Increased Expression of Iron-Regulating Genes in Monkey and Human Glaucoma

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PURPOSE. To understand the mechanisms mediating retinal ganglion cell loss in glaucoma, the gene expression patterns were compared for transferrin, ceruloplasmin, and ferritin between normal and glaucomatous retina in monkey and human eyes.

METHODS. Laser photocoagulation was used to produce unilateral experimental glaucoma in monkeys. Gene expression was assessed by in situ hybridization and quantitative reverse transcription polymerase chain reaction (PCR). Immunohistochemistry was used to examine the retinal expression of iron-related proteins in the retina in experimental monkey glaucoma and human glaucoma.

RESULTS. Comparison of glaucomatous with control monkey retinas demonstrated increased mRNA expression of transferrin, ceruloplasmin, and ferritin heavy and light chains. In situ hybridization localized retinal gene expression of transferrin mainly to the inner nuclear layer and ferritin to both the inner and outer nuclear layers. Immunohistochemical examination of monkey and human glaucoma for these iron-related proteins demonstrated increases at the protein level.

CONCLUSIONS. Increased mRNA and protein levels of the iron-regulating proteins transferrin, ceruloplasmin, and ferritin are present in glaucoma. Together, these results suggest the involvement of iron and copper metabolism and associated antioxidant systems in the pathogenesis of glaucoma. (Invest Ophthalmol Vis Sci. 2004;45:1410–1417) DOI:10.1167/iovs.03-0872

The death of retinal ganglion cells is the hallmark of glaucoma. The level of intraocular pressure (IOP) is a consistent risk factor for glaucoma, with a number of potential mechanisms identified through which retinal ganglion cell (RGC) injury may be mediated. For example, there is evidence of neurotoxicity in glaucoma from neurotrophin deprivation,1 nitric oxide activation,2 glutamate excitotoxicity,3,4 oxidative stress,5,6 and other mechanisms. To uncover the molecular events mediating ganglion cell loss in glaucoma, we initiated a project to compare gene expression patterns in normal retinas with those in glaucomatous retinas. In ongoing work using a custom-made cDNA microarray containing more than 10,000 genes expressed in the retina, we found upregulated transferrin expression in a monkey experimental glaucoma model.

The established properties and disease associations of transferrin and associated iron-regulating proteins provide a rationale supportive of their possible involvement in glaucoma. Transferrin functions as an iron transport molecule, carrying iron both between and within body tissues. Transferrin is crucial for neuronal health, both for the supply of iron it provides, and possibly from independent neurotrophic17,18 and electrophysiological roles.9 Ceruloplasmin promotes iron loading onto transferrin by acting as a ferroxidase, converting the ferrous (Fe(II)) form of iron to the ferric (Fe(III)) form. Transferrin preferentially binds the ferric form of iron. Ferritin serves as the main storage site for organic iron. All three of these enzymes act as antioxidants,10–12 both by tightly restricting reactive iron availability, and in the case of ceruloplasmin and ferritin heavy chain, through intrinsic ferroxidase activity that allows for iron oxidation without free radical release. Tight control of cellular free iron levels is critical to cell viability, since iron catalyzes the generation of reactive oxygen species, leading to such highly destructive molecules as the hydroxyl radical (OH·). Although all cells in the body have an absolute requirement for iron, the retina appears to have a greater need for iron-regulating proteins than do most other tissues. As an illustration of the remarkable importance to the retina of iron regulation, transferrin and ferritin each may account for 0.5% or more of total retinal mRNA production.13 This is on a level similar to that of the visual pigment rhodopsin.14 High levels of transferrin, ceruloplasmin, and ferritin in the retina may be needed as part of an antioxidant system to prevent free radical damage.

Given these facts, together with increasing recent evidence suggesting a role for oxidative stress in glaucoma, we decided to investigate the possible role of iron-regulating proteins in...
glaucoma pathogenesis, studying retinal tissue both from persons with documented histories of open-angle glaucoma and from monkeys that underwent chronic IOP elevation by laser treatment of the trabecular meshwork.

**METHODS**

**Nonhuman Primate Glaucoma Model**

All experiments were conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male juvenile cynomolgus monkeys (Macaca fascicularis) were treated with an argon laser one or more times to scar the trabecular meshwork in one eye, increasing IOP and producing experimental injury similar to that of human glaucoma15 (Table 1). IOP was measured weekly throughout the treatment period with either pneumotonometry or applanation tonometry, and clinical examination of the fundus was performed monthly to monitor changes in the optic disc caused by elevated IOP. After 4 to 16 months, animals were euthanatized by an argon laser one or more times to scar the trabecular meshwork.

**Human Glaucomatous Eyes**

Eyes were obtained from eye banks with informed consent and in accordance with the tenets of the Declaration of Helsinki and approval of the Joint Committee on Clinical Investigation, The Johns Hopkins University School of Medicine. Ophthalmic histories pertaining to glaucoma were collected for all glaucomatous eyes. The retinal specimens from these persons have been reported in published analyses of other tissue features, and based on visual field and/or optic nerve histology, glaucoma severity was estimated as normal in 3 eyes, mild in 10, moderate in 6, severe in 1, and undetermined in 2.17,18 Control eyes, matched for age and sex (Table 2), were selected from persons without a known history of eye disease and were confirmed to be normal by examination of retina, nerve head, and optic nerve area. All eyes were fixed in aqueous formalin within 10 hours of death.

**In Situ Hybridization**

In situ hybridization was performed (Discovery System; Ventana Medical Systems, Tucson AZ) in normal human donor eyes. Eyes were fixed overnight in 10% formalin and then paraffin embedded. Eight-micrometer sections were placed on slides (SuperFrost Plus; Fisher Scientific, Pittsburgh, PA) and incubated overnight at 60°C before hybridization. Probe template was generated by PCR amplification of retinal cDNA with gene-specific primers linked to the T3 (AATTAACCCTCACTAAGGGGAGA) and T7 (TATACGACTCACTATAGGGGAGA) RNA polymerase promoters for transferrin, 5'-T3-CTGCGCAACTTTGTTTGT-G3' and 5'-T7-GATCACTTGCGTTCATCATC3'; and for H-ferritin, 5'-T3-ACCCCATTGTGTGACTCTC3' and 5'-T7-TAAAGGAAAAACCCCACATGC3'. Digoxigenin (DIG)-labeled sense (T3 strand) and antisense (T7 strand) probes were generated from the template in separate reactions by in vitro transcription with a DIG RNA Labeling Kit; Roche Molecular Biochemicals, Indianapolis, IN). Transcript concentration was estimated by dot blot comparison against a DIG-labeled RNA control (Roche Molecular Biochemicals). Automated hybridization on the hybridization machine with 0.5 ng probe per slide for transferrin and 2.0 ng probe per slide for H-ferritin was performed, incorporating Riboprobe, protease treatment P1 for 4 minutes, and a hybridization temperature of 68°C. Biotinylated mouse anti-digoxin primary antibody (Immunogenex, San Ramon, CA) was followed by alkaline phosphatase detection (BlueMap; Ventana) with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate toluidine salt (NBT-BCIP) substrate solution.

**Immunohistochemistry**

Eight-micrometer sections were collected onto slides (SuperFrost Plus; Fisher Scientific) before immunolabeling by the streptavidin-biotin peroxidase technique of Lutty et al.19 Affinity-purified polyclonal antibodies were directed against transferrin (Cappel ICN, Costa Mesa, CA), ferritin (ICN, Irvine CA), and ceruloplasmin (ICN). Western blot analysis confirmed the presence of bands at the appropriate molecular weights. Negative controls included nonimmune serum of the same species as the primary antibody at the same protein concentration and incubation buffer alone. Labeled sections were mounted in glycerol jelly and viewed by microscope equipped with Nomarski optics (Axioskop; Carl Zeiss Meditec, Thornwood, NY) and bright-field illumination.

To decrease experimental variation between paired glaucoma and contralateral control monkey eyes, corresponding sections of glaucomatous and contralateral retina for each monkey were processed into single paraffin blocks for immunostaining. Similarly, for human glaucoma, each slide contained sections from 10 different retinas (5 control, 5 glaucomatous) to decrease slide-to-slide variation arising during immunohistochemical processing.

**Table 1. Characteristics of Glaucomatous Monkeys**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Animal</th>
<th>Glaucoma Severity</th>
<th>Mean IOP (mm Hg)</th>
<th>Duration (mo)</th>
<th>Cup/Disc Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q 941</td>
<td>Moderate</td>
<td>26</td>
<td>16</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Q 948</td>
<td>Severe</td>
<td>38</td>
<td>5</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Q 969</td>
<td>Severe</td>
<td>55</td>
<td>4</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>Q 970</td>
<td>Mild</td>
<td>40</td>
<td>4</td>
<td>NA</td>
<td></td>
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<tr>
<td>Q 971</td>
<td>Mild</td>
<td>28</td>
<td>7</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>I 965</td>
<td>Mild</td>
<td>40</td>
<td>4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>I 968</td>
<td>Severe</td>
<td>45</td>
<td>5</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>I 10512</td>
<td>Severe</td>
<td>27</td>
<td>12</td>
<td>0.8</td>
<td></td>
</tr>
</tbody>
</table>

Glaucoma severity grade based on combined assessment of nerve, disc, and nerve fiber layer examination. Q, quantitative real-time PCR; I, immunohistochemistry; NA, not available.

**Table 2. Characteristics of Human Glaucomatous and Control Eyes**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Glaucoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>23 eyes/22 persons</td>
<td>23 eyes/20 persons</td>
</tr>
<tr>
<td>Mean Age</td>
<td>74 ± 12 y</td>
<td>75 ± 12 y</td>
</tr>
<tr>
<td>Gender</td>
<td>68% male</td>
<td>60% male</td>
</tr>
<tr>
<td>Race</td>
<td>20 white, 1 black, 1 Hispanic</td>
<td>20 white</td>
</tr>
</tbody>
</table>
To estimate differences in antigen concentrations in the retinal sections, a method previously termed the supraoptimal dilution technique was used. This method is based on the careful titration of antibody labeling with dilute primary antisera, so that saturation of labeling is avoided. Although the overall tissue pattern of immunocytochemical labeling does not change at high primary antisera dilutions, tissue areas with relatively lower levels of antigen that would label strongly with less dilute antibody show faint but representative signal intensity.

Image Analysis

Images of retinal sections were captured with a digital camera (Axio-Imager; Carl Zeiss Meditec) with either Kohler optics (color figures) or bright-field illumination (used for further digital image analysis). Photographic and microscopic parameters where kept constant for comparisons between glaucoma and control eyes. Semiquantitative digital image analysis of immunostaining intensity was undertaken for human retinal sections (Metamorph; Universal Imaging Inc., Downingtown, PA). The bright-field light intensity in the region bordering the tissue was assigned a 0 value (no light blocked). The intensity of tissue staining was then calculated as the difference between this 0 value and the gray-scale intensity of light transmitted through the immunostained tissue (with 0 as no stain density, and 255 as highest density, with no light transmitted through the section). The stain density of control tissue (with 0 as no stain density, and 255 as highest density, with no light transmitted through the section). The stain density of control tissue (with 0 as no stain density, and 255 as highest density, with no light transmitted through the section).

Real-Time Quantitative Reverse Transcription–PCR

cDNA was synthesized separately from 1 μg DNase-treated total RNA from left and right eyes of experimental monkeys (Superscript II; Invitrogen, Carlsbad, CA). Primers used are shown in Table 1, and were designed from human sequence information. Real-time quantitative PCR (Q-PCR) reactions were performed with a commercial system (Light-Cycler PCR machine; Roche Diagnostics, Nutley, NJ). PCR products were quantified by using the second derivative maximum values calculated by the system's analysis software. Expression levels were normalized to the average of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and acidic ribosomal phosphoprotein (ARP) mRNA levels. Each gene was tested in triplicate PCR reactions, and the mean of the reactions was used for calculating the expression levels.

RESULTS

mRNA Levels of Iron-Regulating Genes in Monkey Glaucoma

We used Q-PCR to compare the mRNA levels of transferrin, ceruloplasmin, and ferritin in the untreated and treated eyes of monkeys with various degrees of experimentally induced glaucoma. In two monkeys with mild glaucomatous damage, there were no detectable differences in transferrin mRNA levels. In monkeys with moderate or severe unilateral damage, there was a 1.6-fold increase in transferrin. Ceruloplasmin mRNA showed larger increases, approximately sixfold in the monkeys with moderate-severe damage and 1.3-fold in the animals with mild damage. The increase of ferritin mRNA levels was approximately 2.2-fold in both mild and moderate-severe monkey groups. These results at the mRNA level indicated increased endogenous retinal expression of these iron-regulating genes in experimental glaucoma.

In Situ Hybridization of Iron-Regulating Genes

To confirm and localize the production of mRNA for iron-regulating genes in the retina, in situ hybridization was performed for transferrin and ferritin in normal human retina (Fig.)
In our initial observations of transferrin immunostaining, we noticed a strong gradient of increasing immunoreactivity from central to peripheral retina. This gradient was most clearly visualized by immunostaining with nonsaturating dilutions of primary and secondary antibody (monkey, Figs. 2A–2C; human, Fig. 3). Transferrin immunoreactivity was most intense in the NFL and GCL, and extended into the IPL. Intensely stained presumptive Müller cell end feet and processes were observed running from the ILM through the IPL and were especially obvious in human retina (Fig. 3A). Scattered cell bodies were labeled in the INL, with prominent staining of the outer limiting membrane (OLM). This pattern of labeling was consistent with Müller cells being a particular site for high levels of transferrin. In addition, more diffuse transferrin immunoreactivity was present throughout most of the retina, suggesting that other cell types, such as photoreceptors, make or bind the protein. We found that immunostaining for transferrin was increased in glaucomatous monkey retina (n = 3). Labeling was substantially higher in the inner retinal layers, from the macula to the periphery, most strikingly in the most severely glaucomatous regions (Figs. 2D–2F). In glaucoma, labeling was substantially increased, especially in the inner retinal layers.

In human donor eyes, transferrin immunostaining was compared by digital image analysis between donors of glaucomatous and control eyes. Transferrin immunostaining was significantly increased compared with unaffected eyes in the whole retina (1.4-fold, P = 0.0001) and in the inner retinal layers (1.5-fold, P = 0.0001; Fig. 4). Representative sections are shown in Figures 5A–5D (n = 23). There was no significant correlation between transferrin stain intensity and glaucoma severity in human retinas (R² = 0.07, n = 20).

Ceruloplasmin Immunostaining

Ceruloplasmin immunostaining was distributed nearly homogeneously across retinal layers, including the photoreceptor outer segments, although some increased intensity was present in the innermost retinal layers (ILM, NFL, and GCL) and the outer plexiform layer (OPL), especially in the retinal periphery. In glaucomatous monkey retina, immunostaining for ceruloplasmin was increased (n = 3). There was particular intensification of label in the NFL, GCL, and INL and in the plexiform layers. The photoreceptor outer segments were faintly stained in glaucomatous eyes, as in normal eyes. The increase of ceruloplasmin immunostaining was greatest in the most severely affected monkey (Figs. 6D–6F).

In human glaucoma, ceruloplasmin immunostaining was increased compared with control eyes (Figs. 5B, 5E). Comparison of the inner retinal layers (NFL, GCL, and IPL) showed a...
Figure 4. Quantitation of iron-regulating genes in human glaucoma. Box plots showing increased density of immunostaining for transferrin, ceruloplasmin, and ferritin in the inner retina in human glaucoma compared with control eyes (n = 23), glaucoma and control, for each gene. The box represents the 25th to 75th percentiles and whiskers the 10th to 90th percentiles. Squares show mean density, and lines median density. Immunostaining density is expressed on a 255-point gray scale, from zero stain density (0) to complete blockage of bright-field illumination (255).

A statistically significant increase in glaucoma (1.3-fold increase, P = 0.02, Fig. 4), whereas comparison across all retinal layers combined was close to statistical significance (1.2-fold, P = 0.06, n = 23). There was no significant correlation between ceruloplasmin stain intensity and glaucoma severity in human retinas (R² = 0.01, n = 20).

Ferritin Immunostaining

Immunostaining for ferritin was present across all retinal layers, although only faintly in the photoreceptor outer segments (Fig. 7). In the three glaucomatous monkey eyes, the density of ferritin immunostaining was not clearly different from that in control eyes. In contrast, in human glaucomatous eyes (Figs. 5C, 5F), ferritin immunostaining was significantly increased compared with control, both across all retinal layers (1.4-fold increase, P = 0.000004, n = 23), and in inner retinal layers alone (1.2-fold, P = 0.001; Fig. 4). There was no significant correlation between ferritin stain intensity and glaucoma severity in human retinas (R² = 0.02, n = 20).

Discussion

We have shown an increase in the retinal expression of iron-regulating proteins in both monkey and human glaucoma. One of these proteins, ceruloplasmin, is also one of the major copper repositories in the body, thus implicating changes of this reactive metal in glaucoma as well. Transition metals such as iron and copper are key generators of cytotoxic free radicals, whereas iron-regulating proteins such as transferrin, ceruloplasmin, and ferritin counteract their toxic effects, acting as antioxidants. Our results suggest a role for metal-related oxidative stress in the pathogenesis of glaucoma. This conclusion is consistent with accumulating evidence that transition metals are widely involved in a range of neuronal degenerations, possibly as common mediators within neuronal injury pathways. For example, in RGC damage induced by optic nerve crush, retinal expression of ceruloplasmin is increased. During preparation of this manuscript, a microarray analysis of monkey glaucoma was reported, with findings of increased ceruloplasmin.

Figure 5. Iron-regulating genes in human glaucomatous retina. Representative sections of human glaucoma (D–F) and control (A–C) eyes, showing immunostaining for transferrin (A, D), ceruloplasmin (B, E), and ferritin (C, F). The overall pattern of staining in the retina was similar for each gene in both normal and glaucomatous eyes, but with increased density of stain in the glaucomatous eyes. Transferrin immunostaining (A, D) was most intense in the ILM and NFL, as was true to a lesser extent for ceruloplasmin (B, E) and ferritin (C, F).

Figure 6. Ceruloplasmin immunostaining in monkey glaucoma. Corresponding regions of retina, from central (A, D) to peripheral (C, F) are shown for control (A–C) and glaucomatous (D–F) eyes. Ceruloplasmin stain intensity and glaucoma severity in human retinas (R² = 0.02, n = 20).

Figure 7. Ferritin immunostaining in monkey glaucoma. Corresponding regions of retina, from central (A, D) to peripheral (C, F) are shown for control (A–C) and glaucomatous (D–F) eyes. Ferritin immunostaining in normal eyes was greatest in the ILM and NFL and in the plexiform layers. Staining increased from the central to the peripheral retina in both normal (A–C) and glaucomatous (D–F) eyes. In glaucoma, the approximately twofold increase in ferritin detected by Q-PCR was not reflected in ferritin immunostaining.
In retinal diseases unrelated to RGCs, iron and copper are also involved. Iron levels are increased in the RPE in age-related macular degeneration (ARMD) (Hahn P, et al. IOVS 2003;44: ARVO E-Abstract 1733), and ceruloplasmin is increased in animal models of light-induced retinal degeneration. Thus, it seems possible that diverse types of retinal damage may involve changes in transition metal systems.

Changes in iron metabolism also occur as part of normal aging. All four of the genes examined in this study show increases in their RNA levels in the aged human retina, as demonstrated by serial analysis of gene expression (SAGE) and microarray analysis (Chowers I, et al., manuscript submitted). This suggests that iron-regulating systems may be sensitive to age-related perturbations of homeostasis. Outside the eye, changes in iron metabolism are known to occur in common neuronal degenerations, including Alzheimer and Parkinson disease. In fact, evidence supporting the involvement of iron, copper, and zinc in human neurodegenerative diseases has led to clinical trials of metal chelation therapy for Alzheimer disease.

Transferrin has been found to be increased in the aqueous humor in human glaucoma. One explanation for this excess would be increased permeability of the blood–aqueous barrier. This raises the question of whether the increased presence of iron-regulating proteins that we observed in the glaucomatous retinas could also be from increased vascular permeability or from diffusion of proteins produced in the anterior segment or pigment epithelium. Although these mechanisms may have contributed to the increased protein levels that we found, an increase in the endogenous retinal production of these proteins is supported by our finding of increased retinal mRNA levels for transferrin, ceruloplasmin, and ferritin in experimental monkey glaucoma, along with in situ confirmation of retinal mRNA production in human eyes. There was no significant correlation in the monkey data between mRNA and protein levels and the severity of glaucoma (Table 4), although the sample size was small. It seems likely that the relationship between mRNA and protein levels for these genes would be complex, due to factors such as posttranscriptional regulation, as for ferritin, and uptake and release of protein by cells not producing it, as for transferrin. Similar mechanisms probably accounted for some of the differences between the in situ and immunohistochemical results. For example, the dense transferrin immunostaining in the innermost retina probably resulted from protein endogenous to Müller cell end feet (Fig. 3) along with transferrin bound from the vitreous cavity, where it is found in high concentration.

In addition to increased expression of transferrin in glaucoma, we observed an interesting gradient of transferrin expression in both monkey and human eyes, with increased immunostaining in peripheral compared with central retina. This result is consistent with previous SAGE data indicating more transferrin mRNA in the periphery than macula. In contrast, in a detailed study of transferrin and other iron-regulating proteins in the nonfoveal rat retina, no expression gradient was noted. In humans, substances that are present in higher concentrations in the macula compared with the periphery, such as macular pigment, are of particular interest for understanding regional retinal diseases such as macular degeneration. Perhaps the higher concentration of transferrin that we found in the peripheral retina indicates a retinal antioxidant system that runs in an opposite, complementary gradient to that of macular pigment. Only limited studies have been undertaken of the regional distribution of other retinal antioxidants. However, like transferrin, vitamin E is found in higher concentrations in the periphery than the macula.

We used digital image analysis to document increased immunostaining of iron-related proteins in human glaucoma. The ratios generated from this method were more precise than qualitative grading scale values, allowing us to find differences that were not otherwise visually striking. We are aware, however, that the image analytic data are not necessarily a linear quantification of antigen concentration. Attempts at linear quantification of retinal immunostaining have been made, but these require specialized methods that are not readily adaptable to the limitations imposed by human donor tissue. These methods decrease steric interference between antibodies and limit nonlinearity of stain intensity that can occur otherwise from overlapping signal within the tissue section.

Oxidative stress, as an established final common pathway of cell damage, has been implicated in glaucoma’s pathogenesis, both in the anterior segment and in the retina and optic nerve. In the anterior segment, lipid peroxidation may be responsible for trabecular meshwork and Schlemm’s canal damage. Aqueous outflow has been shown to decrease in experimentally induced oxidative damage to the trabecular meshwork. TTR/mucin, the trabecular meshwork inducible glucocorticoid response gene product, increases in trabecular meshwork cells exposed to oxidative stress of hydrogen peroxide. Oxidative DNA damage is increased in the trabecular meshwork of patients with glaucoma. In the retina and optic nerve in glaucoma, oxidative stress is central to the damage produced by glutamate and nitric oxide, two possible mediators of glaucomatous ganglion cell injury. Glutamate damage is mediated in part through free radicals, including nitric oxide (NO). NO, with its unpaired electron, is itself a free radical. Interventions that decrease NO in the retina and optic nerve help prevent glaucomatous damage. A free radical scavenger in combination with brain-derived neurotrophic factor (BDNF) increases the survival of RGCs in glaucoma above that of BDNF alone. Similarly, a free radical scavenger in combination with inhibition of nitric oxide synthase potentiates the neurotrophic effect of BDNF on axotomized RGCs.

Our findings, in addition to suggesting an oxidative damage component in glaucoma pathogenesis, raise many additional questions about the specific role of iron and iron-regulating molecules in glaucoma. Is the increase of iron-regulating genes secondary to iron release from injured ganglion cells? Or could a nonspecific activation of Müller cells in retinal injury from glaucoma include upregulation of iron-regulating genes, similar to the upregulation of glial fibrillary acidic protein (GFAP) in both glaucoma and other retinal insults? Theoretically, it seems likely that the increased levels of iron-regulating proteins in glaucoma are beneficial, because of their ability to limit iron-related oxidation. Our findings might thus represent identification of an endogenous neuroprotective retinal response in glaucoma. It will be important, however, to address this question directly by testing the efficacy of glaucoma therapies that target iron and antioxidant systems. Potentially encouraging is the finding that antioxidant therapy can be beneficial in certain forms of ARMD. However, this hope must be tempered by the report that antioxidant therapy is not effective in age-related cataract, a disease also linked to oxidative stress and iron abnormalities.

Acknowledgments

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References


