Ability of Dorzolamide Hydrochloride and Timolol Maleate to Target Mitochondria in Glaucoma Therapy

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Objective: To test the ability of dorzolamide hydrochloride and timolol maleate to display antioxidant effects.

Methods: Antioxidant activity was tested in whole trabecular meshwork (TM) tissue as collected from corneal donors' biopsy specimens, young (third passage) and old (10th passage) human TM cells, and acellular systems composed of pure DNA and subcellular fractions containing or devoid of mitochondria. Oxidative stress was induced by hydrogen peroxide. Monitored end points included DNA fragmentation as evaluated by the halo test, oxidative DNA damage in terms of 8-hydroxy-2'-deoxyguanosine, and mitochondrial function as evaluated by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide test.

Results: The antioxidant effect of dorzolamide and timolol were observed on TM biopsy specimens and human TM cells exposed to hydrogen peroxide. As evaluated in cell subfractions, timolol displays antioxidant activity regardless of mitochondria presence. Conversely, the antioxidant activity of dorzolamide was maximized in the presence of mitochondria-containing subcellular fractions and in young human TM cells with functional mitochondria.

Conclusions: The antioxidant effect of timolol was direct. The antioxidant effect of dorzolamide involves mitochondria and is likely to be exerted mainly during the early glaucoma phases when the mitochondrial damage in the TM tissue still occurs at low levels.

Clinical Relevance: Timolol has an antioxidant effect on the entire cell, whereas dorzolamide exerts protective activity toward oxidative stress only in the presence of intact mitochondria (ie, in endothelial cells that are younger when the cellular damage is still limited). The important role of mitochondrial damage in primary open-angle glaucoma is supported by the finding that mutant myocilin impairs mitochondrial functions in human TM meshwork cells.


GLAUCOMA, THE MOST COMMON CAUSE OF IRREVERSIBLE BLINDNESS WORLDWIDE,1 IS A SYNDROME CHARACTERIZED BY A PROGRESSIVE OPTIC ATROPHY THAT RESULTS FROM RETINA GANGLION CELL DEATH. THE DEGENERATIVE FORM OF GLAUCOMA (IE, PRIMARY OPEN-ANGLE GLAUCOMA [POAG]) IS A COMPLEX DISEASE THAT AFFECTS VARIOUS EYE STRUCTURES, INCLUDING TRABECULAR MESHWORK (TM) CELLS2,3 AND THEIR EXTRACELLULAR MATRIX,4 BLOOD VESSELS,5 AND NEURONS LOCATED IN THE RETINA AND THE GENICULATE NUCLEI.6 MANY RISK FACTORS AND PATHOGENIC MECHANISMS ARE ABLE TO INDUCE DAMAGE IN THESE STRUCTURES DURING POAG. THESE MECHANISMS INCLUDE INTRAOCULAR PRESSURE (IOP) INCREASE,7 VASCULAR DAMAGE,8 AUTOIMMUNITY,8 METALLOPROTEASE ACTIVATION,9 ENDOThELIN RELEASE,10 AND NITRIC OXIDE DELIVERY.11 RECENT EXPERIMENTAL EVIDENCE SUGGESTS THAT LONG-LASTING OXIDATIVE STRESS IS A MAJOR PATHOGENIC MECHANISM FOR POAG.12,13 FREE RADICALS TRIGGER A VARIETY OF INJURIOUS PATHWAYS, FINALLY RESULTING IN GLAUCOMATOUS OPTIC NEUROPATHY.14 THE ENTIRE WALL OF THE ANTERIOR CHAMBER OF THE EYE, WHICH IS FORMED BY THE CORNEA, THE IRIS, AND THE TM BETWEEN THEM, IS COATED WITH ENDOTHELIAL CELLS.13 THESE CELLS CONSTITUTE THE INNER COMPONENT OF THE TM AND ARE IN DIRECT CONTACT WITH OXIDIZING AGENTS CONTAINED IN THE AQUEOUS HUMOR, SUCH AS HYDROGEN PEROXIDE.14,16,17 THE TM ENDOTHELIAL CELLS REGULATE AQUEOUS OUTFLOW BY ACTIVELY RELEASING LIGANDS THAT, BINDING TO SCHLEMM CANAL ENDOTHELIAL CELLS, INCREASE TRANSENDOTHELIAL FLOW, THEREBY FACILITATING THE EGRESS OF AQUEOUS HUMOR.18 THE TM PORES CONTRIBUTE TO ONLY 10% OF THE TOTAL AQUEOUS OUTFLOW RESISTANCE,19 WITH MOST OF THE AQUEOUS HUMOR OUTFLOW BEING REGULATED BY AN ACTIVE MECHANISM.20 OUR PREVIOUS STUDIES DEMONSTRATED INCREASED OXIDATIVE DNA DAMAGE IN THE TM OF PATIENTS WITH GLAUCOMA COMPARED WITH...
that of unaffected control individuals.\textsuperscript{21,22} Therefore, we decided to investigate whether glaucoma medications such as dorzolamide hydrochloride and timolol maleate have a direct, intrinsic antioxidant effect on the TM.

Dorzolamide is a topical carbonic anhydrase (CA) inhibitor that displays significant IOP-lowering activity and vasoactive effect.\textsuperscript{23} This issue was tested under various experimental conditions including the use of whole TM, human TM (HTM) cells, and mixtures of subcellular components. Experiments were performed directly on HTM fragments collected from corneal donors. To explore the mechanism of the detected antioxidant effect, experiments were also performed in HTM cells, representing the first line of interaction with the aqueous humor in the anterior chamber of the eye. We also performed experiments in mitochondria-containing subcellular fractions because dorzolamide recognizes the CA enzyme as a main target. In humans, 16 different CA isoforms were isolated, which included CAI and CAIL (cytosolic) and CA VA/VB (mitochondrial isoforms).\textsuperscript{24} In particular, the cytosolic enzyme CAIL plays a pivotal role in the regulation of IOP, with its inhibition being an efficient method for regulating aqueous humor dynamics.\textsuperscript{25} The antioxidant activity of dorzolamide was compared with that of a β-blocking agent (ie, timolol), whose antioxidant effects on endothelial cells have been previously reported.\textsuperscript{26}

Dorzolamide and timolol were tested for their ability to counteract oxidative stress as induced by hydrogen peroxide at different doses. The end points monitored included oxidative DNA damage, DNA fragmentation, and mitochondrial function. The level of oxidative DNA damage was tested by analyzing 8-hydroxy-2′-deoxyguanosine (8-oxo-dG), the most abundant DNA oxidative lesions demonstrated in the TM of patients with glaucoma.\textsuperscript{21} The 8-oxo-dG results from the interaction between the hydroxyl radical OH and the C2 of guanine, resulting in a hydroxylated guanine that, if unrepaired by specific glycosylases, may cause G→A transversions.\textsuperscript{27} Fragmentation of DNA was evaluated by the halo test. Mitochondrial function was evaluated by analyzing the reduction of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) salts. The MTT test is an indicator of cell viability and mitochondrial activity, 2 functions that are altered in glaucoma.

**METHODS**

**TM SAMPLE COLLECTION AND TREATMENT**

Fresh ocular specimens collected from 5 corneal donors with no history of ocular diseases were obtained from the Genoa Lions Eye Bank–Melvin Jones Foundation of Genoa, Italy. The human samples were obtained and processed according to the tenets of the Declaration of Helsinki.

The pigmented black stripe containing the complete TM, from the Schwalbe line to the scleral spur and including the Schlemm canal and the TM pigmented band, was cut away (Figure 1). The TM tissue collected from each study participant was divided in 8 fragments and suspended in phosphate-buffered saline (PBS; pH 7.4) containing 10mMD-glucose to evaluate oxidative stress as induced by hydrogen peroxide in the absence or presence of dorzolamide and timolol before treatment for 30 minutes at 37°C.

Dorzolamide was provided in its pure form from Merck & Co, Inc (Rahway, New Jersey). Timolol, devoid of denaturating preservatives such as benzalkonium chloride or similar preservatives, was used as a commercially available drug (Farmila-Thea Pharmaceuticals, Thissen, Belgium). Preliminary dose-response experiments were performed by means of the halo test to determine that dorzolamide and timolol at the dosage used did not induce toxic effects.

The TM tissue fragments as collected from each study participant were treated as follows: group 1, control individuals (untreated); group 2, hydrogen peroxide (25µM and 100µM) for 5 minutes; group 3, timolol (500µM) for 30 minutes fol-
After treatment, the TM fragments were homogenized and DNA extracted (GenePure; Sigma Chemical Company, St Louis, Missouri) was quantified by fiber optic spectrophotometry (Na-

lowed by hydrogen peroxide (50µM and 100µM) for 5 minutes; group 4, dorzolamide (50µM) for 30 minutes followed by hydrogen peroxide (25µM and 100µM) for 5 minutes; and group 5, dorzolamide (500µM) and timolol (50µM) for 30 minutes followed by hydrogen peroxide (25µM and 100µM) for 5 minutes. Monitored end points included oxidative DNA damage as induced by hydrogen peroxide by itself or in the presence of dorzolamide and/or timolol. C. Fragments of TM as evaluated by the halo test as previously reported (Figure 1). After treatments, as previously described, TM fragments were washed twice with PBS. Cells from treated TM fragments were obtained by incubating calf thymus DNA with 1mM copper sulfate and 50mM hydrogen peroxide or using an authentic 8-oxo-dG reference standard (National Cancer Institute Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, Missouri). Samples without DNA were used as the negative control.

**EVALUATION OF DNA FRAGMENTATION BY HALO TEST IN TM FRAGMENTS**

Evaluation by the halo test of DNA fragmentation in TM fragments took place, as described by Sestili and Cantoñi 

**CELL LINE CULTURE AND TREATMENT**

The HTM cells isolated from the juxtacanalicular and corneoscleral region were supplied by ScienCell Research Laboratories (Carlsbad, California). The HTM cells were grown in poly-l-lysine–coated flask culture (2 µg/cm²) in fibroblast medium with 2% (vol/vol) fetal bovine serum, 1% (vol/vol) fibroblast growth supplement, and 1% (vol/vol) penicillin-streptomycin solution. Cells were maintained at 37°C in a humidified atmosphere with 5% carbon dioxide to reach semiconfluence (80%-90%). The medium was supplied with fresh culture medium every 24 hours. When an 80% to 90% of confluence was attained, the monolayers were subcultured or used for experiments by means of trypsin (Sigma Chemical Company).

The cells were pretreated with drugs before oxidative stress as induced by hydrogen peroxide as previously described for HTM biopsies (groups 1-8). However, dorzolamide and timo-

**ANALYSIS OF 8-OXO-dG BY 32P-POSTLABELING**

After treatment, the TM fragments were homogenized and DNA extracted (GenePure; Sigma Chemical Company, St Louis, Missouri) was quantified by fiber optic spectrophotometry (Na-

**Figure 2.** Inhibition of oxidative damage as induced by hydrogen peroxide in trabecular meshwork (TM) specimens by timolol maleate and dorzolomide hydrochloride. A, Histograms indicate mean (SD) 8-hydroxy-2,3-deoxyguanosine (8-oxo-dG) amounts in human TM fragments in the presence of 50µM (light blue columns) and 100µM hydrogen peroxide (dark blue columns). A significant antioxidant effect of dorzolamide and timolol is detected. B, Corresponding oxidative DNA damage (8-oxo-dG) as detected by phosphorus 32–postlabeling in human TM fragments collected from corneal donors under basal conditions or after exposure to hydrogen peroxide by itself or in the presence of dorzolamide and/or timolol. C. Fragments of TM as evaluated by the halo test as previously reported (Figure 1). After treatments, as previously described, TM fragments were washed twice with PBS. Cells from treated TM fragments were obtained by incubating calf thymus DNA with 1mM copper sulfate and 50mM hydrogen peroxide or using an authentic 8-oxo-dG reference standard (National Cancer Institute Chemical Carcinogen Reference Standard Repository, Midwest Research Institute, Kansas City, Missouri). Samples without DNA were used as the negative control.

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loll were used at 250µM and hydrogen peroxide at 10 and 25µM, respectively, for 10 minutes. These doses and experimental conditions have been selected to avoid any toxic effect, as evaluated by the trypsin blue survival test.

After incubation, the medium was removed and cells were washed twice in phosphate buffer, pH 7.4, gently scraped, and suspended (20 × 10^6 cells per well) in complete medium. Monitored end points included DNA fragmentation (evaluated by the halo test), as previously described, and mitochondrial function (per the MTT test). The effects of drugs and oxidizing agents on HTM cells at various aging stages were comparatively evaluated in cells at the third vs 10th passage.

ANALYSIS OF MITOCHONDRIAL FUNCTION BY MTT TEST IN HTM CELLS

Mitochondrial function was evaluated by analyzing the reduction of MTT salts to purple formazan crystals occurring in cells with functional mitochondria owing to a specific dehydrogenase. The HTM cells were seeded in 24-well microtiter tissue culture plates (3 × 10^4 cells per well) and incubated in complete medium. After 48 hours the monolayer cells were pretreated with dorzolamide and timolol, followed by oxidative stress induced by hydrogen peroxide. Experimental group and drug pretreatments were performed as described. After treatment, the medium was removed and the monolayer was washed with PBS solution (pH 7.4) containing 10mM d-glucose. The MTT solution (0.5 mg/mL) was added, and the plates were incubated for 3 hours at 37°C in 5% carbon dioxide. After this period, the supernatant was discarded and the solubilization solution (HEPES buffer [Sigma Chemical Company] 50mM/ethanol 1:9, pH 8) was added. The MTT reduction was detected by quantifying its reduced derivative by evaluating absorbance at 590 nm using a fiber optic spectrophotometer (NanoDrop Technologies, Inc). Results are expressed as the percentage of decrease in the ability of reducing MTT in treated cells compared with 100% referred to untreated controls. Statistical analyses were performed using the t test for unpaired data (StatView software, version 3.0; Abacus Concepts, Berkeley, California). Reported results are mean (SE) of at least 3 independent experiments as performed for each experimental condition tested.

EVALUATION OF ANTIOXIDANT EFFECTS IN CELLULAR SUBFRACTIONS

To obtain cellular subfractions, Sprague Dawley rats (Charles River Laboratories International Inc, Wilmington, Massachusetts) were killed, their livers were removed and homogenized in saccharose Tris 4/1 (vol/vol), and cellular subfraction pellets were collected after 12,000 g centrifugation for 30 minutes at 4°C (S105 fraction). Cellular subfraction pellets collected after 105,000 g centrifugation for 30 minutes at 4°C (S105) were also collected. The ribosomal protein S12 includes mitochondria, whereas S105 is the postmitochondrial cytosolic fraction (ie, microsomes) devoid of mitochondria.

Cellular subfractions and DNA (calf thymus DNA; Sigma Chemical Company) were exposed to oxidative stress in the absence or presence of dorzolamide and timolol. In this manner, we analyzed the antioxidant ability of dorzolamide and timolol in the absence or presence of mitochondria.

In the presence of cellular subfractions, DNA was treated for 5 minutes and calculated after hydrogen peroxide addition at room temperature in a final volume of 1 mL under the following experimental conditions: group 1, untreated DNA (10µg); group 2, untreated DNA (100µg) plus S12 (2-mg proteins); group 3, untreated DNA plus S105 (200-mg proteins); group 4, DNA and hydrogen peroxide (50µM); group 5, DNA, S12, and hydrogen peroxide; group 6, DNA, S105, and hydrogen peroxide; group 7, DNA, hydrogen peroxide, and dorzolamide (500µM); group 8, DNA, S12, hydrogen peroxide, and dorzolamide; group 9, DNA, S105, hydrogen peroxide, and dorzolamide; group 10, DNA, hydrogen peroxide, and timolol (500µM); group 11, DNA, S12, hydrogen peroxide, and timolol; group 12, DNA, S105, hydrogen peroxide, and timolol; group 13, DNA, hydrogen peroxide, dorzolamide, and timolol; group 14, DNA, S12, hydrogen peroxide, dorzolamide, and timolol; and group 15, DNA, S105, hydrogen peroxide, dorzolamide, and timolol.

At the end of the treatments, hydrogen peroxide was evapo-rated by heating at 50°C for 5 minutes and DNA was purified by proteinase K (Boehringer Ingelheim GmbH, Mannheim, Germany) digestion and immunooaffinity column chromatography using a commercially available kit (GenePure) in the presence of antioxidant (dithiothreitol). Extracted DNA was quantified by fiber optic spectrophotometry (NanoDrop Technologies Inc) evaluating absorbance at 260 and 280 nm. Purified DNA samples showed a 260/280 ratio greater than 1.75 and less than 1.90, which was assumed as an indicator of DNA purity. Samples were stored at –80°C until 8-oxo-dG quantification by 32P-postlabeling, as previously described. All reported experiments have been performed in 3 independent analyses, and results are expressed as mean (SD).

RESULTS

EVALUATION OF DRUG ANTIOXIDANT ACTIVITY IN TM BIOPSIES

Figure 2 shows an example of the results obtained by analyzing with 32P-postlabeling 8-oxo-dG formation in TM tissue undergoing oxidative stress and/or drug pretreatments. Quantitative results are also reported in this figure. Dorzolamide was effective in protecting DNA of TM cells from hydrogen peroxide used at high and very high concentrations. Timolol was protective only at high hydrogen peroxide concentrations. Similar results were obtained in TM samples undergoing oxidative stress by analyzing DNA fragmentation by means of the halo test (Figure 3).

EVALUATION OF ANTIOXIDANT ACTIVITY IN HTM CELLS

The HTM cells were severely affected by oxidative damage, as observed by analyzing all monitored end points. Hydrogen peroxide induces oxidative DNA damage in nuclear DNA, as demonstrated by the increased DNA fragmentation (Figure 4). Hydrogen peroxide also induced failure in mitochondrial function, as demonstrated by the decreased ability of these organelles to reduce MTT salts (Table 1).

Dorzolamide and timolol exerted protective effects (Figure 4 and Table 1). The antioxidant effect of dorzolamide at the low dose of hydrogen peroxide (10µM) was higher (P < .01) than that displayed by timolol. At higher doses of hydrogen peroxide (25µM), the protective effect of the 2 drugs was comparable but lower than that displayed by dorzolamide at the low dose of oxidative stress. No addictive effect was observed when the 2 drugs were used in combination. Protective properties displayed by dorzolamide were affected by cell aging because this drug...
was effective in protecting young (third passage) but not old (10th passage) cells.

EVALUATION OF ANTIOXIDANT ACTIVITY ON DNA IN THE PRESENCE OF CELLULAR SUBFRACTIONS

A remarkable oxidative effect on DNA was induced by hydrogen peroxide. This effect was attenuated in the presence of S105 and, to a greater extent, of S12 (Table 2). Dorzolamide and timolol were able to attenuate hydrogen peroxide effects to a similar extent. The antioxidant effect of dorzolamide was remarkably increased in the presence of the mitochondria containing S12 (Table 2). This situation was not observed for timolol, which displayed antioxidant effects independently of the cellular subfraction used. When used in combination, the protective effect of the 2 drugs was slightly increased (Table 2).

DISCUSSION

The results of this study provide evidence that dorzolamide and timolol display antioxidant effects, protecting the whole TM as collected from human donors. Re-

Table 1. Antioxidant Effects of Dorzolamide Hydrochloride and Timolol Maleate vs Hydrogen Peroxide

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>HTM Third Passage, Mean (SD), %</th>
<th>HTM 10th Passage, Mean (SD), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>100 (1.91)</td>
<td>100 (0.95)</td>
</tr>
<tr>
<td>Hydrogen peroxide, 10µM</td>
<td>70.03 (0.78)</td>
<td>60.05 (0.68)</td>
</tr>
<tr>
<td>Hydrogen peroxide, 25µM</td>
<td>65.21 (1.12)</td>
<td>55.11 (0.37)</td>
</tr>
<tr>
<td>Dorzolamide</td>
<td>93.56 (0.33)</td>
<td>101.65 (0.35)</td>
</tr>
<tr>
<td>Timolol</td>
<td>89.27 (0.74)</td>
<td>91.30 (0.60)</td>
</tr>
<tr>
<td>Dorzolamide and timolol</td>
<td>107.14 (2.56)</td>
<td>86.02 (0.67)</td>
</tr>
<tr>
<td>Dorzolamide and hydrogen peroxide, 10µM</td>
<td>88.61 (1.10)</td>
<td>58.17 (0.96)</td>
</tr>
<tr>
<td>Timolol and hydrogen peroxide, 10µM</td>
<td>75.45 (1.13)</td>
<td>54.52 (0.21)</td>
</tr>
<tr>
<td>Dorzolamide, timolol, and hydrogen peroxide, 10µM</td>
<td>87.90 (1.39)</td>
<td>54.05 (0.32)</td>
</tr>
<tr>
<td>Dorzolamide and hydrogen peroxide, 25µM</td>
<td>78.67 (1.43)</td>
<td>51.82 (0.41)</td>
</tr>
<tr>
<td>Timolol and hydrogen peroxide, 25µM</td>
<td>75.75 (0.90)</td>
<td>50.88 (0.28)</td>
</tr>
<tr>
<td>Dorzolamide, timolol, and hydrogen peroxide, 25µM</td>
<td>82.24 (1.76)</td>
<td>52.29 (0.41)</td>
</tr>
</tbody>
</table>

Abbreviations: HTM, human trabecular meshwork; MTT, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide.

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recently, the antioxidant properties of antiglaucomatous drugs have gained attention. Timolol has been reported to display antioxidant protective effects in endothelial cells. These results were recently confirmed by Miyamoto et al, who demonstrated that TM cells showed reduced sensitivity to hydrogen peroxide when cells were treated with timolol. Our study provides evidence that dorzolamide displays antioxidant effects in TM and HTM cells. Dorzolamide exerts more relevant antioxidant properties than timolol when active mitochondria are present in the experimental system. Mitochondria have been suggested to play a major role in glaucoma development. The TM POAG cells display defective mitochondrial function and dysfunction in intracellular calcium regulation. A recent in vivo study performed in 235 individuals demonstrates that mitochondria undergo severe alterations and progressive loss in the TM of patients with glaucoma compared with healthy controls.

Furthermore, the important role of mitochondrial damage in POAG is supported by the finding that mutant myocilin impairs mitochondrial functions in HTM cells and may confer different sensitivity to oxidative stress depending on the mutation. Our data suggest that topical therapy with dorzolamide counteracts the adverse consequences of oxidative damage as occurring in whole TM and in its endothelial component.

The antioxidant effects of dorzolamide are maximized in the presence of intact mitochondria, which are expected to be pivotal intracellular targets for the antioxidant properties of this drug. Dorzolamide displays more remarkable antioxidant effects in young HTM cells, which have good mitochondrial function, than in older cells, which have poor mitochondrial function. Accordingly, it is conceivable that the antioxidant effect of dorzolamide is maximized when the drug targets still-functional TM cells, whereas it is negligible when targeting TM tissue devoid of significant mitochondrial function, which characterizes advanced stages of glaucoma. It is conceivable that dorzolamide therapy at early glaucoma stages fully displays the multiple mechanisms of this drug, including antioxidant effects. Conversely, the same drug could be less effective when administered at late stages of glaucoma.

Dorzolamide exerts its therapeutic effects through multiple mechanisms, including improvement of ocular perfusion, thus decreasing optic nerve sensitivity to IOP-induced damages. Our results indicate that an additional mechanism displayed by this drug is direct protection from oxidative stress exerted, targeting CA and mitochondria. At the level of the central nervous system, CA inhibition exerts a relaxing effect directly on the optic nerve vessels, as demonstrated by the finding that dorzolamide induces dilatation of retinal arterioles. This situation results in the reduction of retinal neural cell damage, thus supporting the view that dorzolamide can be considered a neuroprotectant. This mechanism probably occurs through the protection against the induction of oxidative stress secondary to intracellular pH alterations. Oxidative stress is closely linked to acidification in mitochondria and in the cytoplasm and is able to induce retinal ganglion cell death by apoptosis. Dorzolamide, as an antioxidant, could induce protection against retinal ganglion cell loss. Similar mechanisms could contribute to the explanation of why dorzolamide but not timolol increases blood flow in the optic nerve head and choroid after 6 months of treatment. Timolol antioxidant effects are exerted through their own metabolism by inducing in endothelial cells the expression of peroxiredoxin-2 through the activation of the FOXO3a transcription factor.

Oxidative stress plays a fundamental role in glaucoma pathogenesis, and its effect on TM has been dem-
onstrated. In fact, oxidative DNA damage is remarkably increased in this region in patients with glaucoma, and was proposed to be responsible for diminishing TM cellularity, altering TM intracellular cytoskeletal structures, and contributing to neuronal cell death in the optic nerve head. Neural degeneration extends beyond the retinal ganglion cells, involving neurons of the lateral geniculate nucleus of the brain which, during glaucoma, are affected by peroxynitrite-mediated oxidative cell injury and glutamate toxicity.

Drugs that display antioxidant effects are, in principle, able to counteract many of these oxidative-related mechanisms. Accordingly, evaluating the antioxidant potential of antiglaucomatous drugs is relevant when addressing their clinical use, taking into account that TM is the most sensitive tissue of the anterior chamber to oxidative damage, as evaluated by ex vivo testing with hydrogen peroxide.

In conclusion, reported results indicate that dorzolamide and timolol exert antioxidant protective effects on HTM. The antioxidant properties of these drugs, developed to inhibit aqueous humor production by the ciliary epithelia exerted in the TM, are likely to be a major determinant of their therapeutic properties. However, reported antioxidant effects are drug specific, not class specific. Further experiments will be performed to verify whether these effects are shared by other β-blockers or CA inhibitors. The antioxidant effects of dorzolamide were exerted toward high and low hydrogen peroxide concentrations, whereas timolol was protective only toward low hydrogen peroxide concentrations. This difference is related to the different properties and pharmacokinetic characteristics of these drugs. Timolol has direct antioxidant effects related to its own metabolism. Conversely, dorzolamide exerts protective activity mainly in the presence of intact mitochondria. These findings suggest that dorzolamide should be used in the treatment of glaucoma when TM damage is not advanced and the trabecular cells have a certain viability and intact mitochondrial function.

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**Ophthalmic Images**

**Traumatic Anterior Subluxation of Natural Lens With Aniridia and Blood Lining Descemet Folds**

Mathew Giegengack, MD
Shree K. Kurup, MD

A man was struck with a garden hose in the right eye (Figure). The trauma resulted in limbal laceration, 360° iridodialysis, and expulsion of iris through the wound with anterior lens dislocation. Seven days later, corneal edema with Descemet folds containing blood trapped within were seen.