Cellular osmolytes reduce lens epithelial cell death and alleviate cataract formation in galactosemic rats

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Purpose: Many cataractogenic stresses also induce endoplasmic reticulum (ER) stress in lens epithelial cells (LECs), which appears to be one of the universal inducers of cell death. In galactosemic rats, activation of ER stress results in the activation of the unfolded protein response (UPR)-dependent death pathway, production of reactive oxygen species (ROS), and cell death. All are induced and precede cataract formation. Cellular osmolytes such as 4-phenylbutyric acid (PBA), trimethylamine N-oxide (TMAO), and tauroursodeoxycholic acid (TUDCA) are known to suppress the induction of ER stress. We investigated whether these small molecules prevent cataract formation in galactose-fed rat lenses.

Methods: Cultured LECs were treated with galactose and each cellular osmolyte. Sprague-Dawley rats were fed a 50% galactose chow for 15 days with or without cellular osmolyte treatment. Similarly, selenite was injected subcutaneously into rats with or without cellular osmolytes. Calcein AM and ethidium homodimer-1 (EthD) were used to detect live and dead cells, respectively. The cellular osmolytes, PBA, TMAO, and TUDCA were tested for their ability to suppress LEC death and cataract formation.

Results: Cellular osmolytes rescued cultured human LECs which were treated with the ER stressors. We administered these osmolytes either orally or by injection into galactosemic Sprague-Dawley rats. These rats had significantly reduced LEC death and partially delayed hypermature cataract formation. Since the UPR was not activated in cultured LECs treated with selenite, we used the selenite nuclear cataract as a UPR-independent death pathway control. In selenite-induced nuclear cataract in rats, cellular osmolytes did not prevent LEC death and did not alleviate cataract formation.

Conclusions: These results further establish that ER stress and LEC death play a vital role in certain types of cataract formation. In addition, cellular osmolytes may be potential prophylactic drugs for some types of cataracts.

While cataracts can be successfully treated by surgery, it is important to find a nonsurgical treatment for cataracts. Both epidemiological and experimental studies have shown that noncongenital cataracts can be induced by many stress factors including oxidative stress [1-3], sunlight [4-6], ultraviolet-B (UVB) radiation [7-10], abnormal Ca++ metabolism [11-13], calcimycin [12], selenium [12,14], diabetes [15], and many other factors [16-18]. These stress factors appear to initiate apoptosis of LECs, which interrupts the homeostasis of the lens thus initiating the cataractogenic processes [8]. In our earlier papers, we reported that many of these cataractogenic stresses induce endoplasmic reticulum (ER) stress in lens epithelial cells (LECs) [19,20].

To be functionally active, a protein must acquire a unique three dimensional conformation via a complicated folding pathway. A small error in the folding process results in a misfolded protein structure, which can sometimes be lethal [21]. The rough ER is a principal site for protein synthesis and folding, calcium storage, and calcium signaling. The lumen of the ER has a highly oxidative environment required to carry out these functions efficiently [22-26]. The unfolded protein response (UPR) is an adaptive cellular response involving:

1) attenuation of protein synthesis in order to prevent further protein aggregation and accumulation in the ER, 2) induction of ER-localized chaperone proteins and folding catalysts, and 3) activation of ER-associated protein degradation that enhances elimination of unwanted aggregates [27-29] in the proteasome after ubiquitination of the misfolded protein [30]. The molecular chaperone, Bip, mediates the activation of these three pathways, and Bip is associated with each sensor of protein conformation in the absence of ER stress. As unfolded proteins accumulate in the ER, Bip dissociates from the ER membrane and activates C/EBP homologous protein (CHOP), activating transcription factor 4 (ATF4), and caspase-12 [25,31,32].

Prolonged ER stress induces the unfolded protein response (UPR)-dependent death pathway, which results in LEC death. The results of our cell culture and rat lens organ culture experiments clearly indicate that several cataractogenic stressors induce the UPR, produce reactive oxygen species (ROS) in LECs, and induce apoptosis in LECs, which precede cataract development [19]. In addition, the lenses of galactosemic rats also undergo similar changes which result in cataract formation [19].

Many proteins fold properly by themselves, but some require the assistance of a special kind of ubiquitous molecule, the so-called chaperone [33]. These chaperones can be classified into three groups: molecular chaperones, chemical chaperones, and pharmacological chaperones. Molecular chaper...
ones are composed of several distinct classes of highly conserved proteins like the heat shock family of proteins. The molecular chaperones αA- and αB-crystallins are well-known lens proteins that protect LECs from stress [34,35]. There is no feasible method of delivering these crystallins to the targeted ER of lens epithelial cells, therefore, their use for therapeutic applications is limited. In contrast, chemical chaperones are reported to prevent misfolding or to correct lethal protein conformations to influence the protein-folding environment inside the ER lumen. These low molecular mass compounds such as 4-phenylbutyric acid (PBA), trimethylamine N-oxide (TMAO), and taurododeoxycholic acid (TUDCA) are able to internalize into the ER lumen and stabilize protein conformation, improve ER folding capacity, and facilitate the trafficking of misfolded proteins [21,36,37]. PBA is known to be an ammonia scavenger [38-40]. TUDCA, a derivative of an endogenous bile acid, has a similar structure with cholesterol and is used on patients with cholestatic liver diseases [41,42]. However, TUDCA has not been shown to act as a classical chaperone, therefore, we call PBA, TMAO, and TUDCA cellular osmolytes in this manuscript. TMAO is a macromolecular crystallization reagent designed specifically for the crystallization of proteins, peptides, and nucleic acids. TMAO also counteracts the deleterious effects of the high intercellular concentration of urea in sharks and rays. This suggests that TMAO and PBA may inhibit carbamylation of crystallin proteins, one of the crystallin modification events in uremia [43]. Additionally, these cellular osmolytes have been reported to have outstanding safety profiles [38-40,42] and to have minimal side effects in humans [44]. Pharmacological chaperones such as capsaicin, cyclosporin, vinblastin, and verapamil are also documented as being effective in reversing mutant protein folding, but their use requires very high concentrations [45,46]. In this study, we investigated whether three cellular osmolytes can alleviate ER stress in LECs of galactosemic rats.

METHODS

Materials: PBA was purchased from Fluka (Buchs, Switzerland); TMAO, TUDCA, calcimycin, and homocysteine were purchased from Sigma/Aldrich (St. Louis, MO); tunicamycin was purchased from Alexis Biochemical (San Diego, CA); and galactose was purchased from United State Biochemicals (Cleveland, OH). PBA was dissolved and phosphate buffered saline (PBS) by titration with 5 N NaOH. For injection or gavage, we used 1 g/kg body weight/day of PBA, 1 g/kg body weight/day of TMAO, and 500 mg/kg of body weight/day of TUDCA. For cell culture experiments, we used 1.25 mM PBA, 35 mM TMAO, and 19 mM TUDCA. PBA was initially subcutaneously injected, but half of the animals showed hair loss around the injection site. On the other hand, all animals were healthy when given gavage administration, therefore, gavage was used. Since approximately 90% of TUDCA is removed by a single pass through the liver after being absorbed by the intestinal cells, it was given by subcutaneous injection instead of oral administration.

Animals: Sprague-Dawley rats (65-100 g) were purchased from Charles River Laboratories Inc. (Wilmington, MA) and were fed a 50% galactose diet [47]. The animals were euthanized with carbon dioxide asphyxiation in an uncharged chamber using only bottled carbon dioxide. After euthanasia, the lenses were dissected and photographed in a PBS buffer using a FOTOVIX back light system (Tamron Co, LTD, Torreington, CT). The experimental procedures used on the rats were approved by the University of Nebraska Animal Care and Use Committee and were in compliance with the Animal Welfare Act (Public Law 91-579) as mandated by the NIH Guide for Care and Use of Laboratory Animals. Clinical veterinary services are provided through the University of Nebraska Medical Center. The Association for Research in Vision and Ophthalmology (ARVO) resolution on the use and treatment of animals in ophthalmic and vision research was followed.

Cell culture: Transformed human LECs (from cell line SRA01/04 kindly provided by V. N. Reddy, Kellogg Eye Center, Ann Arbor, MI) were prepared as described [48]. Briefly, LECs (1-5x10^4 cells/ml in 6 cm or 10 cm dishes) were precultured in high sugar (25 mM glucose) Dulbecco’s Modified Eagle’s Medium (DMEM: Invitrogen Corporation, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS: Gemini Bio-Product, Woodland, CA) at 37 °C in a humidified chamber of 5% CO_2. In most assays, we used 100 cm^2 or 60 cm Petri dishes or 24-well culture dishes with cells seeded at a concentration of 1x10^6 cells per 10 ml of growth medium. The culture medium was replaced with a fresh medium every other day. Before reaching confluence, cultures were passaged by dissociation in 0.05% (w/v) trypsin with EDTA in balanced salt solution (BSS). At 70-80% confluence, cells were washed three times with DMEM without serum and transferred into 2% FBS containing DMEM. To determine whether cellular osmolytes suppress LEC death in a culture system, we cultured LECs (5x10^5/ml of medium) in high glucose DMEM overnight then treated them with 250 mM galactose with or without different concentrations of cellular osmolytes for 24 h.

Protein blot analysis: Cultured human LECs or LEC-attached lens capsules were washed three times with PBS then 100 µl of RIPA buffer (1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS in PBS) containing a protease inhibitor mixture (Roche Diagnostics, Mannheim, Germany) was added, and cells were immediately harvested and frozen at -70 °C. Cell lysis was conducted by three rounds of freezing and thawing, and soluble proteins were separated by centrifugation at 12,000 rpm for 15 min at 4 °C, and 20 µg of supernatant was separated by SDS-PAGE. The amounts of soluble protein were quantified by the Bradford method [49]. Primary antibodies included rabbit polyclonal antibodies against GAPDH (Abcam, Cambridge, MA), rabbit polyclonal antibody to Bip (Santa Cruz Biotechnology, Santa Cruz, CA), a rabbit polyclonal antibody to ATF4 (Abcam), a monoclonal antibody to CHOP (ABR Affinity BioReagents, Golden, CO), and rat polyclonal serum against procaspase-12 (Ab-2, Oncogene Research Prod-
ucts, San Diego, CA). Luminol reagent (Santa Cruz Biotechnology) was used to make specific protein bands visible by incubating the filter and exposing it to X-ray film.

**Cell viability/death staining method:** Rats were euthanized and lenses were isolated under a dissection microscope. Whole lenses or cultured LECs were stained with a mixture of two probes, calcein AM, and ethidium homodimer-1 (EthD; Viability/Cytotoxicity Assay Kits; Biotium Inc, Hayward, CA) for 30-45 min according to the company’s protocol. Resultant lenses were washed twice with PBS and observed under a fluorescent microscope (Nikon, Tokyo, Japan) with a filter of green (calcein; for live cell) or red (EthD; for dead cells). Similarly, cultured human LECs were stained with the same dyes and visualized with the same methods.

**Blood glucose and lens water content:** Galactose in serum was quantified by HPLC as described elsewhere [50]. Water content of the lenses was quantified by subtracting total dry lens weight from a total wet lens weight (7-14 lenses). Rat lenses were dried under vacuum at 60 °C for 72 h.

## RESULTS

**Cellular osmolytes promote cell survival in human lens epithelial cells treated with endoplasmic reticulum stressors:** To determine whether cellular osmolytes suppress LEC death in culture, human LECs (5x10^4/ml) were cultured in 25 mM glucose DMEM overnight then treated in DMEM with either 250 mM of galactose, 10 mM homocysteine, or 10 µg/ml tunicamycin with or without different concentrations of each chemical chaperone. The ranges of concentrations for PBA were 1.25, 2.5, 5.0, and 10 mM; for TMAO, they were 10, 30, 100, 300, and 600 mM; and for TUDCA, ranges were 0.1, 0.25, 0.5, and 1.0 mg/ml. The optimal concentrations of each cellular osmolyte for cultured LECs were determined to be 2.5 mM for PBA, 1.0 mg/ml for TUDCA, and 30 mM for TMAO. The LECs were similarly cultured in the presence of optimal concentrations of each cellular osmolyte for 14 h, and an ER stressor (250 mM galactose, 5 mM of homocysteine, or 10 µg/ml of tunicamycin) was added. The LECs were then incubated for an additional 24 h. The death of LECs was suppressed significantly in the presence of each ER stressor but not in the absence of them (Figure 1A,B). Cellular osmolytes suppressed cell death, and more cells survived in the presence of each ER stressor than without osmolytes. Live cell numbers were greater in TUDCA than that in PBA.

**Cellular osmolytes suppressed lens epithelial cell death and alleviated cataract formation:** Female Sprague-Dawley rats weighing 100-120 g were divided into four groups with each group containing six rats. Each group was fed a standard rat diet containing 50% galactose [20] for 15 days. One day prior to the start of the galactose diet, one group of six rats started receiving 1,000 mg/day of PBA that was orally administered by gavage. A second group of six rats received 500 mg/day of TUDCA by intraperitoneal injection, and a third group of six rats received 1,000 mg/day of TMAO by subcutaneous injection. For the control group, six rats were subcutaneously administered an equal volume of phosphate buffered saline (PBS). Respective administration of PBA, TUDCA, TMAO, or PBS was continued twice daily for the duration of the study. The administration of PBA and TMAO was repeated in three separate studies utilizing a total of 18 rats while 12 rats were used for the galactose-fed control without cellular osmolytes and six rats for non galactose-fed control (Table 1). All galactose-fed rats were equally galactosemic with similar body weights among the cellular osmolyte-treated and PBS control-treated groups (Table 1). This suggests that the cellular osmolytes had no detectable effects on serum galactose levels.
levels and that there is no apparent toxicity associated with these osmolytes. In young galactose-fed rats, sugar cataracts begin with the appearance of equatorial vacuoles and swollen cortical fibers which progress to cortical opacities. In the final stage, hypermature cataracts develop as the cortical fibers are destroyed with only a white nuclear protein mass (cherry pit cataract) remaining. In the present study, sugar cataracts rapidly developed in the PBS-control rats with 50% of the eyes developing end-stage hypermature (cherry pit) cataracts with nuclear opacity by day 13 of galactose feeding. The remainder of the rats developed hypermature (cherry pit) cataracts within 15 days of galactose feeding (Table 1 and Figure 2). On the other hand, in the treated groups, only 1 out of 30 rats that were administered PBA or TMAO developed hypermature cortical opacity by day 13. By day 15, cherry pit cataracts Figure 2A,B with nuclear opacity was observed in 14 out of 30 rats treated with PBA, 15 of 30 treated with TMAO, and 3 of 6 treated with TUDCA (Table 1, and Figure 2). The rest of the treated lenses remained relatively clear with prehypermature cataract (Figure 2C-F). In separate experiments, six cellular osmolyte-treated lenses eventually developed hypermature cherry pit cataract after 17-18 days under the galactose diet, suggesting that sugar cataract development was delayed by osmolyte administration. It appears that delivery routes such as oral, subcutaneous, or intraperitoneal did not significantly affect the suppression of cataract among these three cellular osmolytes.

A striking feature of the osmolyte-treated lenses was the relative survival of LECs in the galactose-induced sugar cataracts, suggesting that these compounds suppressed ER stress. Using a cell viability assay kit (a mixture of EthD and calcein) to monitor cell death in the LECs, the majority of LECs appeared to be viable in the control lenses at day 5. However, in our previous publication [19], significant LEC death was observed in the equatorial zone by day 7, and by day 15, less than 10% of the LECs in the central epithelial region remained viable [20]. Control experiments without cellular osmolytes also showed that a significant number of LECs had dropped out from five lenses as shown in Figure 3A, but in one lens, there remained a relatively high number of LECs at day 15. In contrast, greater viability of the central region of LECs was observed at day 15 in the lenses from osmolyte-treated rats (Figure 3B-D). In general, lower concentrations of LECs were found in the central regions of 6 out of 10 cataractous lenses, and four lenses remained about the same as that of controls. Interestingly, much less LEC death was observed in two TUDCA-treated lenses despite hypermature cataract formation with nuclear opacity (Figure 4D). Thus, although there are some inconsistencies, it is apparent that cellular osmolytes delay cataract formation and that lenses treated with these osmolytes retain more live LECs in the central region.

Unfolded protein response specific proteins were not activated in the lens epithelial cells of rats treated with each cellular osmolyte: Biochemical analysis of the LECs from 15 pooled rat lenses treated with PBA or TMAO and five lenses with TUDCA revealed that UPR specific proteins such as Bip/GRP78, CHOP, and ATF4 were not activated. However, these proteins were strongly activated in the LECs from control rats (Figure 4). We pooled 10-13 relatively clear lenses, but some of these lenses (Figure 2C-F) had a mild cortical opacity in the groups of rats. These results suggest that activation of ER stress was suppressed by each of the osmolytes and as a result, more LECs remained viable.

**Cellular osmolytes neither suppressed cell death nor alleviated cataract formation in the selenite nuclear cataract rat model:** We tested whether cellular osmolytes were effective in another cataract model, a selenite nuclear cataract model. Initially, LECs were cultured in the presence of various amount of selenite (Na₂SeO₃) or selenate (Na₂SeO₄) for 24 h and UPR activation was studied. Protein blot analysis clearly indicated that there was no upregulation of detectable Bip levels (data not shown). These results suggest that selenite or selenate did not induce the UPR. Because selenite did not induce the UPR in LECs, this cataract can be used as a non-UPR dependent cataract model.

We predicted that cellular osmolytes could not alleviate cataract formation in selenite cataracts. To study this, four sets of three rats were prepared. One set was injected subcutaneously with 30 µM selenite, a second set was injected with selenite plus PBA, a third set was injected with selenite plus TMAO, and the last set was given selenite plus TUDCA. Delivery routes, doses, and frequency of cellular osmolytes treatment were the same as those of the galactose-fed rats. After six days, all of these rats developed nearly identical cataracts.

**Table 1. Summary of body weights, blood galactose levels, lens water content, and cataract in rats**

<table>
<thead>
<tr>
<th>Condition of Rat</th>
<th>Body Weight (g) (Day 15)</th>
<th>Galactose (mg/ml) (Day 15)</th>
<th>Water content (mg/lens) (Day 15)</th>
<th>Hypermature Cataract (Day 13)</th>
<th>Cataract (Day 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>N/D</td>
<td>N/D</td>
<td>18.69 ± 3.0</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Galactose + No treatment</td>
<td>144±4</td>
<td>1.123±0.78</td>
<td>40.10±6.6</td>
<td>11/22</td>
<td>22/22</td>
</tr>
<tr>
<td>Galactose + PBA (Gavage)</td>
<td>143±3</td>
<td>0.974±0.57</td>
<td>36.69±5.9</td>
<td>1/30</td>
<td>14/30</td>
</tr>
<tr>
<td>Galactose + TMAO (SC)</td>
<td>143±6</td>
<td>1.321±0.06</td>
<td>38.75±6.8</td>
<td>1/30</td>
<td>15/30</td>
</tr>
<tr>
<td>Galactose + TUDCA (IP)</td>
<td>145±7</td>
<td>N/D</td>
<td>N/D</td>
<td>0/6</td>
<td>3/6</td>
</tr>
</tbody>
</table>

Summary of body weights, blood galactose levels, lens water content, and the incidence of hypermature (cherry pit) cataract in rats that were fed a 50% galactose diet for 15 days and treated with either PBA, TMAO, TUDCA, or phosphate buffered saline (PBS; control). SC and IP indicate subcutaneous injection and interperitoneal injection, respectively. N/D indicates not done.
There is neither delay of cataract formation nor morphological difference in these cataracts. In staining these lenses with calcein AM, LEC showed nearly identical patterns among groups, suggesting that cellular osmolytes neither affected the survival of LECs nor delayed cataract formation. In a separate experiment, two sets of two animals/set were treated or untreated with selenite and LECs of lenses at day 3 were prepared. Protein blot analysis showed that Bip levels were not different in selenite-injected animals and control rats (data not shown). These results suggest that cellular osmolytes do not affect the cataract induced by selenite, a non-UPR dependent cataract.

**DISCUSSION**

We have shown that cellular osmolytes suppress LEC death and alleviate cataract formation in galactose-fed rats and LEC cultures in a high galactose DMEM, but not in selenite cataracts. Galactosemic lenses in both the osmolyte-treated and untreated control rats appeared hydrated, consistent with the established galactitol-induced osmotic imbalance known to occur during sugar cataract formation in galactose-fed rats. The water content of the lenses from all galactose-fed rats was double that of normal lenses. A slight (5%) nonsignificant reduction in the water content was observed in age-matched osmolyte-treated lenses (Table 1) [51]. Lens hydration results from the hyperosmotic effects of galactitol accumulation, which is formed by the reduction of galactose by the enzyme aldose reductase that is present in the lens [50,51]. Recently, ER stress has also been observed in classic galactosemia, induced by a deficiency of galactose-1-phosphate uridyltransferase (GULT) activity in humans [52]. Galactose-induced cataract formation is a rare condition in human patients, but galactose-fed rats develop cataract within two weeks and has been used as a model of diabetic cataract [49,50]. Aldose reductase inhibitors (ARIs) block the formation of galactitol and protect the lens from the osmotic changes that result in sugar cataract formation and eventual LEC death [53,54]. In contrast, cellular osmolyte treatment reduced LEC death and alleviated cataract formation without significantly altering lens hydration.

While only aldose reductase inhibitors (ARIs) have been shown to inhibit sugar cataract formation in galactose-fed rats [54], the present study demonstrates by the delay in hypermature (cherry pit) cataract formation that more live LECs with the cellular osmolytes, PBA, TMAO, and TUDCA, also postpones sugar cataract formation by perhaps protecting the LECs from ER stress (Figure 4). These cellular osmolytes have also been reported to reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes [55].

One puzzling question is why LECs are killed in galactosemic rats through ER stress while most retinal cell types are not. To answer this, we speculate that LECs in galactosemic rat lenses have an increased rate of proliferation since it is known that mitotic cells are more susceptible to cell death than nonmitotic cells in islet cells [56]. The first detectable abnormalities occur after 36 h in galactose-fed rats and are limited to the central LECs [57]. Cell edema, the apparent

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**Figure 2.** Appearance of lenses from galactose-fed rats for 15 days photographed without background light. All control rat lenses treated with PBS showed hypermature (cherry pit) cataracts (A). The lenses treated with PBA, TMAO, and TUDCA show either hypermature cataracts (B) or mild pre-hypermature cortical cataracts (C-F). B indicates a lens treated with TUDCA, C indicates a lens treated with PBA, D and E indicate lenses treated with TUDCA, and F indicates a lens treated with TMAO. All lenses in galactosemic rats were hydrated and swollen presumably as a result of the accumulation of galactitol [54].
dilution of cytoplasm, the rounding of nuclei, the aberrant intracellular vacuoles, and the loss of normal cell boundaries are found at day 2 [57]. The serum galactose level reached 25-35 mg/ml after 48 h under a 50% galactose diet [57]. Since LECs contain high aldose reductase levels [58], galactitol must accumulate in the LECs, and during the first few days, ER stress may be induced in these cells but not cause LEC death. Earlier reports [59,60] show that central anterior LECs in galactosemic rat lenses have significantly proliferated by day 4. In two-month-old rats on galactose diets, the proliferating LECs increased by day 2 and reached the highest point by day 4, an almost six-fold increase. This increase was reduced to nearly normal levels by day 7 [59,60]. In lenses of galactose-fed rats, massive cell death was observed from day 5 through day 7 in the periphery of the lenses where the mitotic zone of LECs is located. From day 7 through day 9, most of the central LECs of these lenses were dead, and by day 15, only small numbers of LECs remained under the capsule of these rat lenses [20]. These results suggest that nonproliferative LECs were converted into proliferative cells prior to massive cell death in the galactose-fed rats. In addition, regardless of galactose concentrations in the chow (15%, 25% or 50% galactose), the

Figure 3. Lens epithelial cells from galactose-fed rat lenses stained with a calcein viability dye. Lenses from rats treated with PBS control (A) or the cellular osmolytes (PBA; B; TMAO; C; or TUDCA; D) were obtained after 15 days of galactose feeding. Lenses were isolated from the eyes, then whole lenses were stained with 0.5 ml of calcein for 40 min according to the manufacturer’s protocol. The lenses were washed three times with PBS and photographed by fluorescent microscopy. All pictures were taken from the central region of the LECs where we can focus well. The scale bar indicates 100 µm.
increase in the numbers of mitotic cells was highest by day 4 in these rats [61]. This suggests that the central nonproliferative LECs were converted into the proliferative LECs under galactosemia and that ER stress might have killed those cells. Most retinal cells were not converted into proliferative cells, thus, galactosemia predominantly induces lens-specific destruction in the eyes. Since there is no LEC death in the nuclear cataract in human patients, many lens scientists believe that LEC death is not associated with cataract. Nonnuclear cataract in human patients and cataracts in animal models such as galactose-cataract are associated with the death of LECs [62-65]. These results suggest that death of LECs disturbs lens homeostasis and might induces numerous damages, and results in cataract formation.

Since PBA and TMAO are reported to interact with protein molecules in the highly oxidized lumen of the ER and assist in retaining protein conformation, it is likely that these molecules may protect lens crystallins. TMAO stabilizes the α,-antitrypsin protein inhibitor in an active conformation against heat-induced polymerization [66], onconase against reductive unfolding [67], and prion protein against infectious conformation [68]. TUDCA affects the ER stress-induced caspases-12 activation [69], reduces ER stress [55], and inhibits ER-induced apoptosis caused by α-1 antitrypsin [70]. PBA has been found to have chaperone-like abilities [71]. Thus, it is likely that cellular osmolytes affect the conformation of crystallins if a sufficient amount of cellular osmolytes is delivered to the lens. Alternatively, cellular osmolytes might affect many enzymes in the treated rats and induce abnormal metabolism. These osmolytes might also affect the survival of LEC, resulting in delaying cataract formation. Additionally, it is unlikely but possible that cellular osmolytes directly interact with crystallin proteins to delay protein aggregation. Such aspects have to be further investigated in the future.

Molecular chaperones such as the α-crystallin are well-studied proteins [72] and include the family of heat-shock proteins and α-crystallins. α-Crystallins are especially abundant proteins in the cytoplasm of LECs and fiber cells, but so far, no reports indicate that these chaperones are transported into the ER. It is unlikely but possible that if α-crystallins are transported into the ER, they may act as chaperones to block ER stress.

Selenite cataract is an experimental nuclear cataract model, which can be produced in suckling rats at postnatal 10-14 days [14]. A single injection of selenite induces cataract five to six days after the injection. Although selenite did induce mild LEC death in LEC culture and in rats within the experimental period, the activation of UPR, measured by an elevation of Bip, was not observed in these animals. Furthermore, cellular osmolytes were ineffective, suggesting that selenite might not induce ER stress in LECs under experimental conditions. However, the selenium metabolites such as methylseleninic acid (MSA), selenomethione, and methylselenol are known to induce UPR in prostate cancer cells [73,74].

Currently, the only treatment for cataracts is surgery. It has been estimated that a 10-year delay in the onset and progression of cataract could reduce the need for cataract surgery by 50% [75,76]. The lens is composed of a monolayer of metabolically active epithelial cells that continuously divide at the equator to form lens fiber cells. The present data suggest that cellular osmolytes can delay the progression of cataracts by maintaining the viability of LECs by protecting them from ER stress.

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