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Research article

Does oxidative stress play any role in diabetic cataract formation? ——Re-evaluation using a thioltransferase gene knockout mouse model☆

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ABSTRACT

Oxidative stress is a known risk factor in senile cataract formation. In recent years, it has been suggested that oxidation may also be associated with cataract induced by hyperglycemia, but this concept has not been well examined or validated. Since thioltransferase (TTase) is one of the key enzymes that regulates redox homeostasis and protects against oxidative stress in the lens, we have used TTase gene knockout (KO) mice as a model to examine this new concept. Lenses from 4 months old TTase KO and wild-type (WT) mice were incubated in TC199 culture medium containing 30 mM glucose for 48 h. Each lens was assessed for opacity, graded by LOCSII system, and the wet weight was recorded after which it was homogenized in lysis buffer and analyzed for water-soluble protein and free glutathione (GSH). *In vivo* studies were carried out using 4 months old TTase KO and WT mouse groups. Each mouse received two consecutive days of intraperitoneal streptozotozin (STZ) injections to induce diabetes. The lenses were examined weekly for 4 weeks using a slit-lamp biomicroscope, and then extracted and analyzed for levels of GSH, water-soluble protein, ATP and protein-GSH mixed disulfide (PSSG). TTase KO lenses cultured in high glucose developed a mild cortical opacity but slightly more than that of the WT lenses. Both groups had similar contents of soluble proteins and GSH. Exposure to high glucose did not change the soluble protein level but did suppress GSH by 20% in lenses with or without TTase. STZ-induced diabetic KO mice also developed a higher degree of mild cortical lens opacity compared to that of the diabetic WT controls. Similar 15–20% losses in lens GSH and ATP were found after one-month induced diabetes in WT and KO mice. There was a 20% greater amount of PSSG in the lenses of TTase KO than the WT control. Under diabetic condition, both groups displayed more glutathionylated proteins in the beta-actin (42 kDa) and lens crystallin proteins (18–22 kDa) regions, and some additional modified proteins at 15–17 kDa and 60–70 kDa, with a total 20–30% PSSG increment in both groups. In conclusion, we have found that hyperglycemia induced some oxidative stress-associated biochemical changes with mild lens opacity in both WT and KO mice. However, these changes were only marginally higher in the TTase KO mouse than that of the WT control, suggesting that TTase deletion may only play a minor role in the early stage of hyperglycemia-induced cataract formation in the mice.

1. Introduction

Cataract is a multifactorial eye disease associated with several risk factors such as oxidative stress, abnormality of glucose metabolism, irradiation and toxicity damage. Oxidation of lens protein thiols has been shown in human age-related cataracts (Augusteyn, 1979, 1981; Harding, 1970). Lou et al. (1986) subsequently hypothesized that during aging and/or under oxidative stress

Abbreviation: TTase, Thioltransferase; GSH, glutathione; GSSG, oxidized glutathione; STZ, streptozotozin; PSSG, glutathionylated protein; UV, ultraviolet light; KO, knockout; WT, wild type; AR, aldose reductase.

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conditions, lens glutathione (GSH) could easily being oxidized to oxidized GSH (GSSG) and conjugated with protein thiols, forming protein-GSH mixed disulfide, or glutathionylated proteins (PSSG). This process appeared to initiate the oxidative damage to the lens proteins and is followed by protein-protein disulfide formation and then protein aggregations found in the cataracts. Such findings were substantiated and confirmed in H_2O_2-induced cataract *in vitro* (Lou et al., 1990), in human lens during aging (Lou and Dickerson, 1992), and in human cataractous lenses (Lou et al., 1989a,b, 1999). Most importantly, the accumulation of PSSG was directly proportional to the lens nuclear opacity, and pigmentation (Truscott and Augusteyn, 1977; Harding, 1970; Lou, 2003). Because PSSG accumulation could be reversed in the H_2O_2-exposed rat lenses after timely removal of the oxidant in organ culture (Cui and Lou, 1993), an intrinsic oxidation defense enzyme, thioltransferase (TTase), was identified in the lens (Raghavachari and Lou, 1996).

TTase, also known as glutaredoxin, is present ubiquitously in prokaryotes and eukaryotes (Holmgren, 1979), and it can specifically reduce PSSG to protein-SH, with GSH as its cofactor. It is a small, 11.8-kDa heat-stable cytosolic protein with multiple catalytic functions for many biochemical processes. Our previous study has shown that TTase can repair the H_2O_2 -damaged glyceraldehyde 3-phospho-dehydrogenase, a key glycolytic enzyme for ATP production, in human lens epithelial cells (Xing and Lou, 2002). Along with alpha-crystallin, TTase can partially revive glutathione reductase activity in opaque or clear old human lenses (Yan et al., 2007). Apparently the powerful oxidation defense of TTase can be achieved through its upregulated gene expression during the early stage of oxidative stress. Such a unique phenomenon was demonstrated in the human lens epithelial cells under H_2O_2 stress (Xing and Lou, 2002), in pig lens exposed to H_2O_2 in culture (Wang et al., 1997; Moon et al., 2005) or in mouse lens with ultraviolet (UV) radiation (Zhang et al., 2012). The importance of TTase in lens protection was further established in a TTase gene knockout (KO) mouse model. These mice developed an earlier and more severe cataract during aging, with various biochemical changes including the extensive accumulation of PSSG in comparison with that of the wild type mice (Lou et al., 2004; Zhang et al., 2011).

Besides age-related cataract, hyperglycemic-induced cataract or diabetic cataract is a common cause of vision impairment. Several mechanisms have been proposed and studied, including protein glycation (Stevens et al., 1978), osmotic stress (Kinoshita et al., 1962) and more recently oxidative stress (Hegde et al., 2003). The latter two will be the focus of this report. The mechanism of osmotic stress has been well established in animal models. The high level of aldose reductase (AR) and the activated polyol pathway in the rat lens exposed to high glucose *in vitro* or *in vivo* have long been considered as the main culprits in causing high accumulation of sugar alcohols, whose cell impermeability cause osmotic stress to the lens with eventual cataract formation (Van Heyningen, 1959; Kinoshita, 1990; Kador et al, 1986). This hypothesis was confirmed by the total elimination of lens pathology in the rat when an aldose reductase inhibitor was used (Kador et al., 1985; Lou et al., 1989a). Mice on the other hand, have a low level of AR (Varma and Kinoshita, 1974). Thus they do not show a profound hyperglycemic effect on the lens in comparison with the rat. However, Lee et al. (1995) used AR transgenic mice and demonstrated that hyperglycemia could induce osmotic stress and cataract in these AR-enriched mice as easily as in rats.

Because the human lens has a low level of AR, mechanisms other than osmotic stress have been the subject of new enthusiastic research interest in recent years. Several reports (Hegde et al., 2003; Chan et al., 2008; Drel et al., 2009) suggested that oxidative stress, either alone or in combination with osmotic stress might play major roles in hyperglycemic-induced cataracts. In this study we intend to evaluate this possibility by using a unique mouse model, the TTase gene knockout (KO) mice. The benefit of this model is that the mouse lens has a low risk of damage from inducible osmotic stress, but it is very sensitive to oxidative stress in the absence of TTase antioxidant protection. Our findings suggest that oxidative stress is induced in the lens during the process of hyperglycemia cataract formation but TTase appears to play a minor role in such lens pathogenesis.

2. Materials and methods

2.1. Materials

Glutathione (GSH), 5′, 5′-dithiobis (2-nitribanzoic acid) (DTNB), dithiothreitol (DTT), NADPH, and ATP were all from Sigma Chemical Co. (St. Louis, MO). Bicinchoninic acid (BCA) protein assay reagent and chemiluminescent substrate were from Pierce (Rockford, IL). The specific antibody for GSH was purchased from ViroGen Co. (Watertown, MA) and the antibody for beta-actin was made by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). All other chemicals and reagents were of analytical grade.

2.2. TTase KO mouse model

A global TTase gene KO mouse model was developed in Dr. Y-S Ho's laboratory at the Wayne State University. The TTase gene consists of three exons, in which the 2nd exon that contains the coding region for GSH binding was targeted for deletion. The global TTase deletion proved to have eliminated TTase expression in various tissues including the lens (Ho et al., 2007). This TTase KO model along with the wild type (WT) of matching background of C57BL/6 were kept in germ-free facility with up to 5 per cage and fed with regular rodent chow and water at lib. The Institutional Animal Care and Use Committees approved the use of mice for this study. We chose 4 month-old WT and KO animals to conduct the following *in vitro* and *in vivo* studies.

2.3. In vitro hyperglycemic cataract studies

Eight (8) mice from each of the TTase KO and WT groups (4-month old) were sacrificed with CO_2 asphyxiation following the guidelines of the ARVO Resolution on the Use of Animals in Research. The eyes were immediately enucleated and each lens was surgically removed under a dissecting microscope with great care to avoid any damage. Fresh mouse lenses were first pre-incubated in TC-199 medium for 8 h in a CO_2 incubator (95% air and 5% CO_2). The clear lenses were selected for experiment by transferring into 24-well plates. Half of the lenses were incubated for 48 h in 1 ml TC-199 medium with normal glucose content (6.4 mM), while the other half were incubated in 30 mM glucose-containing medium. At the end of the incubation, each lens was carefully removed, examined under a dissecting microscope and weighed. They were photographed using dark field illumination to record all the morphological changes.

2.4. In vivo diabetic studies

Diabetes was induced in 9 TTase KO mice (4-month old) through intraperitoneal injection of streptozotozin (STZ) at 100 mg/kg/day for 2 consecutive days. Eleven wild type mice with the same age and background as the KO mice were also induced into diabetes for comparison. Mice of both KO (8) and WT (10) were given STZ vehicle (100 mM sterile citrate buffer, pH 4.2) injection in the same manner, and used as the non-diabetic control. Blood glucose level was measured by a glucometer with blood taken from the tail vein.