Taurine Prevents Oxidative Damage of High Glucose-Induced Cataractogenesis in Isolated Rat Lenses

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Summary Diabetic cataract is an ocular disease represented as blindness by lens opacification. Oxidative as well as osmotic stress caused by accumulation of polyols within the lens has been shown to be associated with glucose-induced cataractogenesis. Taurine has an antioxidant capacity and its level in diabetic cataractous lens is markedly decreased. Therefore, we investigated whether taurine is a part of antioxidative defense mechanism involved in protecting the lens against high glucose-induced oxidative stress and tissue damage. Lenses were isolated from male Sprague-Dawley rats weighing about 180–200 g and cultured in high glucose medium (55.6 mM) for 6 d as a model of high glucose-induced cataractogenesis. To investigate the antioxidative effect of taurine, 30 mM taurine was added in normal medium for 2 d before the addition of high glucose. The culture of lenses in high glucose medium increased the weight and opacity of lenses of and the carbonylated protein level, and decreased glutathione (GSH) content. Although there were no significant effects of taurine on the weight or opacity of lenses, pretreatment of lenses with 30 mM taurine significantly reversed the level of protein carbonylation and GSH to those of controls. Therefore, taurine might spare GSH and protect the lens from oxidative stress induced by a high concentration of glucose.

Key Words cataract, oxidative stress, glucose, taurine, GSH

Diabetic cataract, one of the secondary complications of diabetes, is the major cause of blindness due to the opacification of the lens. The lens is the only transparent organ in the body and focuses light on the retina. The main components of the lens are about 63% of water and 35% of protein which is composed of over 90% of crystallins (1). Because the turnover rate of crystallins is very slow compared to many other organs, the reversal of protein modification by several factors is slow and it can be thought to induce cataract (2). The mechanism of cataractogenesis is not clear, but the osmotic stress and the oxidative stress have been thought of as the major causes of cataractogenesis (3). The osmotic stress by excessive accumulation of sorbitol formed from elevated glucose has been reported as the main mechanism of diabetic cataract. But several studies indicated that oxidative stress may be the major contributor to a sugar-induced cataractogenesis, which can be prevented or attenuated by antioxidants such as vitamin E (4), glutathione (GSH) (5), vitamin C (6), and N-acetylcysteine (7). It is shown that levels of major antioxidants were reduced in cataractogenesis by osmotic compensation against sorbitol accumulation (8, 9). Along with the loss of antioxidants, the increase of glucose concentration in the lens would result in higher free radical generation and nonenzymatic glycation (10, 11).

Taurine (2-aminoethanesulfonic acid) has a sulphonic acid group, which is not found in other amino acids. Because this amino acid is not used for the synthesis of protein, it is abundant as a free amino acid in the range of 5–50 mM with especially high concentrations in the heart, retina, brain, and neutrophils (12). Taurine is involved in several physiological reactions such as bile acid conjugation, osmoregulation, calcium transport regulation, and cell membrane stabilization. It is also known to protect tissues against oxidative damage by scavenging hypochlorous acid (HOCl), although antioxidant action of taurine remains unestablished (13, 14). Several studies showed that the levels of taurine and GSH were reduced significantly in diabetic cataractous lens (15, 16) and dietary supplementation with taurine reduced tissue damage and mortality rate resulting from diabetes (8, 17, 18). The present study was therefore carried out to investigate whether pretreatment with taurine can protect the lens against oxidative stress and cataract formation in glucose-induced cataractogenesis. Lenses were cultured in 55.6 mM glucose-containing medium for 6 d as a model of diabetic cataractogenesis and several parameters were measured to verify our hypothesis.

MATERIALS AND METHODS

Chemicals. Most reagents were obtained from Sigma...
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Chemical Co. (St. Louis, MO, USA), unless otherwise stated. Medium 199 (M199), glutamine, gentamycin, and fungizone were purchased from Invitrogen (Carlsbad, CA, USA).

Lent organ culture. Male Sprague-Dawley rats weighing about 180–200 g were killed by CO2 inhalation and the eyes were extracted. The rats were maintained and handled according to the policies and procedures of the Institutional Animal Care and Use Committee of the Seoul National University. The lenses were carefully removed by a posterior approach and immediately incubated in 24-well cluster plates containing Medium 199 based on the method of Spector et al. (19). The final preparation had an osmolality of 290–300 mOsm. Lens culture was maintained at 37°C with an atmosphere of 95% air/5% CO2. No metal instruments that could cause damage were used to handle the lenses. Damaged lenses which rapidly lost their transparency, were excluded from the experiments and lens viability was also checked by measuring protein leakage in incubation media after 24 h of incubation (20). Lenses were divided randomly into four groups. The lenses from the first group were incubated in a normal medium (M199) served as a control (n=13). The second group of lens was incubated in a normal medium for 6 h after being incubated in a medium containing 30 mM taurine for 2 d (n=12). The third group was incubated in a normal medium for 2 d and then in a medium containing 55.6 mM glucose for 6 d (n=12). The fourth group was incubated in a medium containing 30 mM taurine for 2 d and then in a medium containing 55.6 mM glucose for 6 d (n=13). The medium was changed every 24 h. Glucose-treated lenses turned opaque within 6 d. After culture, lenses of each group were divided into three sets (n=3–4 each set) and were used for biochemical measurements.

Measurement of the lenticular opacity. Photographs of the lens were taken using a dark-field set up with illumination from a circular light situated above the lens by microscope equipped with a CCD camera (Olympus, Japan). The outline of the lens image was determined by selecting 4 points on the image, and then the transparent area within the outline and thread layer were set automatically by the software (21). The total area of opacity, in pixels, was analyzed by a computer using Image Pro Plus system (Media Cybernetics, USA). The lenses were classified into 6 grades according to the density of opacity, which is expressed as an arbitrary unit of pixels (Grade I: <20, Grade II: 20–30, Grade III: 30–40, Grade IV: 40–50, Grade V: 50–60, Grade VI: >60).

Determination of the lenticular GSH level. The GSH level in the lens was determined by Ellman’s reaction (22). Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0. Proteins was precipitated by centrifugation after the addition of an equal volume of a 20% TCA solution. The supernatant was then mixed with eight-times volume of 0.3 M sodium phosphate and an equal volume of 5,5’dithiobis(2-nitrobenzoic acid) (DTNB) solution prepared by dissolving 4 mg of DTNB in 10 mL of 1% trisodium citrate solution. The absorbance was measured spectrophotometrically at 410 nm and GSH level was calculated with reference to the standards.

Determination of the lenticular taurine level. The level of taurine in the lens was determined by HPLC. Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0 and homogenate was centrifuged at 10,000 ×g for 5 min. Proteins were precipitated by centrifugation after the addition of acetoni-trile. The supernatant was incubated with dabsyl chloride, a derivatizer, at 70°C for 10 min and then filtered. The column was Waters symmetry C18 (3.9×150 mm) and mobile phases were composed of solution A (20 mM sodium acetate, 0.05% triethylamine, pH 6.5) and solution B (acetoniitril) (73 : 27). The mobile rate was 1.0 mL/min.

Determination of glutathione peroxidase activity. The glutathione peroxidase activity was determined by the procedure of Tappel (23). Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0 and homogenate was centrifuged at 10,000 ×g for 20 min. Twenty microliters of the supernatant were then mixed with 310 μL of the assay mixture (50 mM Tris, pH 7.6, 0.1 mM EDTA, 0.25 mM GSH, 0.12 mM NADPH, and 1 U/mL glutathione reductase). The reaction was initiated by addition of 10 μL of cumene hydroperoxide and absorbance at 340 nm was recorded. The activity was calculated using a molar extinction coefficient for NADPH of 6.22 μmol·cm−1·L−1 at 340 nm and expressed in units per milligram of protein.

Determination of glutathione reductase activity. The glutathione reductase activity was determined by the procedure of Carlberg and Mannervik (24). Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0 and the homogenate was centrifuged at 10,000 ×g for 20 min. Twenty microliters of the supernatant were then mixed with 400 μL of the assay mixture (0.1 M potassium phosphate buffer, pH 7.0, 1 mM EDTA, 0.1 mM NADPH, and 1 mM oxidized glutathione) and incubated at 30°C. The absorbance at 340 nm was recorded and the activity was expressed in units per milligram of protein. To calculate the specific enzyme activity, protein in each sample was measured by the method of Lowry et al. (25).

Determination of carbonylated protein level. Protein carbonyl content in the lens was analyzed as described by Levine et al. (26). Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0 and were further diluted to 0.5 mg protein/mL in 0.1 M potassium phosphate buffer, pH 7.0. Proteins were precipitated by centrifugation after the addition of an equal volume of a 20% TCA solution and the pellet was mixed with 1 mL of 10 mM 2,4-dinitrophenyldiazine (DNPH) in 2 N HCl and kept at room temperature for 1 h with vortexing every 10 min. Five hundred microliters of 20% TCA was added and the precipitated proteins were subsequently washed three times with ethanol : ethylacetate (1 : 1). The final pellet was redis-

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solved in 1 mL of 6 M guanidine solution, pH 2.3. After removing any insoluble material by centrifugation at 10,000 × g for 10 min, the absorption of the supernatant was measured spectrophotometrically at 370 nm. The blank of each sample was prepared by treatment with 2 N HCl instead of 10 mM 2,4-DNPH in 2 N HCl.

**Determination of TBARS level.** The level of thiobarbituric acid reactive substances (TBARS) in the lens homogenate was analyzed according to the method of Ohkawa et al. (27). Briefly, lenses were homogenized in 10 volumes of 0.1 M potassium phosphate buffer, pH 7.0 and were further diluted to 2 mg protein/mL in 0.1 M potassium phosphate buffer, pH 7.0. Five hundred microliters of homogenate were incubated at 37°C for 1 h, and then 500 μL of 10% (w/v) TCA and 500 μL of 0.67% thiobarbituric acid were added to the reaction mixture. After vortexing vigorously for 30 s and the reaction mixture was boiled for 15 min, cooled, and then centrifuged at 2,500 × g for 15 min. Finally, the absorption of the supernatant was measured spectrophotometrically at 535 nm. As a standard, 1,1,3,3-tetraethoxypropane was used.

**Statistical method.** Statistical analyses were performed using SAS statistical programs. Values were expressed as the mean ± SE. Analysis of variance coupled with Duncan’s new multiple-range test was used to detect the mean differences among groups. A value of p<0.05 was considered statistically significant.

**RESULTS**

Rat lenses were pre-cultured in 30 mM of taurine in this study and the antioxidative effect of 30 mM taurine measured by chemiluminescence assay using an ABEL-microperoxidase-H2O2 system showed 34.8% inhibition. The wet weights of lenses cultured in the different media were measured (Fig. 1). It has been known that the wet weight of lenses increases progressively with the stage of maturation of cataract (28). The weight of lenses incubated in high glucose medium was significantly increased compared to that of control lenses (p<0.05). After being cultured in a medium with taurine, high glucose-treated lenses were still significantly heavier than those of the control group (p<0.05). This result showed that the high concentration of glucose increased the weight of the lenses and taurine had no effect on decreasing that weight.

To examine whether there is a change in the lens opacity from a high concentration of glucose and the addition of taurine, the opacity of the lenses was measured individually using the Image pro analysis system (Fig. 2). An increase in grade indicates an increase in the opacity of lens. The control group was distributed at II–III, while the high glucose group without taurine was at III–V, showing opacity of the glucose-treated lenses increased markedly. The level of opacity of high glucose group with 30 mM taurine was located at II–V. With the change of the lenticular weight, the opacity of the lens clearly increased with a high concentration of glucose, but taurine did not interfere with this increase of the opacity of the lens.

The lens has several enzymatic and non-enzymatic antioxidants to protect it against ROS-mediated damage (14). The anti-cataract effect of a variety of natural and synthetic compounds has been attributed to their antioxidant properties (29); therefore, we measured several biomarkers of oxidative stress in the lens. Figure 3A presents the GSH levels of lenses after being cultured in different media for 8 d. The result showed that the GSH level in the high glucose only group was significantly decreased compared to that in the control group. The value of the taurine-supplemented group was significantly higher than the value of the group treated with glucose only, indicating that the addition of taurine to the medium of the high-glucose group reversed the
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![Graph A](image1)

![Graph B](image2)

Fig. 3. The levels of GSH (A) and taurine (B) of rat lenses cultured in the presence of 30 mM taurine and/or high (55.6 mM) glucose medium. The experiment group is described in Fig. 1. Values are mean±SE (n=3-4). Means with different superscripts are significantly different at p<0.05.

Table 1. The activities of glutathione peroxidase and glutathione reductase of rat lenses cultured in M199 in the presence of 30 mM taurine and/or high (55.6 mM) glucose medium.

<table>
<thead>
<tr>
<th></th>
<th>Glutathione peroxidase (μmol NADPH oxidized/min/mg protein)</th>
<th>Glutathione reductase (μmol NADPH oxidized/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56.40±2.37NS</td>
<td>14.93±0.72NS</td>
</tr>
<tr>
<td>Control + taurine</td>
<td>60.12±1.88</td>
<td>12.08±1.43</td>
</tr>
<tr>
<td>High glucose</td>
<td>56.18±5.57</td>
<td>12.88±3.05</td>
</tr>
<tr>
<td>High glucose + taurine</td>
<td>61.86±3.04</td>
<td>15.72±1.11</td>
</tr>
</tbody>
</table>

Lenses were cultured in the media as follows. Control: Lenses were cultured in 5.6 mM glucose medium (M199) for 8 d; Control + taurine: Lenses were cultured in M199 for 6 d after being cultured in 30 mM taurine-supplemented M199 for 2 d; High glucose: Lenses were cultured in 55.6 mM glucose medium (HGC) for 6 d after being cultured in M199 for 2 d; High glucose + taurine: Lenses were cultured in HGC for 6 d after being cultured in 30 mM taurine-supplemented M199 for 2 d. Values are mean±SE (n=3-4). NS: Means are not significantly different.

GSH level to that of the control group. To determine the fate of taurine after exhibiting antioxidative activity, we measured the level of lenticular taurine (Fig. 3B). Taurine concentration in glucose-treated lenses decreased distinctly compared to the control group (p<0.05). The level of taurine in the taurine-pretreated group was also significantly decreased compared to that of the control group (p<0.05) and was not different from that of the high glucose group without taurine. Therefore, GSH is thought to be an important factor in cellular function.
and defense against oxidative stress. We also measured total protein content per lens to determine the possibility of protein leakage due to the loss of cell viability. Although the levels of GSH and taurine were significantly decreased, there was no significant decrease in total protein content in the high-glucose treated group (data not shown), suggesting the cultured lenses retained their viability. The activities of the antioxidative enzymes, glutathione peroxidase and glutathione reductase, were not significantly changed by the addition of either glucose or taurine (Table 1).

The levels of carbonylated protein (Fig. 4A) and TBARS (Fig. 4B) were measured to investigate whether lenses were damaged by free radicals or not. A carbonyl group can be introduced into proteins by glycation and glycoxidation reactions. Therefore, protein carbonyl groups provide a reasonable marker for free radical-induced protein oxidation (30). The level of carbonylated protein in the high glucose with taurine group was significantly lower than that in the high glucose without taurine group and was reversed to that of the control group (p<0.05). Although the TBARS level was not significantly changed by the treatment of high glucose or taurine supplementation, protein peroxidation and lipid peroxidation progressed together with a highly positive correlation (r=0.8086, p=0.0001), suggesting that oxidative stress induced by the high concentration of glucose was decreased by taurine.

DISCUSSION

Here, we show that taurine prevents oxidative damage of high glucose-induced cataractogenesis in isolated rat lenses. Oxidative stress has been suggested as a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the lens has been shown to prevent or delay cataract. The ubiquitously found β-amino acid taurine has been shown to possess a number of cytoprotective properties through its actions as an antioxidant, osmoregulator, and intracellular Ca²⁺ flux regulator (31). Although taurine is considered as an inert compound chemically, taurine is capable of converting reactive hypochlorous acid into...
N-chlorotaurine (32). In addition, taurine was found to be the most reactive amino acid towards carbonyl groups, therefore, taurine could be expected to scavenge the reactive carbonyl and glycation intermediates formed intracellularly (32).

In the present study, the presence of high glucose in the medium induced a significant physiological stress to lenses, as evidenced by the increase in lens weight and opacity. This result is consistent with previous studies in which the lens was cultured in 30 mM xylose (33) or 30 mM galactose medium (34). These changes in lenses are thought to be due to the water absorption into the cells because the lenticular membrane was damaged by oxidative stress, and osmotic imbalance was caused by polyol pathway activity (28). However, in the present study, the pretreatment of lenses with 30 mM taurine could not reverse the changes in lenticular weight or opacity induced by cataract development.

Among the systems that defend against ROS in the lens are a high level of GSH, abundant antioxidant enzymes, and the chaperone-like functions of crystal-lins. The oxidative stress response of the lens is characterized by a diminishing level of GSH and reduced activities of antioxidant enzymes (35, 36). The GSH concentration in the high glucose group decreased significantly compared to that in the control group as shown in previous studies (16, 37). The decrease of GSH in the high glucose group is thought to be due to the faster GSH efflux under hyperosmotic conditions caused by an accumulation of sorbitol. In addition, the synthesis of GSH in the lens could be also decreased because the concentration of amino acids in the lens decreased as a function of osmotic pressure (15, 37). GSH is synthesized by a chain of processes from amino acids absorbed through the lens membrane (38). GSH in the cultured lenses was about 40% lower than that in non-cultured lenses within 24 h and 60% lower within 72 h, indicating that GSH concentration was sensitive to oxidative stress (39). The activities of glutathione peroxidase and glutathione reductase were not changed significantly by the treatment with glucose and/or taurine in our study. Lou et al. (37) found that under hyperglycemic conditions, there was no change in the enzyme glutatione reductase activity. They concluded that the decreased membrane transport of amino acids which are needed for GSH biosynthesis and the simultaneous loss of GSH through leaky membranes, initiated by the polyol pathway, can be responsible for the drastic GSH depletion. In a recent study using streptozotocin-induced diabetic rats, the levels of gamma-glutamylcysteine synthetase mRNA and protein were not reduced in the diabetic rats (40), suggesting that the availability of substrate may have an important role in the synthesis of GSH, rather than the level of involved enzyme.

The level of GSH in a taurine-treated high glucose group increased significantly compared to the high glucose group without taurine and was similar to the control group, indicating that taurine is involved in the action of GSH. It has been shown that the addition of taurine increased the level of GSH in the lens oxidatively stressed by menadione (14), suggesting that taurine could spare GSH. In fact, we measured the lenticular taurine content after culturing for 8 d and found that taurine content in the glucose-treated group with or without taurine decreased significantly compared to that in the control. But the taurine content of the lenses cultured in taurine-supplemented medium for 2 d was higher than that of the control lenses, indicating that taurine added to the high-glucose medium may be used in protecting the lens against oxidative stress. Other studies also suggested that the decrease of taurine in diabetic cataract lenses might be directly due to the oxidative stress (15, 34). In addition, intracellular accumulation of sorbitol is most likely to cause depletion of taurine, since specific transporter systems exist for taurine, not for sorbitol (32).

To investigate the antioxidant activity of taurine, levels of TBARS and carbonylated protein were also measured. It is thought that the lens is sensitive to oxidative stress and the increase of oxidative stress by high glucose could be a major cause of cataract. Many studies have reported the increase of MDA concentration in cataractous lenses. MDA concentration of rabbit lenses treated in 30 mM galactose for 3 d increased significantly (34). It was also reported that the concentration of carbonylated protein in diabetic lenses increased compared to normal lenses (41, 42). Taurine also functions as a modulator of intracellular Ca²⁺, which is also involved in oxidative stress-mediated cell injury (31). A recent study also showed that inhibition of peroxidation markers and upregulation of antioxidant activity in rat tissues by taurine signify the potential utility of taurine as an adjunct in the treatment of insulin resistance (43).

In the present study, taurine reversed the levels of oxidative stress and kept the GSH level high, although the physiological characteristics of the cataracts were not changed by the addition of taurine. Therefore, taurine seems to act as an antioxidant with GSH in high glucose-induced cataracts and further studies on the significance of taurine in lens physiology and cataract formation will be needed.

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