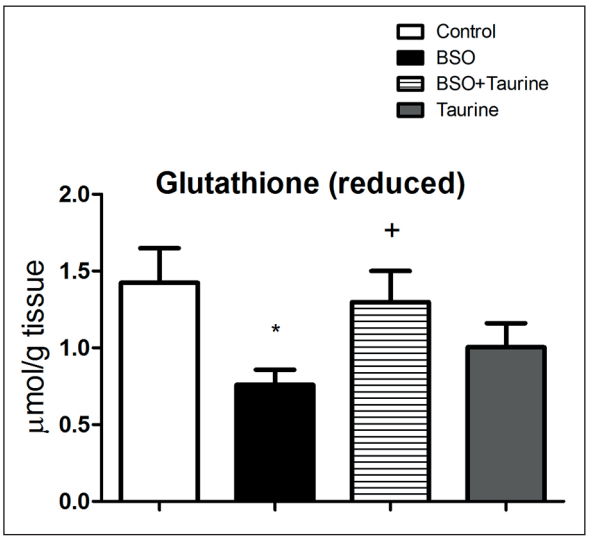

Figure 3. Levels of reduced glutathione (GSH) in rabbit lenses of control animals and animals treated with BSO, BSO+Taurine or Taurine. Data are means ± SEM of values from five rabbits from each group. *p<0.05 Control *vs.* BSO +p<0.05 BSO *vs.* BSO+Taurine.

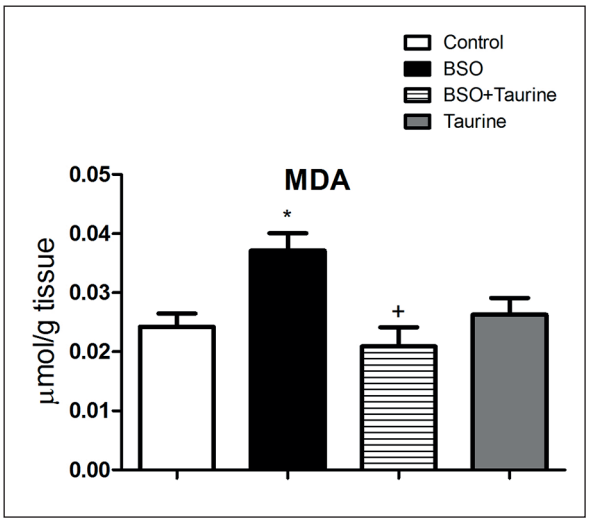

Figure 5. Levels of MDA in rabbit lenses of control animals and animals treated with BSO, BSO+Taurine or Taurine. Data are means ± SEM of values from five rabbits from each group. *p<0.05 Control *vs.* BSO +p<0.05 BSO *vs.* BSO+Taurine.

versed back (Figure 5). Taurine alone had no effect on MDA levels when compared to control group (Figure 5).

Discussion

At present, the treatment of cataracts requires removal of the opacified lens through surgery and replacing it with a synthetic lens to restore the transparency of the lens for a better vision. Although cataract surgery is considered to be a relatively safe procedure, it is expensive, and it can cause postsurgical complications such as posterior capsular opacification[33]. Therefore, alternative preventive therapies which will cause less complications for the patient and which will have less economic burden are needed to be investigated.

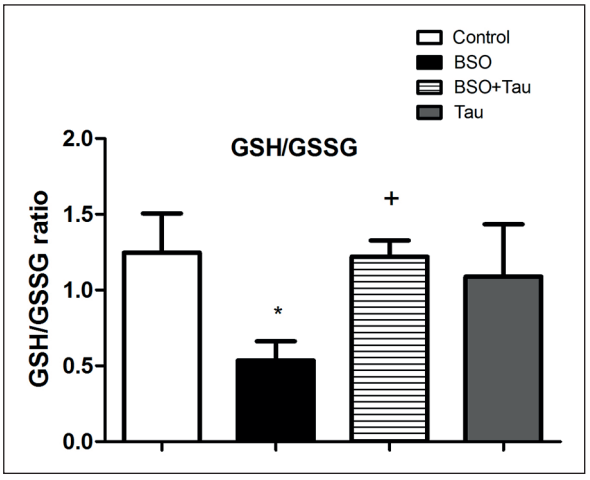

Figure 4. The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in rabbit lenses of control animals and animals treated with BSO, BSO+Taurine or Taurine. Data are means ± SEM of values from five rabbits from each group. *p<0.05 Control *vs.* BSO +p<0.05 BSO *vs.* BSO+Taurine.

GSH concentration is crucial in lens tissues, thus GSH levels can markedly affect the direction of oxidation processes. GSH above 1 mM inhibits hydroxyl radical formation whereas concentration below 1 mM accelerates its production[3]. According to oxidative stress hypothesis, age is the most common risk factor for cataracts and age-related nuclear cataract (ARN) is associated with a loss of glutathione in the centre of the lens and extensive modification of the nuclear proteins[1,6]. Oxidative stress associated with increased reactive oxygen species is known to accelerate cataract formation[2]. UVB irradiation of the albino rabbit lenses resulted in a significant decrease in the concentrations of glutathione and taurine[34]. There are several animal models used to mimic ARN cataract such as diabetic cataract, UV-induced, steroid-induced or hyperbaric oxygen-induced cataracts none of which could represent fully the ARN cataract[35-37].

In early ages, although a number of antioxidant defence mechanisms such as superoxide dismutase, catalase and antioxidants such as vi-

tamin E (α-tocopherol), lutein and zeaxanthin are present to protect against ROS-mediated tissue damage, glutathione (GSH) is the principal antioxidant. However, in older ages glutathione levels become significantly depleted which leads to lens opacification[38]. Although the concentrations of other antioxidants which are present in lens tissue such as vitamin E, lutein and zeaxanthin contents do not reveal any difference between normal and cataractous lenses, GSH levels are depleted[38,39]. This depletion of GSH is not uniform and occurs in the nucleus of the lens with advancing age[3]. A reduction in the ability of circulation system to deliver GSH to the lens and/or a modification in the transporters in the lens nucleus are the proposed mechanisms which lead to a reduction of GSH delivery and uptake in the lens nucleus[38]. Therefore, *in vivo* GSH depletion model with BSO which is used in this study might be a better model to represent ARN cataract.

In our study, *in vivo* taurine supplementation replenished the GSH levels and decreased the MDA levels which is an indicator of lipid peroxidation. In another *in vivo* study by Carey et al[40], N-acetylcysteine amide inhibited BSO induced cataract, reversed back GSH level decreased by BSO and inhibited lipid peroxidation. The results of both studies which are based on glutathione depletion are in agreement, although the antioxidant agents that are used are different. On the other hand, taurine is found at particularly high concentrations in tissues exposed to elevated levels of oxidants, suggesting its role in the attenuation of oxidative stress[24]. The effects of taurine in lens have been demonstrated by other investigators. Taurine is found to be protective against UV-B induced apoptosis in lens epithelial cells[41]. Devamanoharan et al[27] showed that incubation of rat lenses by an oxidant menadione decreased GSH and co-incubation with taurine increased GSH. In another study[30], taurine prevented oxidative damage induced by high glucose in isolated rat lenses whereas it did not change the opacity induced by high glucose. The effect of dietary taurine supplementation on GSH and lipid peroxidation was revealed in streptozotocin-diabetic rats fed with taurine supplemented diet[29]. Likewise, taurine is also found to be protective against alloxan-induced diabetic cataracts in New Zealand White rabbits[28]. The results of all these studies support our findings on GSH levels and lipid peroxidation which is represented by MDA levels. However, the levels of taurine in the lenses significantly decrease in senile cataracts compared with normal lenses. Senile cataracts are believed to have an oxidative mechanism rather than the osmotic mechanism of diabetic cataracts[28,42]. In this sense, glutathione depletion model which was used in this study might be a better *in vivo* model representing the oxidative stress induced in ARN cataract to observe the beneficial effects of taurine which is another important player in lens tissue that reduces with age.

Conclusions

This study reflects two points. Both glutathione and taurine are crucial for the maintenance of transparency of lens. One point is, as the lens age, the balance between reduced and oxidized forms of glutathione is disrupted in favour of oxidized glutathione. Therefore, the glutathione depletion model which is used in this study could be a good approach in representing the clinical phenomenon of senile cataract formation. The second point is that the level of taurine decreases with age in lens tissue. The protective role of taurine in re-establishing the glutathione balance reveals its importance as an antioxidant in lens tissues. Therefore, supplementation of taurine, an important antioxidant in lens tissue which normally decreases with age, may have a therapeutic role in decelerating the process of cataract formation. In future studies, the effects of long-term taurine supplementation on lens opacification in age-related human cataracts are needed to be investigated.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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