Activity Loss of Glutathione Synthesis Enzymes Associated with Human Subcapsular Cataract

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Purpose. To assess the activities of the two enzymes required for glutathione synthesis, γ-glutamylcysteine synthetase and glutathione synthetase, in various forms of human cataracts.

Methods. The Cooperative Cataract Research Group cataract classification method and standard enzyme assay procedures were used.

Results. An inverse relationship was shown between residual activity of each of the glutathione synthesis enzymes and degree of subcapsular cataract. A weaker inverse relationship existed between glutathione synthetase activity and supranuclear and nuclear cataracts. No other parameters yielded comparable correlations with the activity of either enzyme.

Conclusions. Activity loss of the glutathione synthesis enzymes is associated with human subcapsular cataract formation. Invest Ophthalmol Vis Sci 1993;34:2049-2054

The requirement of a high level of glutathione for maintenance of clear lenses is universally recognized. After the 1912 report of Reis1 concerning human cataracts, very low levels of glutathione have consistently been reported in cataracts. Experimentally, this low content occurs before irreversible cataract formation regardless of the cataract-inducing agent.2 However, the cause of the low glutathione levels has not been determined for any form of cataract.

One possible function that might be damaged by cataractogenic agents is the biosynthesis of glutathione. Two enzymes are required for glutathione biosynthesis in the lens. Those two enzymes are γ-glutamylcysteine synthetase (γ-GCS, E.C.6.3.2.2) and glutathione synthetase (GSHS, E.C.6.3.2.3). Synthesis of glutathione must be active to replace that which is destroyed; the half-life of glutathione was estimated as varying from 23 to 90 hr in rat, rabbit, and human cultured lenses.3-5

Despite continuous interest concerning glutathione content in cataract, activity measurements of the two biosynthetic enzymes in human cataracts have been reported only twice. Reduction in γ-glutamylcysteine synthetase activity was reported for whole human cataracts and of glutathione synthetase activity in nuclei derived from extracapsular extractions.6-7

Potts stated that the term “cataract” was a semantic trap. “By using the single word ‘cataract’ for what we well know to be fifteen or twenty disease entities, we trap ourselves into thought processes which are self defeating.”8 Risk factors for age-related cataract development were shown to vary with the morphologic location of the cataract.9-11 Cortical cataract formation was related to increased ultraviolet light exposure due to glare on water.12

It is the purpose of this article to demonstrate the inverse relationship between decreasing residual activity of the glutathione synthesis enzymes and increasing subcapsular opacity in human lenses.
MATERIALS AND METHODS

Materials

The following reagents were obtained commercially: disodium adenosine triphosphate, Tris (trizma base), L-glutamic acid, L-cysteine, glycine and EDTA (tetrasodium salt) (Sigma Chemical Company, St. Louis, MO); L-γ-glutamyl-L-α-aminobutyric acid (Bachem Fine Chemicals, Inc., Philadelphia, PA). All other chemicals were of reagent grade.

The tenets of Declaration of Helsinki were followed. Institutional permission was granted by the Committee on the Use of Human Subjects in Research for this work. Informed consent was obtained from cataract donors after the proposed use of extracted cataracts was explained to them. Cataractous lenses were obtained from the operating rooms of the University of Minnesota Hospitals immediately after excision. A series of 8–12 colored stereophotographs—required by the Cooperative Cataract Research Group method and employing various set-ups, lighting, and magnification—were taken immediately using the modified operating microscope (Zeiss, Thornwood, NY) as described for Cooperative Cataract Research Group classification. Stereophotographs were submitted to Leo T. Chylack, Jr., for cataract classification. After being photographed, all lenses were stored at −74°C until classification results were received. Criteria for exclusion of cataracts from laboratory analysis included broken capsule, grossly misshaped lens, unusually long time at room temperature, and photographic slides yielding inadequate classification data. Lenses were accepted solely on the presence of opacity, not possible clinical causes of the opacities because the focus of the study was on the relationship of the glutathione synthesis system and opacity location. Traumatic cataracts were excluded with one exception. The age range was 37–82 yr, with a median age of 68 ± 11 yr.

Methods

Thawed lenses were homogenized on ice in all-glass homogenizers in cold 0.02 M Tris-sulfate buffer containing 0.003 M EDTA, pH 7.6. The average volume of buffer used for homogenization was 1.0 ml per 0.1 g of lens. Homogenates were centrifuged for 25 min at 15,000 g (4°C) and the supernatant fluids were dialyzed with stirring at 4°C against 4 l of the homogenizing buffer with one buffer change. Dialyzed supernatants were stored at −74°C until assayed for enzyme activities. Enzyme assays were conducted within a time period of a few days to a few months after receipt of the lenses. Identical procedures, including storage time and enzyme activity assays, were used in the study of clear human lenses previously carried out in this laboratory. The activities of γ-glutamyl-cysteine synthetase and glutathione synthetase were previously found to be stable to limited freezing and thawing and for periods exceeding 1 yr when stored at −74°C.

γ-Glutamylcysteine synthetase and glutathione synthetase activities were determined by inorganic phosphate formation from adenosine triphosphate and unit definitions were as previously described. Activities of the two enzymes were determined on the same lenses. All assays were performed using a Beckman Kinetic VII or DU-70 spectrophotometer (Beckman, Fullerton, CA). Analysis of the results was performed using the Cricket Graph program for the Macintosh computer (Apple Computer, Cupertino, CA).

Although experience showed that the activity of the enzymes would be little affected by a few hours of storage at room temperature, metabolite concentrations were likely to be changed greatly. Therefore, because times from excision to freezing of the cataractous lens varied widely, glutathione was not determined in this work.

Mature cataracts were considered to be 100% opaque in all regions. Subcapsular cataracts were estimated as a percentage of the total subcapsular area being considered. Degree of apparent opacity in cortical, supranuclear, and nuclear cataracts was graded 0–4, with 4 being the most severe. Lens color was graded as follows: pale yellow = 1, yellow = 2, dark yellow = 3, very dark yellow = 4, brown = 5, black = 6.

The enzyme activity values are expressed as enzyme activity units/lens. Although much tighter data groupings are obtained when data are expressed either as units/g tissue or specific activity such references for data expression are not appropriate with cataracts that both imbibe water and leak proteins.

RESULTS

γ-Glutamylcysteine synthetase activity

Activity of γ-glutamylcysteine synthetase was demonstrable in all cataracts; however, the activity was variable (Table 1). In part, some of the variability was attributable to the extremely low activity of this enzyme and significant blank values of human cataracts. The residual activity of this enzyme was inversely correlated with anterior subcapsular opacity ($R^2 = 0.486$, Fig. 1A), posterior subcapsular opacity ($R^2 = 0.481$, Fig. 1B) and in combined forms of subcapsular cataract ($R^2 = 0.558$, Fig. 1C). A weak inverse correlation was shown with supranuclear cataract ($R^2 = 0.305$, Table 2, detailed data not shown).

Tables 1 and 2 illustrate a variety of parameters of the cataracts versus the activity of both enzymes. γ-Glutamylcysteine synthetase residual activity showed no significant correlation with the degree of cortical cataract, nuclear cataract, age, or lens color.
TABLE 1. Human Cataract Morphology and GSH-Synthesizing Enzymes

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<th>ID No.</th>
<th>Age (Yr)</th>
<th>SCA (%)</th>
<th>SCP (%)</th>
<th>CxA</th>
<th>Cxe</th>
<th>CXP</th>
<th>SN</th>
<th>Color</th>
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<th>GSHS (U/Lens)</th>
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SCA = subcapsular anterior; CxA = cortical anterior; SN = supranuclear; SCP = subcapsular posterior; Cxe = cortical equatorial; N = nuclear; CXP = cortical posterior.

When morphologic subtypes were combined for graphing, the highest value of the morphologic area represented the opacity of the area. Subcapsular graded by estimated percent of three-dimensional lens area opacified; other areas graded by 0 to 4 opacity index; color graded 1 (pale yellow) through 6 (black).

* Data for this vitrectomy-induced cataract were not included in computing logarithmic curves.

GSH Synthesis and Subcapsular Cataract

Glutathione synthetase residual activity was inversely correlated with anterior subcapsular opacity (R² = 0.586, Fig. 2A), posterior subcapsular opacity (R² = 0.593, Fig. 2B), and in all combined forms of subcapsular cataract (R² = 0.645, Fig. 2C).

TABLE 2. Enzyme Activity Versus Lens Variables

<table>
<thead>
<tr>
<th>Lens Region or Characteristic</th>
<th>R² Values of Logarithmic Plot*</th>
<th>γ-GCS</th>
<th>GSHS</th>
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<td>Subcapsular (combined)†</td>
<td>0.558</td>
<td>0.645</td>
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<tr>
<td>Cortical (combined)†</td>
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<td>0.070</td>
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<tr>
<td>Supranuclear</td>
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<tr>
<td>Nuclear</td>
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<td>Age</td>
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</table>

* Statistics computed from curves omitting lens number 199.
† Data concerning anterior and posterior opacities were combined for the subcapsular region; similarly, data for anterior, equatorial, and posterior regions of the cortex were combined.

Much weaker inverse correlations were shown with supranuclear opacity (R² = 0.271), nuclear opacity (R² = 0.181), and age (R² = 0.205). Tables 1 and 2 show no correlation of glutathione synthetase residual activity with the degree of cortical cataract or lens color.

Simultaneous Presence of Supranuclear and Subcapsular Opacities

Supranuclear opacities frequently appeared in lenses with subcapsular cataracts. A plot of the incidence of each against the other showed a weak positive correlation (R² = 0.351, data not shown).

DISCUSSION

Subcapsular Cataract of this Study

The Cooperative Cataract Research Group classification scheme yields six graded levels of subcapsular opacity. If it were practical to have estimated finer gradations the data of Figures 1 and 2 would reflect that. However, the classification scheme allowed demonstration of inverse relationships between loss of the...
two glutathione synthesis enzyme activities and degree of subcapsular cataract (Figs. 1 and 2). Considerably weaker relationships of both enzyme activities were shown with supranuclear cataract (Table 2). In marked contrast to these results is the lack of correlation of γ-glutamylcysteine synthetase activity with all other morphologic locations of cataract and color. These correlation comparisons strengthen the probability that damage of the glutathione synthesis system is associated with subcapsular cataract.

The activity of glutathione synthetase (units/lens) was less in 23 of 24 cataracts than regression line values of age-matched clear lenses previously assayed in this laboratory using the same analytic and lens storage procedures. The activity of γ-glutamylcysteine synthetase (units/lens) was less in 15 of 24 cataracts than published regression line values. Eight of the nine exceptions that were not lowered exhibited subcapsular opacities of 0–25%. Therefore, simultaneous lower activities of both enzyme activities was not always observed. The ninth exception was the cataract of patient 199, which was the apparent result of vitrectomy. The activities of both enzymes in this cataract are similar to those of clear lenses. This suggests that causes of this vitrectomy-associated cataract were different than those of the other subcapsular cataracts of this study.

Supporting Work

Xie et al assessed activity of these two enzymes and determined glutathione content in nuclei derived from extracapsular extractions of human cataracts. Low nuclear glutathione synthetase activity characterized only intumescent, intense subcapsular, and mature cataract. These were also the cataract types showing a loss of glutathione synthetase activity in the current study. Pau et al demonstrated that the lowest levels of glutathione characterized lenses with subcapsular and primary subcapsular with secondary nuclear cataracts.

Cataract has been induced in suckling rodents by inhibition of glutathione synthesis by buthionine sulfoximine. The initial damage site was variously re-
ported as being the epithelium or the outermost anterior cortical cell layer. Simultaneous administration of glutathione monoester prevented the symptoms in the suckling rat model. Thus, the data of these various research groups concerning subcapsular opacity and low levels of glutathione or its biosynthetic enzymes are consistent with the findings of the current study.

**Spacial Distribution of GSH and GSH Biosynthetic System**

The data of the current work correlates opacity formation in the superficial cortex with activity loss of the glutathione synthesizing system. Only gross data have been published concerning the spacial distribution of each enzyme activity within the lens, however. Activity of human \(\gamma\)-glutamylcysteine synthetase has been reported to be confined to the epithelial/cortical fraction because activity in the nucleus was not detectable. The distribution of glutathione synthetase activity was similar: 98.6% in the epithelial/cortical fraction and 1.4% in the nucleus. The current work suggests that a future spacial distribution study within the epithelium and cortex (via microdissection) of the glutathione biosynthetic system might be desirable.

To hypothesize that the glutathione biosynthetic system is greatly localized in the superficial cortical layers is unnecessary. L-Cysteine derived from transport from the aqueous humor is the putative major source of L-cysteine used in the synthetic scheme. The low \(K_m\) value of L-cysteine coupled with the extreme intracellular paucity of this amino acid ensures its immediate incorporation into glutathione. Therefore, an appreciable proportion of L-cysteine derived from the aqueous humor is unlikely to pass beyond the superficial cortical cells to the deeper layers of the cortex. This suggests that the highest concentration of glutathione should be found in these outermost cell layers. Data consistent with this hypothesis were shown for the epithelial cell layer of a rabbit lens that had a glutathione concentration sixfold higher than the deeper cortical layers.

Some of the major functions of glutathione include maintenance of the plasma membrane and participation in some phases of active transport through this membrane. Membrane maintenance may be especially critical in the relatively oxygen-rich, metabolically active cells of the subcapsular layer as they form the initial site for removal of water and aqueous-derived \(\mathrm{H}_2\mathrm{O}_2\) from the lens, and for nutrient transport. If a high glutathione content is a requirement for proper function of this cell layer then it is not surprising that the subcapsular cells should be among the first to be affected adversely by glutathione deficiency and to develop apparent opacity caused by water influx.

**Additional Vulnerable Sites that May Affect GSH Content**

The current work indicates that low activity levels of the two synthesis enzymes are associated with some forms of subcapsular cataract. This work does not exclude other factors that may simultaneously influence glutathione content in subcapsular cataract. In contrast, because of lack of correlation, low activity levels of this biosynthetic system are unlikely to be major factors causing low glutathione levels in other forms of cataract. A variety of other possibilities exist. Transport of L-cysteine, the rate-limiting substrate of glutathione synthesis, was shown to diminish 70% with aging in cultured human lenses. The rate of glutathione synthesis was shown to be proportional to the uptake of L-cysteine from the medium. Damage to this vulnerable transport system by various agents would supplement aging effects and result in low lenticular glutathione. Conditions that increase the ratio of oxidized glutathione to reduced glutathione increase the chances for loss of oxidized glutathione through the plasma membrane and so to the aqueous and vitreous humors. Such conditions might include lowering the available nicotinamide adenine dinucleotide phosphate and/or damage to enzymes of the glutathione redox cycle. However, oxidation may also be a mechanism for distribution of glutathione from the superficial cortical areas with high glutathione concentrations to areas of low concentrations in the deeper layers of the lens. Additionally, electrophilic compounds capable of conjugating with glutathione cause degradation of glutathione through the mercapturic acid pathway thereby yielding lower concentrations of the tripeptide. For example, naphthalene epoxide, a metabolite of naphthalene, lowers the intracellular glutathione concentration through S-conjugation as shown in the lens and elsewhere.

**Key Words**

human cataract, glutathione, \(\gamma\)-glutamylcysteine synthetase, glutathione synthetase, subcapsular cataract

**Acknowledgment**

The authors are indebted to Leo T. Chylack, Jr. for classification of the cataracts used in this study.

**References**


