Impact of Corneal Endothelial Dysfunctions on Intraocular Oxygen Levels in Human Eyes

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PURPOSE. We studied the implications of corneal endothelial dysfunctions on oxidative stress in the anterior segment via in vivo measurements of oxygen partial pressure (pO2) in the anterior chamber (AC) of human eyes.

METHODS. We recruited 51 patients undergoing cataract surgery and/or endothelial keratoplasty (EK). Endothelial cell density (ECD; n = 33) and central corneal thickness (CCT; n = 41) were measured on patients with relatively clear corneas. Before surgery, an oxygen sensor was introduced into the AC via a peripheral corneal paracentesis. In all patients, seven measurements of pO2 were obtained by positioning the flexible tip near the endothelium at the central cornea, at four cardinal subendothelial locations near the midperipheral cornea, and in the mid-AC and AC angle. In patients with pseudophakia or eyes undergoing cataract surgery, pO2 also was measured near the lens surface and in the posterior chamber.

RESULTS. Consistent with our previous reports, a steep oxygen gradient was noted in the anterior segment of normal controls (n = 24). In patients with endothelial dysfunctions (n = 27), there was a significant increase of pO2 at all five subendothelial locations without a significant increase of pO2 in the AC angle. By regression analyses, subendothelial pO2 correlated inversely with ECD and positively with CCT in patients with endothelial dysfunctions.

CONCLUSIONS. This study demonstrates an even steeper intraocular oxygen gradient in eyes with corneal endothelial dysfunctions. It suggests that the reduced oxygen consumption in corneal endothelial cells may increase oxidative stress in the AC and the existence of an alternative aqueous inflow pathway that maintains a relatively low and constant pO2 at the AC angle.

Keywords: corneal edema, corneal endothelial cells, oxidative damage, aqueous flow

Cataract and glaucoma are two of the most common ocular morbidities. Previous studies by our group and others have suggested that exposure of the crystalline lens to excessive oxygen may have a critical role in the pathogenesis of age-related nuclear cataracts. Specifically, the oxygen level is relatively low in the vitreous gel and nuclear cataracts are prone to develop after vitrectomy by increasing lens exposure to oxygen from the posterior segment altering the normally hypoxic environment around the lens. Furthermore, Chang reported an increased risk of open angle glaucoma developing after vitrectomy and subsequent cataract surgery. The presence of the natural lens at the time of vitreectomy was associated with a 28-month delay in the onset of glaucoma. We hypothesized that exposure of the anterior segment to oxygen may increase the risk of glaucoma.

A large body of evidence indicates that increased oxidative stress or the accumulation of oxidative damage contributes to the pathogenesis of open angle glaucoma. Recent studies by our group on oxygen distribution in the human eyes clearly demonstrated that the oxygen level was significantly elevated in aqueous humor near the trabecular meshwork (TM) after combined vitrectomy and cataract surgery. Therefore, it further supports the notion that the elevated oxygen near the TM may contribute to the development of open-angle glaucoma.

Our most recent study also has revealed a steep oxygen gradient in the anterior segment of human eyes with an inverse correlation between central corneal thickness (CCT) and oxygen partial pressure (pO2) exclusively in the anterior chamber (AC) angle. As the corneal endothelial pumps require active oxygen consumption, we speculated that corneal endothelial dysfunctions could impact the oxygen tension in the anterior segment of human eyes. This study further explored the oxygen consumption by corneal endothelium and its implications for oxidative stress in the anterior segment. Measurements of pO2 were performed in the anterior segment of patients undergoing either cataract surgery (normal control) or Descemet’s stripping automated endothelial keratoplasty (DSAEK) due to Fuchs’ endothelial corneal dystrophy (FECD) or pseudophakic bullous keratopathy (PBK).

METHODS

Study Design

A cross-sectional study was designed to compare the oxygen distribution in different intraocular locations in three groups of patients. Patients scheduled to undergo routine phacoemulsi-
Corneal Endothelial Dysfunctions

**Table 1.** Demographic Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, y, Mean ± SD</th>
<th>Sex, % Male</th>
<th>Race % Caucasian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, n = 24</td>
<td>70 ± 9</td>
<td>45.8</td>
<td>66.7*</td>
</tr>
<tr>
<td>FECD, n = 11</td>
<td>64 ± 8*</td>
<td>54.5</td>
<td>100*</td>
</tr>
<tr>
<td>PBK, n = 16</td>
<td>75 ± 12*</td>
<td>62.5</td>
<td>75.0</td>
</tr>
</tbody>
</table>

ANOVA with multiple comparisons is used for the data analysis. Control, patients with normal cornea and cataract.

* P < 0.05.

Patients and pO2 Measurements

We recruited 51 patients undergoing cataract surgery and/or DSAEK by AJWH for this study. Patients were excluded from the study if they had a history of prior ocular surgery other than cataract surgery, ischemic ocular disease, AC angle closure, inflammatory disease, ocular neoplasia, or monocular status. General medical and ophthalmic histories were obtained as well as a comprehensive ophthalmic examination. Preoperatively, semiautomated estimate of endothelial cell density (ECD) and CCT were measured on patients with relatively clear cornea using confocal specular microscopy (n = 33; Confoscan 4; Nidek, Freemont, CA, USA) and Pentacam HR (n = 41; Oculus, Lynnwood, WA, USA). Race was self-reported by a standardized questionnaire used in our previous studies.19-22

As per our routine protocol for intraocular surgeries, the patient was placed in the supine position and a peribulbar injection of 4 mL of 2% lidocaine and 0.375% bupivacaine (50:50) solution was performed to provide local anesthesia under intravenous sedation. Supplemental oxygen was provided by nasal cannula. After the surgical eye was prepped and draped, a lid speculum was placed. The surgical field was completely separated from the cannula using an adhesive surgical drape to avoid any additional oxygen exposure to the eye. Blood oxygen saturation (SaO2) was continuously monitored by pulse oximetry and maintained between 95% and 100%

Before the planned surgical procedure, a peripheral corneal paracentesis was performed with a 30-gauge needle near the limbus for entry into the AC. A flexible Oxylab pO2 optical oxygen sensor (Optode; Oxford Optronix, Oxford, UK) was introduced carefully into the AC without leakage of aqueous humor. Instrument calibration was checked against solutions equilibrated to 0% and 5% oxygen before and following each set of measurements. On the basis of previous studies,19-22 we set the temperature compensation to a constant 32°C to minimize any variation of the measurements.

The tip of the flexible fiberoptic probe was positioned in the AC for pO2 measurements in all patients by the surgeon: (1) in the anterior AC subendothelially at the central cornea and four cardinal locations in the midperiphery, (2) in the mid-AC, and (3) in the AC angle. In patients with pseudophakia and undergoing cataract extraction (control eyes or phakic FECD undergoing combined DSAEK and cataract surgery), 2 additional measurements were taken (4) at the anterior surface of the crystalline lens or intraocular lens and (5) in the posterior chamber just behind the iris. In patients with PBK undergoing DSAEK, pO2 was measured under the pseudophakic condition at the anterior surface of the IOL. Special care was taken to avoid damage to the lens in patients who were to remain phakic after the operative procedure. Patients were monitored postoperatively for any complications.

Statistical Analysis

Results are expressed as mean values ± SD. Statistical analyses were performed using SPSS software Version 17.0 (SPSS, Inc., Chicago, IL, USA). Student’s t-test or ANOVA with multiple comparisons was performed to compare each variable among the groups. Multivariate regression analyses were performed with adjustment for all potential confounding variables measured. Probability values less than 0.05 were considered statistically significant.

Results

We included 51 eyes (one eye each from 51 patients) in the study. Table 1 shows the demographic characteristics of three groups (normal control, FECD, and PBK). Patients with FECD were all Caucasians. Patients with PBK were statistically older than those with FECD. The reference control group comprises patients undergoing cataract surgery with normal CCT (mean 551 μm) and ECD (mean 2508 cells/mm²), whereas the patients with FECD or PBK undergoing DSAEK surgery or combined with cataract surgery had statistically thicker CCT (mean 705 and 764 μm for FECD and PBK, respectively) and ECD below 500 cells/mm² (Table 2).

The pO2 measurements at five different intraocular locations of each group are listed in Table 3. The mean intraocular pO2 levels in the normal controls (n = 24) were 20.9 mm Hg near the central cornea, 7.9 mm Hg in the mid-AC, 13.0 mm Hg in the AC angle, 2.4 mm Hg at the anterior surface of the crystalline lens, and 3.1 mm Hg in the PC. In patients with FECD (n = 11) and PBK (n = 16), there was a statistically significant increase of pO2 near the central cornea (42.7 and 13.0 mm Hg in the AC angle, 2.4 mm Hg at the anterior surface of the IOL, and in the PC (18.0, 11.8, and 6.9 mm Hg, respectively) in patients with PBK.

The respective intraocular oxygen gradients of these three groups in Table 3 are further illustrated in Figure 1. Consistent
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DISCUSSION

The findings in this study are consistent with our previous reports10–22 that the normal human eye has a steep oxygen gradient in the aqueous humor of the AC from the subendothelial regions to the anterior surface of the lens. We found that there is a significant increase of pO2 in the subendothelial AC of FECD and PBK without impacting the pO2 in the AC angle. These observations may provide further insights into the oxidative stress induced by endothelial dysfunctions and its implications on aqueous humor dynamics.

Progressive or accelerated loss of corneal endothelium can lead to corneal decompensation with resultant corneal edema and vision impairment.23,24 Various endothelial keratoplasties, including DSAEK, have been developed to manage corneal endothelial dysfunctions effectively as seen in patients with FECD or PBK. Consistent with the prevailing knowledge, we demonstrated in this study that there is a significant decrease of ECD with a related increase of CCT in patients with FECD or PBK undergoing DSAEK for visual rehabilitation. Our in vivo pO2 measurements confirmed the steep oxygen gradient in normal human anterior segment and further supported our previous conclusion that oxygen consumption by crystalline lens significantly reduces intraocular oxidative stress. Our data also demonstrated that there is a generalized increase of subendothelial pO2 and an even steeper intraocular oxygen gradient in eyes with corneal endothelial dysfunctions. Thus, our data suggested that reduced oxygen consumption due to corneal endothelial dysfunctions increases the possible source of reactive oxygen species (ROS), molecular oxygen, in the AC.

The implication of oxidative damage in corneal endothelial dysfunctions has not been thoroughly investigated to our knowledge; however, evidence indicates that multiple processes, such as oxidative stress, unfolded protein response, and apoptosis, have a role in accelerated corneal endothelial cell loss.25–29 The generalized increase of subendothelial pO2 observed in FECD and PBK in this study further substantiated the presence of potential sources of ROS in endothelial dysfunctions. As corneal endothelial pumps are the major site of oxygen consumption in the anterior segment of the human eye, endothelial loss will result in reduced oxygen consumption,23,24 various endothelial keratoplasties, including DSAEK, have been developed to manage corneal endothelial dysfunctions effectively as seen in patients with FECD or PBK. Consistent with the prevailing knowledge, we demonstrated in this study that there is a significant decrease of ECD with a related increase of CCT in patients with FECD or PBK undergoing DSAEK for visual rehabilitation. Our in vivo pO2 measurements confirmed the steep oxygen gradient in normal human anterior segment and further supported our previous conclusion that oxygen consumption by crystalline lens significantly reduces intraocular oxidative stress. Our data also demonstrated that there is a generalized increase of subendothelial pO2 and an even steeper intraocular oxygen gradient in eyes with corneal endothelial dysfunctions. Thus, our data suggested that reduced oxygen consumption due to corneal endothelial dysfunctions increases the possible source of reactive oxygen species (ROS), molecular oxygen, in the AC.

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<table>
<thead>
<tr>
<th>Group</th>
<th>Cornea</th>
<th>Mid-AC</th>
<th>Lens</th>
<th>Angle</th>
<th>PC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.9 ± 5.8†</td>
<td>7.9 ± 3.9†</td>
<td>2.4 ± 2.3†</td>
<td>13.0 ± 3.7</td>
<td>3.1 ± 2.6‡</td>
</tr>
<tr>
<td>FECD</td>
<td>42.7 ± 12.4‡</td>
<td>13.5 ± 3.3</td>
<td>6.6 ± 6.1</td>
<td>15.9 ± 4.3</td>
<td>5.5 ± 4.8</td>
</tr>
<tr>
<td>PBK</td>
<td>45.8 ± 6.5†</td>
<td>18.0 ± 9.1†</td>
<td>11.8 ± 7.3†</td>
<td>14.9 ± 5.6</td>
<td>6.9 ± 4.1‡</td>
</tr>
</tbody>
</table>

ANOVA with multiple comparisons is used for the data analysis. Mid-AC, midlocation of AC; Lens, cataract in control and FECD, IOL in PBK; PC, posterior chamber. *,† P < 0.001. ‡ P < 0.05.
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Multivariate regression analyses were performed to evaluate the correlations between the subendothelial pO2 and CCT or endothelial cell density. Each model is adjusted for age.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variable</th>
<th>β</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCT</td>
<td>ECD</td>
<td>−0.061</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cornea pO2</td>
<td>ECD</td>
<td>−0.013</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Cornea pO2</td>
<td>CCT</td>
<td>0.088</td>
<td>&lt;0.00001</td>
</tr>
</tbody>
</table>

Multivariate regression analyses were performed to evaluate the correlations between the subendothelial pO2 and CCT or endothelial cell density. Each model is adjusted for age.
Our data demonstrated that pO2 measurements in the AC angle remain relatively low in all three groups and are independent of oxygen levels in the AC. We propose the presence of an alternative flow pathway in the AC angle as illustrated in this diagram. The *sage arrow* indicates the classic pathway of aqueous humor flowing from the ciliary epithelium to the angle. The *yellow arrow* indicates the alternative pathway of plasma from the ciliary body vasculature. The *light yellow "fog"* around the trabecular meshwork indicates that plasma from the alternative pathway may have a critical role in maintaining a relatively stable oxygen level at the angle independent of the oxygen level in the aqueous humor.

stress in the TM. Even though we did not notice significant increase of pO2 in the AC angle in our patients with FECD and PBK, we speculated that extensive ocular surgery, such as penetrating keratoplasty or implantation of a keratoprosthesis, may affect the aqueous humor homeostasis at the AC angle and increase oxygen exposure to the TM, thereby increasing the risk of developing secondary glaucoma.

In summary, our current study revealed a close relationship between subendothelial intraocular oxygen tension and the health of the corneal endothelium. To the best of our knowledge, this is the first report demonstrating the differences in subendothelial oxygen tension in vivo between human eyes with normal corneas and corneas with severe endothelial dysfunctions. It suggested that the pO2 near the corneal inner surface is regulated by oxygen diffusion from the air across the cornea as well as by corneal oxygen consumption and not directly regulated by the circulation in aqueous humor. We further demonstrated that pO2 in the AC angle is unaffected by the significant increase of in the subendothelial AC and mid-AC in patients with corneal decompensation, such as FECD or PBK. With an increasingly oxidized environment in the AC with FECD or PBK, oxidative stress may result in potential extracellular matrix overproduction in the Descemet’s membrane of patients with FECD and reach a critical level of activating cellular apoptosis as reported previously by Junkunas et al. Further study exploring the role of oxidative stress in the TM induced by corneal endothelial dysfunctions in the development of open angle glaucoma is indicated.

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