Cysteamine Prevents the Development of Lens Opacity in a Rat Model of Selenite-Induced Cataract

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PURPOSE. The activation of transglutaminase 2 (TG2) by oxidative stress through TGFβ has been reported to play a crucial role in cataract formation. The authors investigated whether TG2 is involved in selenite-induced cataract formation in rats and whether cysteamine, a chemical inhibitor of TG2, can prevent cataract formation in this model.

METHODS. Intracellular TG2 activity was monitored in a human lens epithelial cell (HLE-B3) line and cultured rat lenses after treatment with selenite. Rat pups (13 days old) were injected subcutaneously with sodium selenite (Na₂SeO₃; 20 µmol/kg) and intraperitoneally with cysteamine (30, 40, and 60 mg/kg) for 14 days. Lenses were evaluated photographically at days 7 and 14. The concentrations of malondialdehyde and glutathione in the lenses were determined.

RESULTS. In HLE-B3 cells or rat lenses, selenite induced intracellular TG activity, which was inhibited by cysteamine. In selenite-treated rats, the rate of cataract formation was significantly reduced by cysteamine (P < 0.001). The mean cataract area in the lenses of cysteamine-treated rats was smaller than that of control rats (P < 0.01). The levels of total and reduced glutathione in the lenses of cysteamine-treated rats extracted at day 14 were higher than those of control rats.

CONCLUSIONS. Cysteamine suppresses cataract formation induced by selenite in rats, suggesting that cysteamine can be used as a pharmaceutical intervention to prevent or delay cataract formation. (Invest Ophthalmol Vis Sci. 2012;53:1452-1459) DOI:10.1167/iovs.11-8636

Cataract is a disease of increasing lens opacity that is mainly associated with aging. Cataract affects more than 20 million people worldwide and is the leading cause of blindness, particularly in underdeveloped countries.1,2 Age, smoking, excessive UV-B exposure, diabetes, and steroid treatment are known cataract risk factors, suggesting that increased oxidative stress is implicated with the mechanism underlying cataract formation.3 However, no antioxidant medications are confirmed to prevent or slow cataract formation.4,5

Analysis of proteins extracted from lenses with cataract revealed that crystallins undergo extensive posttranslational modifications, a wide variety of irreversible modifications caused by oxidative stress, including deamidation, oxidation, intermolecular disulfide cross-linking, carbamylation, glycation, phosphorylation, thiolation, and racemization.6,7 However, these modifications can also be found in normal aging lenses.6 Thus, the modifications that are specific and associated with cataract formation have not been identified.

Transglutaminase 2 (TG2) is a calcium-dependent enzyme that mediates the posttranslational modification of substrate proteins by catalyzing acyl transfer reactions between the γ-carboxamide group of glutamine residues and the ε-amino group of lysine residues, producing cross-linked, polyamminated, or deamidated proteins.8,9 TG2 is a ubiquitously expressed enzyme. By catalyzing transamidation reactions, TG2 is involved in apoptosis, differentiation, and extracellular matrix formation.9 TG2 expression is upregulated by various stimuli, including glutamate, calcium influx, oxidative stress, UV irradiation, and inflammatory cytokines.10 These findings suggest that overstimulation or aberrant activity of TG2 may cause the aggregation of proteins observed in several degenerative diseases, whereas the cross-linking activity of TG2 could be relevant to biological processes.10

Recently, we reported that the intracellular activity of TG2 is latent and activated by treatment with H₂O₂ or UV irradiation.11 In a human lens epithelial cell line (HLE-B3), TGFβ mediates oxidative stress-induced TG2 activation through the nuclear translocation of Smad3.8 Under oxidative stress conditions, TG2-mediated protein modification results in a decrease in protein solubility and a collapse of the intermediate filament network, which lead to protein aggregation.8 Moreover, TGFβ, a potent cataractogenic cytokine, failed to induce opacity in ex vivo cultured lenses from TG2-deficient mice.8 Therefore, TG2 activity is critical for oxidative stress-induced cataract formation.

Cysteamine (2-mercaptoethylamine), a decarboxylated form of cysteine, inhibits TG2 through a thiolate-disulfide exchange reaction with cysteine residues in the active site of this enzyme.12,13 In epithelial cells, cysteamine is produced by pantetheinase encoded in the Vanin-1 gene from pantetheine or coenzyme A.14 It plays a role as an antioxidant finely tuned with glutathione through stress-induced biphasic expression of...
Vanin-1.14,15 In neural tissue, cysteamine serves as a precursor for taurine and hypotaurine, which exert neuroprotective actions against ischemia and oxidative stress.16,17 Cysteamine also causes the depletion of somatostatin, prolactin, and noradrenaline in the brain and peripheral tissues. In addition, cysteamine is involved in the regulation of apoptosis and the differentiation of epithelial cells by inhibiting caspase-3 and PPAR-γ.18–20

In the clinical field, cysteamine bitartrate (approved by the US Food and Drug Administration in 1994 as Cystagon [Mylan Pharmaceuticals, Morgantown, WV]) is a treatment of choice for patients with cystinosis, a rare autosomal recessive disorder caused by impaired transport of amino acid cystine from cellular lysosomes.21 Topical cysteamine eyedrops (0.55%) are used to dissolve corneal cystine crystals in cystinosis.22 Cysteamine is also used for the detoxification of acetaminophen poisoning. In this study, we evaluated the in vivo effect of cysteamine on cataractogenesis by using a rat model of selenite-induced cataract formation to test its feasibility as a new medication for cataract prevention. Our results revealed that cysteamine suppresses selenite-induced cataract formation in rats.

**Materials and Methods**

**Animals**

Pregnant Sprague-Dawley rats were obtained from Orientbio Inc. (Seongnam-si, Korea). After delivery, male and female 13-day-old pups were used for experiments. All pups were housed with their mothers in a cage maintained at the animal facility of Seoul National University College of Medicine. For anesthesia, 2% xylazine hydrochloride (Rompun; Bayer Korea Ltd., Seoul, Korea) and a combination anesthetic drug (tiletamine 125 mg/ml and zolazepam 125 mg/ml; Zoletil; Virbac, Carros, France) were used. All animal experiments were performed with the approval of the Institutional Animal Care and Use Committee at Seoul National University and according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Induction of Cataract Formation and Cysteamine Treatment**

Cataract formation was induced in rat pups by a single subcutaneous injection of sodium selenite (Na2SeO3, 20 μmol/kg body weight [BW]; Sigma-Aldrich, St. Louis, MO) in 85 to 125 μL phosphate-buffered saline (PBS) on postpartum day 13, as previously described.22 To examine the effect of cysteamine, pups were injected intraperitoneally 2 hours before selenite injection and daily for 14 days thereafter with various concentrations of cysteamine (PBS only; 30, 40, and 60 μg/kg BW; Sigma-Aldrich).

**Measurement of Cataract Area**

To evaluate cataract formation, anterior segment photographs were taken under a surgical microscope on days 7 and 14. After euthanization, photographs of dissected lenses were also taken. Before the photographs were taken, pups were anesthetized and treated with a topical ophthalmic solution for mydriasis containing phenylephrine hydrochloride and tropicamide (Mydrin-P; Santen, Osaka, Japan). To eliminate analytical bias, photographs were randomly named and sent to two analysts. The cataract area (CA) was measured using NIH ImageJ software. The whole lens area was selected, and the mean gray value was measured. Lens opacity was shown as fold increase compared with the value of the lens without selenite treatment after background subtraction.

**Measurement of Glutathione and Malondialdehyde in Rat Lens**

Lenses were homogenized by vortexing (5000 rpm, 20 seconds, 5 times) with glass beads in a buffer (50 mM Tris-Cl, pH 6.8; 150 mM NaCl, 1 mM EDTA; 1% Triton X-100) containing protease inhibitors (Roche Diagnostics, Mannheim, Germany). Lens homogenates were treated with 10% metaphosphoric acid and 4 M triethanolamine. After centrifugation at 12,000 g for 10 minutes at 4°C, the concentration of total glutathione was determined using a glutathione assay kit according to the manufacturer’s instructions (catalog no. 703002; Cayman Chemical Co., Ann Arbor, MI). To measure the concentration of oxidized glutathione, homogenates were treated with 1 M 2-vinylpyridine to remove reduced glutathione. The concentration of reduced glutathione was calculated by subtracting the oxidized glutathione from total glutathione. Concentrations of malondialdehyde (MDA) in the lenses were determined using a thiobarbituric acid reactive substances assay kit according to the manufacturer’s instructions (Catalog No. 10000955; Cayman Chemical Co.). The BCA method was used to quantify the protein in the samples.

**Cell Culture**

Human lens epithelial cells (HLE-B3) were maintained in MEM containing 20% fetal bovine serum in a humidified atmosphere with 5% CO2 at 37°C. Cells were incubated in serum-free medium for 12 hours at 37°C and exposed to medium containing sodium selenite (0, 0.125, 0.25, 0.5, and 1 μM) for 48 hours.

**TG2 Activity Assay**

Cells or lenses were labeled with 3 mM biotinylated pentaethylene (BP; Pierce, Rockford, IL) for 1 hour in serum-free medium before harvesting, and the homogenates were prepared by sonication, followed by centrifugation (12,000 g, 10 minutes at 4°C). Cell and lens extracts were assayed for intracellular TG2 activity by determining the amount of BP incorporated into proteins using two different methods, as described previously.13 In brief, for Western blot analysis, the cell extracts (30 μg) were subjected to 10% SDS-PAGE, and the proteins were transferred to nitrocellulose membrane. The BP-incorporated proteins were probed with horseradish peroxidase (HRP)–conjugated streptavidin (Zymed Laboratories, San Francisco, CA), followed by enhanced chemiluminescence detection (Pierce). For solid-phase microplate assay, cell or tissue extracts (0.1 mg/mL 50 μL/well) in coating buffer (50 mM Tris-Cl, pH 7.5; 150 mM NaCl, 5 mM EDTA) were coated into each well of a 96-well plate (Immuno 96 Well/ Nunc, Naperville, IL) by overnight incubation of the plates at 4°C. Then the plates were blocked by 5% bovine serum albumin in PBS containing 0.1% Tween-20 at room temperature for 2 hours. The proteins incorporated with BP were probed with HRP-conjugated streptavidin, followed by reaction with o-phenylenediamine dihydrochloride (Sigma-Aldrich). After the reaction was stopped with the addition of 1 N H2SO4, TG2 activity was determined by measuring the absorbance at 492 nm on a microplate spectrophotometer (Bio-Rad, Hercules, CA). TG2 activity was expressed as fold activation compared with the activity in the samples without selenite treatment after sub-
tracting the values obtained in the absence of BP, which represent endogenous biotin-conjugated proteins.

Protein levels of actin and TG2 were assessed by probing with monoclonal antibodies specific for actin (Sigma), and TG2 was generated using recombinant human TG2 as an antigen. Cysteamine (0.5 mM; Fluka, Milwaukee, WI) and a pan-specific neutralizing antibody for TGFβ (30 µg/mL; R&D Systems, Minneapolis, MN) were used to evaluate the inhibitory effects of intracellular TG2 activity and TGFβ signaling pathway, respectively, in HLE-B3 cells and cultured lenses.

**Statistical Analysis**

Statistical significance was determined with the χ² test for grade analysis, one-way analysis of variance (ANOVA) with Bonferroni’s adjustment for multigroup comparison of mean CA analysis, and Mann-Whitney U test for comparison of glutathione and MDA between groups (SPSS, version 16.0; SPSS Inc., Chicago, IL). P < 0.05 was considered statistically significant.

**RESULTS**

**Selenite Activates Intracellular TG2 in a Lens Epithelial Cell Line**

Selenite-induced lens opacity in rats, a well-known model of cataract, has been used to screen the efficacy of new drug candidates for cataract prevention. However, the molecular mechanism by which selenite induces the development of cataract is not clearly defined. Because selenite generates reactive oxygen species (ROS) and TG2 activation is critical for oxidative stress-induced cataract formation, we reasoned that TG2 is involved in the process of selenite-induced lens opacity. To test the hypothesis, we examined the effect of selenite on intracellular TG2 activity in a human lens epithelial cell line (HLE-B3). Treatment with selenite resulted in a concentration-dependent increase in intracellular TG2 activity, as measured by a well-plate assay and by Western blot analysis that plateaued at 0.5 µM sodium selenite (Fig. 1A, top and bottom, SA). The protein levels of TG2 were not changed by treatment with selenite (Fig. 1A, bottom, TG2). Because low levels of oxidative stress induce the delayed activation of TG2, we evaluated time-dependent changes of TG2 activity. In HLE-B3 cells treated with 0.5 µM sodium selenite, intracellular TG2 activity peaked (~2-fold increase) after 36 hours and then decreased (Fig. 1B).

A role for TGFβ released under oxidative stressed conditions in the activation of intracellular TG2 has been demonstrated previously. To test the involvement of TGFβ in selenite-induced TG2 activation, we examined the effect of a TGFβ-neutralizing antibody on TG2 activity in selenite-treated HLE-B3 cells.

**FIGURE 1.** Treatment with sodium selenite activates intracellular TG2 in a concentration- and a time-dependent manner through TGFβ in HLE-B3 cells. (A) HLE-B3 cells were treated with increasing concentrations of sodium selenite and cultured for 48 hours before analysis. (B) HLE-B3 cells were treated with 0.5 µM sodium selenite for 6, 12, 24, 36, 48, or 60 hours. (C) HLE-B3 cells were treated with sodium selenite (0.5 µM) for 48 hours in the absence or presence of cysteamine (CSH, 0.5 mM) and TGFβ neutralizing antibody (TGFβ Ab, 30 µg/mL). Intracellular TG2 activity was measured by a well-plate assay method (top) and was visualized by Western blot analysis by probing with HRP-conjugated streptavidin (SA, bottom). TG2 activity is expressed as a relative value to that in untreated cells. Protein levels of TG2 and actin were evaluated by Western blot analysis (TG2, bottom). Data represent mean ± SD based on three independent experiments. *P < 0.05.
cells. Treatment of cells with 0.5 μM sodium selenite increased intracellular TG2 activity by 1.89-fold after 48 hours. The addition of TGFB-neutralizing antibody or cysteamine, a chemical inhibitor of TG2, into the culture medium decreased TG2 activity by 1.23- and 1.27-fold, respectively, compared with that in untreated cells (Fig. 1C). These results indicate that selenite activates TG2 in HLE-B3 through the release of TGFB into culture medium.

**Cysteamine Suppresses Selenite-Induced Lens Opacity in Ex Vivo Rat Lens**

We next investigated whether the inhibition of TG2 activity by cysteamine prevents the development of selenite-induced opacity in the rat lens. Exposure of ex vivo–cultured rat lenses to 100 μM selenite caused a gradual increase in lens opacity over 8 days (Fig. 2). Under the same experimental conditions, the addition of cysteamine (0.125, 0.25, 0.5, 0.75, 1 mM) into the culture medium significantly inhibited or delayed the development of selenite-induced lens opacity, most effectively at the concentration of 0.5 mM (Fig. 2). Calculation of lens opacity showed 17.62 ± 1.17-fold increase in the positive control group and 13.39 ± 1.42-fold increase in the 0.5 mM cysteamine-treated group at day 8 (n = 4, P = 0.029; Mann-Whitney U test; Fig. 2B). At concentrations lower than 0.5 mM, cysteamine exhibited a concentration-dependent inhibitory effect on selenite-induced lens opacity (Fig. 2). To confirm these results, we measured the TG2 activity of rat lenses. Treatment of rat lenses with 100 μM selenite caused 1.85 ± 0.15- and 4.27 ± 0.74-fold increases in intracellular TG2 activity on days 2 and 5, respectively, compared with that in untreated rat lenses. The addition of 0.5 mM cysteamine inhibited selenite-induced TG2 activity to the levels observed in untreated lenses (Fig. 3). Moreover, the addition of pan-specific neutralizing antibody for TGFB (30 μg/mL) reduced the lens opacity and TG2 activity in selenite-treated rat lenses, though reduction levels were not statistically significant (Supplementary Fig. S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental).

Separately, one group of 50 rat pups was treated with cysteamine only (60 mg/kg BW) as a control. Through daily examination of their eyes, we observed that lens opacity appeared at day 4 and was not altered after day 6 of selenite injection. Thus, anterior segment photographs were taken under a surgical microscope on days 7 and 14 after selenite injection for documentation and analysis. Photographs of dissected lenses were also taken after euthanization. To quantitate the severity of cataract, the ratio of the area of lens opacity to the corneal area (defined as CA) was measured (Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental).

None of the lenses exhibited cataract in rat pups that received daily doses of 60 mg/kg BW cysteamine for 14 consecutive days without selenite (Supplementary Fig. S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental). In rat pups pretreated with PBS...
only, subcutaneous injection of selenite caused the development of cataract in 94% of eyes after 7 days. Treatment with 30, 40, and 60 mg/kg BW cysteamine resulted in a significant decrease in the percentage of lens with cataract (CA >1%) induced by selenite injection compared with that in PBS-treated rats (*P < 0.001 for rats treated with 30 and 60 mg/kg BW; †P < 0.01 for rats treated with 40 mg/kg BW; Fig. 4A and Supplementary Fig. S3, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental). Interestingly, the inhibitory effect of cysteamine on the rate of cataract formation induced by selenite was not dose dependent. In addition, CA analysis of lenses revealed that the percentage of lenses with CA between 10% and 30% was significantly decreased by cysteamine treatment, whereas the percentage of lenses with CA exceeding 30% was not affected in all groups of rats (Fig 4A, Table 1). When the lenses were examined on day 14 after selenite injection, the rate of cataract formation was further decreased by cysteamine treatment (*P < 0.001 for all groups; Fig. 4B, Table 1), whereas the rate of cataract formation in selenite-treated rat pups remained similar to that on day 7. These results demonstrated that cysteamine treatment is effective for preventing the development of cataract.

Oxidation of lens proteins plays an important role in cataract formation. Because cysteamine is a thiol compound, we asked whether cysteamine can act as an antioxidant. To test this possibility, we measured the level of glutathione in ex vivo–cultured rat lenses and in lenses from rat pups on day 14 after selenite injection. Treatment of rat lenses with 100 μM selenite for 8 days caused the depletion of total and reduced glutathione. Under the same experimental conditions, the addition of 0.5 mM cysteamine showed sparing effect on total and reduced glutathione (Supplementary Figs. S4A, S4B, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental). Similarly, we found a significant decrease in the concentration of total glutathione (Fig. 5A) and reduced glutathione (Fig. 5B) in lenses from selenite-treated rat pups (n = 6; *P < 0.05). This effect was abrogated by treatment with 30 mg/kg cysteamine. We also evaluated the level of MDA in the rat lenses. We observed a decrease in the concentration of MDA in cultured rat lenses treated with cysteamine (Supplementary Fig. S4C, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.11-8636/-/DCSupplemental) and in lenses from cysteamine-treated rats compared with that in selenite only–treated animals (Fig. 5C). These results indicate that cysteamine prevents selenite-induced cataract formation by acting as an antioxidant as well as a TG2 inhibitor.

**DISCUSSION**

Our data indicate that the activation of intracellular TG2 induced by selenite treatment led to the development of lens opacity in a lens organ culture system, pointing to the essential role of TG2 activation in cataract formation in response to oxidative stress. We also demonstrated that the administration of cysteamine, a chemical inhibitor of TG2, can protect against the development of cataract in a rat model of selenite-induced cataract formation.

**FIGURE 3.** Cysteamine (CSH) inhibits intracellular transglutaminase 2 (TG2) activity in selenite-treated rat lens. Explanted rat lenses were treated with sodium selenite (100 μM) in the presence of CSH (0.5 mM). Intracellular TG2 activity was determined on days (A) 2 and (B) 5 after selenite treatment. Data represent the mean ± SD based on three experiments. TG2 activity is expressed as a relative value to that in untreated lenses.

**FIGURE 4.** Cysteamine (CSH) prevents the development of cataract in selenite-treated rats. Rat pups (13 days old) were treated with selenite (20 μmol/kg) subcutaneously. CSH (0, 30, 40, or 60 mg/kg BW) was injected intraperitoneally 2 hours before selenite injections and then daily for 14 days. Anterior segment photographs for each eye were taken under a surgical microscope on days ? (A) and 14 (B) after selenite injection. The severity of cataract was assessed by measuring the ratio of area of lens opacity to CA using NIH ImageJ software. Figures show the percentage of lenses with different grades of CA. Rats treated with cysteamine were protected from the development of cataract (n = 78–82 for each group; day 7 (A): *P < 0.001; †P = 0.008; day 14 (B): *P < 0.001, χ² test).
Epidemiologic studies indicate that aging, exposure to UV light, smoking, and diabetes are major risk factors for cataract formation, suggesting a causative role for oxidative stress in the aggregation of lens proteins through protein modification. Thus, a number of studies have investigated the possibility of slowing cataractogenesis by administration of antioxidants or by dietary supplementation with micronutrients that have antioxidant capabilities, including vitamin C and vitamin E. However, large-scale prospective studies revealed that long-term vitamin C or vitamin E supplementation did not exhibit any significant effect on cataract risk. These observations suggest that mechanisms in addition to the direct oxidation of lens proteins by ROS may be involved in the process of cataract formation, such as signaling pathways activated by oxidative stress.

TG2 has been proposed to be implicated in cataract formation because of its catalyzation of γ-glutamyl-e-lysine isopeptide bonds, producing cross-linking, or polyaminated proteins. Moreover, structural proteins and intermediate filament proteins in the lens, such as α- and β-crystallin and vimentin, are the substrates for TG2. Significant increases in TG activity were observed in young rats homozygous for the radiation-induced recessive cataract mutation cat-2 and in the age-related cataract formation associated with a persistent hyaloid vascular system in senescence-accelerated mice and in ICR/f hereditary cataractous rats. In humans, the expression of TG2 mRNA and protein was markedly increased in lens epithelial cells from patients with anterior polar cataracts. Although the enzymatic properties of TG2 contribute to cataract formation, how TG2 is related to the known risk factors for cataract formation remained to be established. Recently, we demonstrated that ROS induce the release of TGFβ and the increase of intracellular calcium levels, which subsequently activate TG2, leading to the aggregation of lens proteins. The observation that TGFβ-induced cataract formation was completely blocked in ex vivo lenses from TG2-deficient mice confirmed a critical role of the TGFβ-TG2 axis in ROS-induced cataract formation. In this study using a rat model, however, it was difficult to monitor the intracellular TG activity of lens epithelial cells because of a lack of information, such as tissue distribution, metabolism, crossing the blood-aqueous barrier of BP, the high concentration of polyamine in the blood that inhibits the transport of BP into the cells, and the activation of TG2 in the lens by tissue damage that inevitably occurs during surgical procedures. Therefore, the ex vivo lens was used to monitor the inhibitory effect of cysteamine on TG2 activity and lens opacity in selenite-treated lenses. Moreover, we demonstrated that the inhibition of TG2 by cysteamine administration is effective in preventing cataract progression. Thus, our results suggest that cysteamine could be a novel pharmacologic strategy to delay or prevent cataract formation.

The human lens contains a high concentration of glutathione, the most important nonprotein antioxidant in mammalian cells. Glutathione levels in the lens decrease with age and are lower in cataractous lenses than in normal lenses, suggesting a critical role of cellular antioxidants in cataract formation. Our data indicated that cysteamine administration increased glutathione levels in the rat lens, which was depleted by treatment with selenite. Thus, in addition to the inhibition of TG2, cysteamine suppresses the development of cataract by increasing glutathione levels in a murine model of selenite-induced cataract. It should be noted, however, that cysteamine is known to inhibit γ-glutamylcysteine synthetase (γGCS), an enzyme required for the production of glutathione.
sensible for glutathione synthesis, through sulphhydril-disul-
fide exchange reactions between cysteine residues in the en-
zyme active site and cysteamine.40 In fact, Vanin 1-null mice
exhibited markedly elevated levels of glutathione,41 suggesting
that cysteamine is involved in the regulation of glutathione
synthesis. In our experiments, we found that the protective
effect of cysteamine was not dose dependent, particularly
when high doses of cysteamine were injected. Thus, it is
probable that such dose independence may be due to the
inhibition of γGCS by cysteamine at high concentration.

Sodium selenite has been used to induce cataract formation
in animal studies and in in vitro models using lens organ culture
or cell culture, though the mechanism for selenite-induced cataract
is not fully understood.22,28-42 In this study, we con-
firmed that selenite induces cataract formation only in 13-days-
old rat pups but not in 12- or 14-day-old pups, suggesting that
postpartum age is critical for selenite-induced cataract forma-
tion. We also found that the minimum concentration of sele-
nite required for inducing cataract formation in all 13-day-old
pups was 20 μmol/kg BW. In the explanted rat lens, by con-
trast, the optimum concentration of selenite to induce opaci-
fication was reported to be 100 μM.32 Although the reason for
this difference between ex vivo and in vivo experiments is un-
clear, 100 μM selenite was used in this study for comparison
of inhibitory potency with other reports.

Sodium selenite is known to generate ROS. In the present
study, we found that selenite activates intracellular TG2, which
contributes to cataract formation through the cross-linking or
polyamination of substrate proteins. Moreover, consistent with
findings of a previous report,8 we found that TGFβ mediates
selenite-induced TG2 activation. Our data indicate that TG2
activation is one of the mechanisms underlying selenite-
duced cataract formation. However, as demonstrated in our ex-
vivo and in vivo experiments, cysteamine treatment resulted in
a nearly complete inhibition of TG2 activity, but cysteamine
exhibited limited efficacy to prevent cataract development.
This observation suggests that other factors may be involved in
selenite-induced cataract formation. Indeed, treatment with
selenite causes the oxidation of sulphhydryl groups of proteins
and the activation of m-calpain (also known as milipalpain or
calpain II), another calcium-dependent enzyme, which leads to
prolylosis and the loss of N-terminal extensions on β-crystal-
lin.25-44 Thus, the activity of m-calpain together with TG2
results in the insolubilization of proteolyzed β-crystallins and
the coprecipitation of α- and γ-crystallins, leading to lens opac-
ity. Nonetheless, these results indicate that TG2 is a common
factor for the pathogenesis of oxidative stress–induced cataract
formation.

Surgical removal of the lens and implantation of an artificial
intraocular lens are the mainstay for the management of cata-
ракt. However, surgical management inevitably has resulted in
a number of complications, such as bullous keratopathy, post-
operative glaucoma, retinal detachment, loss of accommoda-
tive power, and endophthalmitis.46 Moreover, cataract also
imposes a severe strain on global health care budgets because of
an increasingly aged population.2 Thus, medical treatment
is needed to prevent or delay the progression of cataract. At
present, however, there is no effective drug to prevent the
onset of cataract formation. Our data indicate that cysteamine
administration can effectively suppress cataract formation in a
rat model. Because cysteamine is a natural product of metab-
olism and importantly is now under use as an eyedrop for the
treatment of cystinosis, the evaluation of cysteamine as a phar-
macological intervention in humans merits further investigation.

In conclusion, we have demonstrated that the inhibition of
intracellular TG2 activity using cysteamine can suppress cata-
ракt formation in a rat model. In addition, the mechanism-based
inhibitors we investigated might provide a basis for the devel-
oment of new compounds.

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