Corneal Acidosis During Contact Lens Wear: Effects of Hypoxia and CO₂

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The effects of tear-film hypoxia and contact lens wear on human in vivo stromal pH was tested using a non-invasive fluorometric technique. Hypoxia was produced by exposing the normal open eye to 100% nitrogen gas passed through tight-fitting goggles. Stromal pH dropped from $7.53 \pm 0.02$ to $7.34 \pm 0.03$ (n = 12, ±SD) within 90 min of nitrogen gas exposure, $t_{1/2} = 20$ min. After removing the goggles, stromal pH returned to baseline in 35 min, $t_{1/2} = 10$ min. Wearing a thick hydrogel contact lens which caused a tear $P_{O_2} \leq 2$ mm Hg with the eyes open, reduced stromal pH from $7.55 \pm 0.02$ to $7.15 \pm 0.04$ (n = 12, ±SD) in 80 min, $t_{1/2} = 9.5$ min. After removing the lens, baseline pH was reached in 40 min, $t_{1/2} = 4.5$ min. The stromal pH differences between hypoxia ($N_2$ only) and contact lens wear were not due to differences in tear temperature between the two procedures (contact lens wear $32 \pm 1.5^\circ C$, goggles $33 \pm 1.0^\circ C$). However exposing the eye to 95% nitrogen-5% carbon dioxide reduced stromal pH to $7.16 \pm 0.05$ (n = 7, ±SD) in 80 min, $t_{1/2} = 8$ min, which was similar to that produced during contact lens wear. These experiments show that contact lens wear causes corneal acidosis by: (1) the production of protons from hypoxic metabolism; and (2) the accumulation of carbon dioxide behind the lens due to low lens CO₂ transmissibility.

Clinicians have observed that contact lenses worn for extended wear may result in several corneal complications such as ulcerative keratitis, 1 neovascularization, 2 epithelial microcysts, 3, 4 and endothelial polymegathism. 3, 5 Other studies have shown that contact lens wear can slow epithelial mitotic activity 6 and reduce the rate of epithelial wound healing. 7 A likely contributing factor to this altered physiology is the corneal hypoxia that occurs during lens wear, 8 especially when the eyes are closed. 9 It is known that hypoxia increases epithelial lactate production, 10 which in turn causes stromal and epithelial edema. 10, 11 This hypoxia would also be expected to reduce intracellular and extracellular pH, 12 which may be a fundamental factor underlying the changes in cell morphology and alterations in corneal function accompanying extended wear.

This pH effect would not be unexpected since studies have demonstrated that intracellular pH (pHi) can control many cellular functions including glycolysis, 13 membrane ion secretion, 14 and mitotic activity. 15 An important factor directly affecting pHi is extracellular pH (pHo). 14 In the cornea, in vitro studies have shown that decreases in pHo can reduce endothelial pHi, 16 barrier function, 17 transendothelial potential, 18, 19 fluid transport, 18 and epithelial Cl⁻ secretion. 20–22 Recent human in vivo studies 23 have demonstrated that the formation of transient endothelial morphological changes, observed shortly after insertion of contact lenses, are most likely due to a reduction in corneal pH.

It therefore seems plausible that when a contact lens causes tear-film hypoxia, there will be a drop in corneal pH and that some physiological and morphological changes will occur. What has not been possible is to verify that in vivo corneal pH is altered during contact lens wear. Recently, we reported on the use of a non-invasive fluorometric technique for measurement of human in vivo stromal pH. 24 We determined the normal open eye stromal pH to be 7.54 which fell to 7.39 after lid closure as a result of a rise in corneal $P_{CO_2}$.

In the present paper, we report the effects of tear-film hypoxia and contact lens wear on stromal pH. The data indicate that contact lens wear can substantially reduce stromal pH by: (1) the effects of hypoxia; and (2) the accumulation of carbon dioxide when the lens CO₂ transmissibility is low. We also determined the stromal buffering capacity which provides an estimation of the acid load to the stroma during human in vivo tear-film hypoxia.

Materials and Methods

Measurement of Stromal pH

Fluorescein was used as a pH-sensitive stromal fluorescent probe. When the dye is excited at two wave-
lengths (490 and 450 nm) alternately in rapid succession, the ratio of fluorescence intensities is a function of pH, independent of dye concentration and path-length. Calibration procedures and fluorophotometer instrumentation have been previously described. Briefly, a slit-lamp fluorophotometer light source was modified to accommodate two interference filters (490 and 450 nm), which were alternately placed in the light path by a motorized shuttle every 0.6 seconds. To calibrate the fluorescence intensity ratio as a function of pH, rabbit, bovine and human stromas were incubated in buffered fluorescein solutions (pH 6.0-7.7). The fluorescence ratio of each stroma was then measured and a plot of ratio versus pH was constructed. The fluorescence ratio was found to be unaffected by: (1) whether bovine, rabbit, or human stroma was used; (2) raising the temperature of the stroma to 35°C; (3) dye concentration over a wide range; or (4) choice of buffer. However, the ratio was slightly affected by stromal hydration. In vitro sensitivity was ±0.02 pH units or better. To measure in vivo human stromal pH, fluorescein was loaded into the stroma by iontophoresis. Sensitivity in vivo was ±0.04 pH units between pH 7.4-7.6 and ±0.03 below pH 7.4.

Subjects

Twelve human subjects (seven males, five females, mean age 27, range 23-30), who were free of ocular disease and were not wearing contact lenses, participated in the study. Informed consent was given by each subject prior to undertaking the study.

Procedures

Background stromal fluorescence was measured in each eye with and without experimental goggles or contact lenses in place. Following these measurements, fluorescein was loaded into the stromas of each eye by iontophoresis. The eyes were rinsed with non-preserved sterile saline to remove any residual dye from the tears. Two hours elapsed before further measurements were made to allow adequate time for the dye to diffuse more uniformly across the stroma.

Following this 2 hr period, tear film hypoxia was produced by passing 100% nitrogen gas through one side of tight-fitting goggles while measuring stromal fluorescence through the goggles. Air was passed through the contralateral side, which served as a control. After 1 week, a thick (0.4 mm), 38% water content, plano hydrogel lens (Polymacon, Bausch and Lomb, Rochester, NY) was placed on one eye and stromal fluorescence was measured through the lens. The contact lens had an oxygen transmissibility of

\[2.4 \times 10^{-9} \text{ (cm/s)} \times (\text{ml O}_2/\text{ml} \times \text{mm Hg})\]

which leads to a tear film oxygen tension of ±2 mm Hg. Fluorescein was also measured in the contralateral non-lens-wearing eye. The stromal fluorescence ratio was measured until a new steady-state was reached. At that point the goggles or lens was removed and the fluorescence ratio was again measured until a steady-state was attained. For both procedures (N₂ and contact lens), the eyes remained open and subjects were instructed to blink normally.

The stromal fluorescence ratio can be affected by increases in stromal hydration. Therefore, corneal swelling was monitored by measuring central corneal thickness at the beginning and end of the hypoxic period. Thickness measurements were made directly through the goggles using an optical pachometer, which has been previously described. Percent swelling was calculated by dividing the change in corneal thickness by the starting thickness and multiplying by 100.

Statistics

The rate of pH change was expressed as the time for half of the total change to occur, t_{1/2}. All mean values reported are ±1 SD. Where indicated, student t-test was used to test differences in rate or final pH.

Results

When the eye is exposed to an anoxic environment, there is a drop in stromal pH. Figure 1 shows the pH changes that occurred in 12 subjects over a 90 min exposure to nitrogen gas. As nitrogen gas was applied to the cornea, the stromal pH slowly declined from pH 7.53 ± 0.02 to a new steady-state at 7.34 ± 0.03, t_{1/2} = 20 min. Corneal thickness increased 26 ± 4 µm or 5.2 ± 0.8%. When the goggles were removed, stromal pH returned to 7.53 in approximately 35 min, t_{1/2} = 10 min. Stromal pH in the eye exposed to air did not change.

Figure 2 shows the effect of contact lens wear on stromal pH. Following 80 min of lens wear the pH dropped from 7.55 ± 0.02 to a new steady-state at pH 7.15 ± 0.04 (n = 12), t_{1/2} = 9.5 min. This represents a two-fold greater reduction in stromal pH and two-fold faster change in pH compared to nitrogen gas. Return to baseline pH after removing the lens took approximately 40 min, t_{1/2} = 4.5 min. During the contact lens experiment corneal thickness was not measured, since this required removing the lens which would interfere with continual pH measurements. However, previous studies using this lens have shown that after 3 hr of open eye wear there is approximately 8% swelling (Bonanno and Polse, unpublished observations). Thus, in both the contact lens and nitrogen experiments, the corneal thickness did not increase above the 13% threshold at which a
Fig. 1. Effect of tear-film hypoxia on human in vivo stromal pH. Fluorescein was loaded into the corneal stromas of 12 subjects. Two hours later, nitrogen gas was passed over one eye and air over the contralateral eye through tight fitting goggles. The fluorescence intensity ratio from excitation at 490 and 450 nm was measured through the goggles. Arrows indicate gas on and gas off. Filled circles: nitrogen gas; open circles: control eye exposed to air. Error bars indicate standard deviation. Half-time of pH reduction was 20 min; half-time of pH recovery was 10 min.

small pH correction for increased stromal hydration would be needed.24

To explain the differences in pH and half-times between the lens wear and nitrogen gas experiments, we tested whether lenses could trap carbon dioxide or raise the tear-film temperature. Six of the 12 subjects were still available for these experiments. In addition, one new subject was recruited. To measure tear temperature, the cornea was anesthetized with one drop of proparacaine HCl and a small temperature probe (YSI #511, 0.5 mm diameter, Yellow Springs Instrument Co., Yellow Springs, OH) was placed in the tears on the central area of the cornea. With the goggles in place and gas flowing through them, the probe was brought between the skin and the goggles and placed on the central cornea of the open eye. With the contact lens on the eye the probe was "threaded" underneath the lens edge and placed on the central cornea. Temperature equilibrated within 5–10 seconds of placing the probe in the tears. Tear temperature in the open eye was 32 ± 0.5°C. Temperature between the contact lens and the cornea in the open eye was 32 ± 1.5°C, and wearing the goggles warmed the tears to 33 ± 1°C. The temperature difference between goggles and lens wear was not significant (2-tailed paired t-test, P > 0.05).

To test whether carbon dioxide contributes to the acidosis during lens wear, CO2 was mixed with nitrogen gas and passed through the goggles. The carbon dioxide transmissibility of the lens, approximately 4.9 × 10^-8 (cm/s) (ml O2/ml × mm Hg), is low enough that it will act essentially as an impermeable barrier to CO2 diffusion.29 Thus the [CO2] at the epithelial surface will equilibrate with that in the aqueous.29 Assuming an aqueous [CO2] of 5%, a mixture of 95% N2-5% CO2 was applied to one eye and air to the contralateral control eye. Figure 3 shows the mean pH change for seven subjects over a 90 min exposure to the 95% N2-5% CO2. The stromal pH dropped from 7.53 ± 0.02 to 7.16 ± 0.05, t1/2 = 8 min. Corneal thickness increased 30 ± 4 μm or 6 ± 0.8%. Recovery of pH after removing the goggles took 40 min, t1/2 = 6 min. Figure 4 shows the results of the gas mixture application and contact lens wear.
experiment for one representative subject. The extent of the pH decline due to lens wear and 95% N₂-5% CO₂ exposure was similar and followed similar time courses. For the six subjects who participated in both experiments (contact lens and N₂-CO₂ mixture), the final pH and half-time values were not significantly different (paired t-test, P < 0.05). Table 1 summarizes the results for the three experimental conditions.

Hypoxic Acid Load

The extent of the stromal pH change due to hypoxia (0.19 pH units for nitrogen gas) is determined by the amount of acid added to the stroma and the total buffering capacity, βₜ, of the stroma. βₜ in the open eye was estimated to be 56.4 mM/pH (see appendix). From the relation, Δ[acid] = (Δ pH) (βₜ),¹⁴ we estimate the acid load to be 10.7 mM. Since one proton is produced for every lactate,⁵,¹⁴ Δ[acid] and Δ[lactate] should be similar. Stromal [lactate] in the in vitro rabbit cornea¹⁰ and the in vivo guinea pig cornea³² have been shown to double following tear side hypoxia. In the in vitro rabbit cornea, 2 hr of CN⁻ treatment raised the stroma [lactate] from 4 to 9 mM.¹⁰ However, in the same study the normal in vivo rabbit stroma [lactate] was found to be 14 mM. Thus the change in the in vivo stroma [lactate] following tear-film hypoxia should be between 5 and 14 mM. Therefore, these estimates of hypoxic acid load and lactate accumulation are in reasonable agreement.

Discussion

This study demonstrates that corneal acidosis can result from tear-film hypoxia. Cellular hypoxia stimulates glycolysis and reduces the consumption of metabolically derived protons leading to greater lactate production and a reduction in pHᵢ, respectively.³¹ Analysis of the stoichiometry has shown that one net H⁺ is produced for every lactate.³¹ In the cornea, these ions leave the epithelial cells and diffuse to the stroma, where lactate accumulation leads to swelling¹⁰ and H⁺ cause the pH to drop. The extent of the stromal pH drop will be determined by the total stromal buffering capacity, which we have estimated to be 56.4 mM/pH in the open eye. From the relation βₜ = Δ[acid]/Δ pH¹⁴ we have calculated that the acid load to the stroma is 10.7 mM which is in close agreement with previous estimates of changes in [lactate] following tear-film hypoxia.¹⁰,³²

Contact lenses reduce stromal pH more quickly and to a greater extent than does simple hypoxia, although the steady-state times are similar. At least two mechanisms might explain this result: (1) temperature differences between the two procedures (higher temperature-greater metabolic rate); or (2) accumulation of carbon dioxide at the tear-lens interface due to low lens CO₂ transmissibility. We found no significant difference in tear temperature between the two procedures. This finding is in agree-
Contact lens wear can also affect epithelial and endothelial morphology. A recent report by Holden et al. suggests that a drop in corneal pH may be the cause of transient endothelial morphological changes that appear shortly after contact lens insertion. Furthermore, several studies have reported that extended wear contact lenses can cause endothelial polymegathism, the severity of which correlates with the length of contact lens wear and degree of long-term hypoxia. This is a clinical concern since increased polymegathism may indicate a reduction in endothelial function. Although the endothelial PO₂ is probably only slightly reduced during use of extended-wear contact lenses, there is most certainly some chronic acidosis. This pH shift may be a possible factor affecting endothelial morphology and function. Additional studies on the effects of long-term reductions in ambient pH on endothelial morphology and function are needed to determine the possible consequences of chronic corneal acidosis.

Key words: corneal acidosis, contact lenses, hypoxia, carbon dioxide, buffering capacity

Appendix

Stromal Buffering Capacity

Buffering power formulation and the method for its measurement was taken from Roos and Boron. A brief summary is included. Buffering power, β, (mM/pH) is defined as the amount of acid or base added to a sample divided by the change in pH caused by the acid or base (β = Δ[acid]/ΔpH). Total buffering is the sum of non-CO₂ buffering (intrinsic buffering) and CO₂ buffering, βT = β + βCO₂. Carbon dioxide application is a convenient and rapid method for measuring β directly. When using CO₂ one proton is released for every HCO₃⁻ released, so the acid load is equivalent to the change in [HCO₃⁻] and βT = ∆[HCO₃⁻]/ΔpH. The ∆[HCO₃⁻] is calculated from the initial and final values of pH and PCO₂ using the Henderson-Hasselbalch equation: [HCO₃⁻] = s(PCO₂)10(ΔpH/pK), where s is the CO₂ solubility in stroma.

The cornea was extracted, the epithelium and endothelium were lightly scraped off, and a 9 mm button was cut. A 500 µm trephine was used to obtain a stromal button. The button was then incubated in unbuffered fluorescein, 0.5 µM, 150 mM NaCl, pH 6.65–7.70, at 35°C for 2 hr. The button was then placed on a clear perforated polymethylmethacrylate disc which was mounted inside a lucite box.

Table 1. Summary of pH changes and half-times for N₂ gas exposure, contact lens wear, and N₂-CO₂ mixture

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH</th>
<th>ΔpH</th>
<th>Decline</th>
<th>Return</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% N₂</td>
<td>7.34 ± .03</td>
<td>0.19</td>
<td>20.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Contact lens</td>
<td>7.15 ± .04</td>
<td>0.40</td>
<td>9.5</td>
<td>4.5</td>
</tr>
<tr>
<td>95% N₂-5% CO₂</td>
<td>7.16 ± .05</td>
<td>0.37</td>
<td>8.0</td>
<td>6.0</td>
</tr>
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</table>

Values are means ± SD. Number of subjects is indicated in parentheses.
Appendix

Table 1. In vitro stromal buffering capacity

<table>
<thead>
<tr>
<th>% CO₂</th>
<th>Δ pH</th>
<th>Final pH</th>
<th>β₁ (mM)</th>
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<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.44</td>
<td>7.00</td>
<td>26.2</td>
</tr>
<tr>
<td>5</td>
<td>0.48</td>
<td>7.05</td>
<td>24.1</td>
</tr>
<tr>
<td>5</td>
<td>0.34</td>
<td>6.79</td>
<td>18.8</td>
</tr>
<tr>
<td>5</td>
<td>0.37</td>
<td>6.74</td>
<td>15.3</td>
</tr>
<tr>
<td>5</td>
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<td>6.57</td>
<td>14.6</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>6.40</td>
<td>10.9</td>
</tr>
<tr>
<td>5</td>
<td>0.33</td>
<td>6.37</td>
<td>7.5</td>
</tr>
<tr>
<td>10</td>
<td>0.68</td>
<td>6.88</td>
<td>22.9</td>
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<tr>
<td>10</td>
<td>0.62</td>
<td>6.67</td>
<td>15.1</td>
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<tr>
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<tr>
<td>10</td>
<td>0.73</td>
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<td>6.8</td>
</tr>
<tr>
<td>10</td>
<td>0.45</td>
<td>6.21</td>
<td>7.2</td>
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<tr>
<td>35°C</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>7.25</td>
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</tr>
<tr>
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<td>6.98</td>
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<td>6.68</td>
<td>14.4</td>
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Bovine stromas were incubated in unbuffered fluorescein, 0.5 μM, 150 mM NaCl, at 60 mm Hg swelling pressure. The stromal fluorescence ratio (Ex 490/450) was measured in air and then following exposure to 5 or 10% CO₂. See Appendix for further details. Intrinsic buffering capacity, βᵰ, was calculated from the equation: β (mM/pH) = ∆[HCO₃]/∆pH. ∆[HCO₃] was calculated from the Henderson-Hasselbalch equation at pH 7.54 with stromal Pco₂ = 19 mm Hg, the [HCO₃] is 15.5 mM. At pH 7.38 and stromal Pco₂ = 38 mm Hg, the [HCO₃] is 21.5 mM. Therefore, ∆[HCO₃] = 6 mM and βᵰ = (6/16) or 37.5 mM/pH, which agrees closely with bovine stromal β₁ at pH 7.38 (35 mM/pH).

Knowledge of βᵰ will allow an estimation of the acid load to the stroma following in vivo tear film hypoxia. Stromal pH dropped to 7.34 from 7.53 following 90 min of N₂ gas exposure; thus ΔpH is 0.19. Stromal βᵰ at 35°C, pH 7.34 is 33.9 mM/pH. However in the in vivo cornea, bicarbonate buffering is also available. Again assuming an average stromal Pco₂ of 19 mm Hg, at pH 7.34, the [HCO₃] is 9.8 mM. The buffering power of bicarbonate at constant Pco₂ is 2.3 times the bicarbonate concentration. Therefore, β[HCO₃] is 22.5 mM/pH and total buffering, β₇, is 56.4 mM/pH.

References


