Contact Lens-Induced Edema in Vitro

Ion Transport and Metabolic Considerations

Joseph W. Huff

The relationship of contact lens-induced edema to epithelial and endothelial function was determined in isolated superfused rabbit corneas. Placement of a polymethyl methacrylate (PMMA) contact lens on the cornea caused swelling rates of 15–28 μm/hr compared to 0–6 μm/hr in paired control corneas. The edema increased with temperature \((P < 0.01)\). PMMA-induced swelling was significant in: 1) bicarbonate-free Ringer’s solution; 2) chloride-free Ringer’s; 3) 0.3 mM furosemide-treated corneas; and 4) deepithelialized corneas. The swelling did not occur in corneas with silicone oil replacing the endothelium to block fluid uptake. The effluent aqueous bathing fluid from edematous corneas did not induce edema in normoxic corneas. These studies demonstrate that contact lens-induced edema depends on metabolism, involves a significant stromal contribution, and requires fluid absorption across the endothelial layer, but is not a direct result of epithelial and endothelial ion transport inhibition.


Attention to contact lens-induced edema has returned with the popularity of extended-wear contact lenses.1-2 The literature suggests that contact lens-induced edema results from corneal hypoxia, and the most accepted hypothesis to date has been refined for corneal hypoxia, by Klyce3: as a result of hypoxia, epithelial glycolysis is stimulated by way of the Pasteur effect, leading to stromal lactate accumulation. Stromal lactate accumulation osmotically increases stromal hydration.

Besides stromal edema, other effects of contact lens wear have been reported. Hamano et al4 demonstrated a decrease in the transepithelial potential difference in rabbits during lens wear, which suggests that the edema mechanism involves inhibition of epithelial ion transport. Epithelial ion transport inhibition may be expected, since contact lens-induced hypoxia depletes epithelial glycogen reserves.5-7 Lens wear also can decrease the stromal oxygen tension \((PO_2)\)8 and even can reduce the anterior chamber \(PO_2\)9,10 suggesting that endothelial transport inhibition can occur as well. Therefore, it is speculated that if the lens-induced hypoxia is serious enough, then epithelial or endothelial transport may be compromised, as occurs in other hypoxic or ischemic tissues.11-13 To determine more clearly the dependence of contact lens-induced edema on epithelial and endothelial function, this study used a system allowing physiologic and pharmacologic manipulation of the epithelial, stromal, and endothelial layers.14

In this study, contact lens-induced edema was examined using large-diameter polymethyl methacrylate (PMMA) lenses and a nonblinking eye. The study was designed to examine further the metabolic dependencies of the edema; to determine the dependency of the edema on epithelial, stromal, and endothelial functions; and to explore whether edematous corneas release an edema-producing autacoid.

Materials and Methods

Male and female New Zealand white rabbits (2–3 kg, Doe Valley Farms, Bentonville, AK) were sacrificed with a pentobarbital overdose via the marginal ear vein and were enucleated. This investigation adhered to the ARVO Resolution on the Use of Animals in Research. The corneas were mounted on Teflon® rings for specular microscopy as described by Dikstein and Maurice.15 On the aqueous side, they were maintained with a pressure of 20 cm H₂O and a flow rate of 0.5–1.0 ml/hr (posterior chamber volume = 0.25 ml). The tears-side bath (0.5 ml) was changed every 30 min. The bathing solutions (Table 1) were bubbled with 95% air and 5% CO₂ to maintain a pH of 7.3–7.4 on the aqueous side, and 7.3–7.8 on the tears side. The bathing solutions used (Table 1) were

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Table 1. Composition of bathing solutions*†

<table>
<thead>
<tr>
<th>Solute</th>
<th>Ringer</th>
<th>Cl-free ringer</th>
<th>HCO3-free ringer†</th>
<th>TC 199‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>118.1</td>
<td>—</td>
<td>143.1</td>
<td>124.4</td>
</tr>
<tr>
<td>Na gluconate</td>
<td>—</td>
<td>118.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>KCl</td>
<td>4.7</td>
<td>—</td>
<td>4.7</td>
<td>4.9</td>
</tr>
<tr>
<td>K gluconate</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CaCl2•2H2O</td>
<td>1.9</td>
<td>—</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Ca digluconate</td>
<td>—</td>
<td>1.9</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Na2HPO4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.2</td>
</tr>
<tr>
<td>KH2PO4</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.2</td>
</tr>
<tr>
<td>MgSO4•7H2O</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>—</td>
<td>1.2</td>
<td>1.2</td>
<td>0.8</td>
</tr>
<tr>
<td>NaHCO3</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>279 ± 5</td>
<td>285 ± 5</td>
<td>274 ± 4</td>
<td>286 ± 3</td>
</tr>
</tbody>
</table>

* All concentrations are millimolar.
† Solutions were bubbled with 95% air/5% CO2, except HCO3-free Ringer’s solution, which was bubbled with air.
‡ TC 199 contains supplementary nutrients, as described by its manufacturer (see Materials and Methods).

Krebs-bicarbonate Ringer’s solution for ion substitution experiments or TC 199 medium with 15 mM bicarbonate and phenol red (Medium 199, #M03935; Sigma, St. Louis, MO). All reagents except furos­semide and silicone oil were obtained from Fisher (St. Louis, MO). Furosemide was obtained from Sigma and 20-cs silicone oil was purchased from Dow Corning Service to Medical Research (Midland, MI). Osmometry was performed on each bathing solution with a Precision Systems Osmette A Osmometer (Sudbury, MA). All bathing solutions were made weekly, and all added drugs were prepared on the day of use.

After a 1.5-hr equilibration period, corneal thickness was monitored for a 3-hr period. At the beginning of the 3-hr period (zero time), paired corneas from the same animal typically differed in thickness by less than 10 μm. However, if this difference exceeded 30 μm, the pair was rejected based on previously described differences in initial corneal swelling pressure.16 At zero time, a PMMA contact lens was placed on the experimental cornea. Thicknesses of each cornea were measured at zero time and every 30 min in duplicate (a total of seven observations in duplicate for each cornea). The swelling rates for each group of five to eight corneas were determined by least squares linear regression analysis. In some experiments, corneal thickness was measured both immediately before and after lens placement, to examine whether an apparent thickness change resulted from the optics of the lens or of the tears between the lens and cornea. With the lens in place, the measured thicknesses typically differed by less than 8 μm (a marked apparent increase was associated with a steep lens, indicating the need for a larger base-curve radius). Corneal swelling rates for each group were compared by analysis of covariance17 at the P < 0.05 level of significance, as reported previously.18
PMMA contact lenses (7.0-, 7.2-, or 7.4-mm base curve, 11-mm peripheral curve, -2D power, 9–10-mm optic zone, and 10–11-mm diameter) were fit within 0.2 mm of the in vivo keratometry readings. The marked asphericity of the isolated corneas tied to the 12-mm Teflon® rings prevented a tight lens, allowing some fluid movement between the lens and epithelium. With the regular replacement of the tears-side bath, and with regular specular microscopy measurements, some lens movement occurred, suggesting at least a limited circulation of fluid between the lens and epithelium.

In experiments involving reduced temperature, the desired temperature was obtained in control and experimental corneas 35–45 min before zero time. Otherwise, corneas were maintained at 34°C throughout the equilibration and experimental periods. In experiments involving ion substitution, control and experimental corneas were preequilibrated for 1–1.5 hr with the appropriate bathing solution placed on both sides of the corneas. With furosemide, the agent was added to the aqueous-side bath of control and experimental corneas 1 hr before zero time. In experiments where the epithelium or endothelium were removed, silicone oil was placed on the stromal surface at the beginning of equilibration to prevent fluid imbibition.

**Results**

As demonstrated previously, control corneas bathed in Krebs-bicarbonate Ringer's typically swelled 0–5 μm/hr over the measurement period (Fig. 1). The epithelial thickness was unaffected by lens placement (Fig. 2). Because of the time requirements for accurate epithelial thickness measurements, these were not conducted throughout all experiments. PMMA lens placement caused statistically significant swelling of corneas in Ringer's (Fig. 1) and in TC 199 (Fig. 3). The reversibility of edema after 1.5 hr is demonstrated in the inset of Figure 1. Comparisons between figures is discouraged, particularly where ini-
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Fig. 8. Effects of lens placement on deepithelialized corneas bathed in TC 199 medium. Silicone oil covered the deepithelialized stroma to prevent fluid uptake (n = 6). **, P < 0.01.

At 34°C (Fig. 3) and 13°C (Fig. 4), corneas without lenses maintained a fairly constant thickness, and PMMA lens placement caused significant swelling. At 5°C the swelling rates of controls and PMMA-treated corneas were identical (Fig. 5), indicating a significant temperature dependency.

In the presence of 3 × 10⁻⁴ M furosemide (Fig. 6), or in the absence of chloride (Fig. 7; gluconate substituted Ringer’s), PMMA lens placement caused significant edema. PMMA lens placement also caused swelling in deepithelialized corneas (Fig. 8), clearly demonstrating that the edema can occur independently of the epithelium.

In bicarbonate-free Ringer’s solution (Fig. 9), corneas swelled as reported previously,¹⁹,²⁰ and PMMA-treated corneas swelled significantly more, demonstrating that contact lens-induced edema is additive with endothelial transport inhibition. Fig. 10 shows a negligible effect of PMMA lens placement in deendothelialized corneas. Because of the nonlinear slope of the PMMA-treated group (ie, due to heterogeneous variances of residuals), slope comparisons could not be made statistically, but at each time point during the 3-hr period, control and experimental thickness changes were never significantly different using a pooled t-test.

The possibility of autacoid release as an edema-causing factor also was considered (Fig. 11). Normal corneas perfused on the aqueous side with effluent aqueous bathing media from other controls or from PMMA-treated corneas did not develop edema. This suggests that the aqueous effluent contains no edema-causing substance (ie, that the edema probably is not transferred by means of autacoid release).

Discussion

Figures 1 and 3 illustrate, as described previously,¹⁴ that the edema occurs in either Ringer’s or TC 199
media. Like clinical edema, it is reversible soon after lens removal (Fig. 1, inset), and like its in vivo counterpart, a marked variability of edema reversal (deswelling) exists among corneas, reflected in the standard error for each point after lens removal.

Temperature Dependency

Although Klyce demonstrated the temperature dependency of hypoxic edema in vitro, no study has examined the temperature dependency of contact lens-induced edema. The edema due to lens placement was seen at 34 and 13°C, but not at 5°C, which is consistent with a hypoxic mechanism involving lactate production. Control corneas swelled at reduced temperatures as described by Bito et al and Hodson.

Epithelial Considerations

The furosemide-treated controls and the chloride-free controls of Figure 5 and 6 suggest a minimal contribution by active chloride transport to corneal hydration, as described by Riley and by Beekhuis and McCarey, and more importantly, demonstrate that lens-induced edema can occur whether chloride transport is compromised or not. What is more surprising is the finding that lens-induced edema occurred even when the epithelial layer was absent and replaced by silicone oil (Fig. 8). This indicates that lens-induced edema can occur regardless of either epithelial transport or epithelial metabolic state, and that the edema does not necessarily depend on epithelial trauma or hypoxia. However, these findings do not contradict the suggestion that lens placement can cause transport inhibition or epithelial trauma: Hamano et al demonstrated a decreased transepithelial potential difference due to lens wear, and Thoft and Friend demonstrated that acute mechanical trauma can deplete epithelial ATP and glycogen.

Endothelial Considerations

That lens-induced edema occurs in bicarbonate-free Ringer’s (Fig. 9) indicates that it involves a mechanism independent of transport inhibition. Since lens-induced edema is not significant in deendothelialized corneas (Fig. 10), it appears that its development requires fluid uptake across the endothelium. This contrasts slightly with Klyce’s findings during epithelial hypoxia: in Klyce’s study, isolated superfused corneas made hypoxic on their epithelial side swelled whether the endothelium was bathed in Ringer’s or blocked with silicone oil. Therefore, edema could occur via fluid uptake across the epithelial surface. In the current study, because of the presence of a static contact lens, fluid diffusion across the epithelium (and thus edema) would be expected to be reduced.

Figure 11 suggests the absence of a role for massive autacoid or toxin release, because effluent aqueous superfusates from lens-wearing corneas did not induce edema in normoxic corneas. This agrees with a clinical report that the prostaglandin synthesis inhibitor Naproxen does not ameliorate lens-induced edema in humans. There is overwhelming evidence, however, that lens placement can cause immediate endothelial (bleb) responses in humans and cats but not in rabbits (Holden, personal communication), probably as a result of corneal acidosis. Since bleb responses were not consistently noted in these experiments, it appears that bleb responses are not a requirement for edema. The importance of acidosis deserves further study, however, since buffering by the bathing media may obscure its role in the edematous mechanism. Alternatively, autacoids that either are rapidly metabolized or are nonpolar may be involved.

Probable Mechanism

The current study used an in vitro “worst case” model for better determining the mechanism of contact lens-induced edema, and may have a number of implications for edema due to daily and extended lens wear, as follows. 1) Lens-induced edema occurred in the absence of epithelial or endothelial anion transport, suggesting that the mechanism does not require corneal ion transport inhibition. 2) Edema occurred in deepithelialized corneas (with oil replacing the denuded surface), suggesting a significant role for the stroma in its origin. 3) The temperature dependency and the amelioration by lactate dehydrogenase inhibitors support the idea that the edema is a result of the osmotic load of stromal lactate accumulation, as suggested previously. 4) Edema was not significant when the endothelium was replaced with silicone oil, suggesting that fluid uptake across the endothelium is required for the development of edema during static lens wear.

As suggested by Klyce, stromal lactate accumulation may account for osmotic stromal swelling in response to contact lens wear. Our findings substantiate this, but the origin of the accumulated lactate deserves more study because relative epithelial and stromal lactate concentrations have not been measured during hypoxia. The current study suggests a significant role for the stroma in lens-induced edema. The hypoxic threshold for stimulation of stromal lactate production is not well-documented; however, it has been determined that the stromal cell volume is
roughly equal to epithelial cell volume\textsuperscript{30,32}, the epithelial lactate production (in normoxia) is equal to stromal production\textsuperscript{33}, and the stroma has a lower PO\textsubscript{2} than the epithelium during contact lens wear.\textsuperscript{8} Additionally, the isoenzyme profile of lactate dehydrogenase favors conversion of pyruvate to lactate more in the stroma than in the epithelium.\textsuperscript{34} Thus, lactate formation due to kerocyte or neuronal hypoxia may equal or even outweigh the epithelial contribution, and warrants further study.

**Key words:** contact lens, corneal edema, ion transport, metabolism, rabbit

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**References**