Contact Lens-Induced Edema In Vitro

Pharmacology and Metabolic Considerations

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The effects of physiologic and pharmacologic manipulations on contact lens-induced edema were studied. In isolated superfused rabbit corneas bathed in Ringer's solution and covered with large-diameter polymethylmethacrylate (PMMA) lenses, corneal swelling rates of 17-26 μm/hr (versus -5-5 μm/hr in paired controls) were observed. Neither the calcium antagonist diltiazem (10⁻⁴ M), the glucocorticoid dexamethasone (10⁻⁷ M), the glucose substitute fructose (20 mM), nor 0.5 mM adenosine and 0.3 mM reduced glutathione mitigated the edema. Lens-induced edema was 25 μm/hr in corneas bathed at pH 8.2 and decreased to 9 μm/hr at pH 7.0. In corneas without lenses, however, decreasing the pH from 7.4-7.0 caused significant swelling (P < 0.05). The pyruvate dehydrogenase stimulant sodium dichloroacetate (3.2 mM) on the tears side ameliorated the edema, and its congener, 3.2 mM 2-chloropropionate, was less effective. These latter agents are known to relieve lactic acidosis systemically and had no significant effect on corneas without lenses. In tissues bathed with 20 mM lactate Ringer's, normal thickness was maintained in both control and PMMA-treated corneas throughout the 3-hr period. These findings suggest that the contact lens-induced edema does not involve the acute cytotoxic mechanisms seen in severe tissue ischemia or hypoxia. The edema appears to result in part from acidosis but mainly from stromal lactate accumulation. Invest Ophthalmol Vis Sci 32:346-353, 1991

Contact lens-induced edema is thought to result from corneal hypoxia. Klyce demonstrated that during hypoxia, increased stromal lactate accumulation may account osmotically for the stromal edema. The best current therapy for contact lens-induced edema is to increase the oxygen availability to the hypoxic cornea by either lens removal or changing the lens materials or design. However, some practitioners have ignored edema, and still others have advocated topical osmotic therapy as a partial remedy for the problem. Contact lens-induced edema has also been associated with decompensation of epithelial ion-transport processes, endothelial hypoxia, and morphologic changes, but dissociation of these events from edema has been difficult clinically. Because corneal edema is a significant problem, a better understanding of its mechanisms and response to pharmacologic and metabolic manipulation is desirable.

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This study further explored the mechanism(s) of contact lens-induced edema in vitro using eight approaches useful for treating or characterizing ischemia, hypoxia, and lactic acidosis in other tissues. We used static (nonmoving) polymethylmethacrylate (PMMA) lenses as a “worst case” model for the edema. The therapeutic approaches were: (1) a calcium blocker to alter the possible calcium-mediated consequences of hypoxia, (2) a glucocorticoid to suppress phospholipase A₂ and thereby inhibit autacoid production via the arachidonic acid cascade, (3) Ringer’s solution containing fructose (instead of glucose) as an alternative energy source, to prevent glucose-6-phosphate-induced inhibition of glycolysis, (4) adenosine/glutathione Ringer’s, which protects corneal and other tissues by several mechanisms, (5) alkaline (pH 8.2) media to prevent acidosis, (6) acidic (pH 7.0) media to inhibit lactate production and/or to promote acidosis, (7) the pyruvate dehydrogenase stimulants, dichloroacetate and 2-chloropropionate, to inhibit lactate formation, and (8) preequilibration and bathing in 20 mM lactate Ringer’s to minimize osmotic gradients of lactate between the stroma and the bathing media.

Materials and Methods

This investigation adhered to the ARVO Resolution on the Use of Animals in Research. We killed...
male and female New Zealand white rabbits (2–3 kg in weight; Doe Valley Farms, Bentonville, AK) with a pentobarbital overdose administered via the marginal ear vein and enucleated their eyes. The corneas were mounted on Teflon rings for specular microscopy as described by Dikstein et al. The aqueous side bath (chamber volume, 0.25 ml) was maintained with a pressure of 20 cm H₂O and a flow rate of 0.5–1.5 ml/hr, and the tears side bath (chamber volume, 0.5 ml) was changed every 30 min. The bathing solutions were bubbled with 95% air and 5% CO₂ and monitored continuously to maintain a pH of 7.3–7.4 on the aqueous side and 7.3–7.8 on the tears side (except where pH was intentionally adjusted to 7.0 or 8.2). For experiments at pH 7.0, the solutions were bubbled with 20% CO₂:20% O₂:60% N₂, and for experiments at pH 8.2, the solutions were equilibrated with air and further adjusted with 1 M NaOH. The bathing solutions used were Krebs-bicarbonate Ringer’s (118 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 25 mM NaHCO₃, and 5 mM glucose) and 20 mM lactate Ringer’s (identical in all respects except that 20 mM NaCl in the solution was substituted with 20 mM sodium lactate). Each solution had an osmolality of 279 ± 5 mOsm/kg. Ringer’s containing pH neutralized 3.2 mM dichloroacetate and 3.2 mM 2-chloropropionic acid had an osmolality which was increased by 7–9 mOsm/kg. We confirmed all osmolalities with a Precision Systems Osmette A Osmometer (Sudberry, MA). All bathing solutions were made weekly, and all added experimental agents were dissolved and added on the day of use. The reagents were obtained from Fisher (St. Louis, MO) except for adenosine and reduced glutathione (Sigma, St. Louis, MO) and diltiazem hydrochloride (Marion, Kansas City, MO).

After a 1.5-hr equilibration in Ringer’s, corneal thicknesses were measured for a 3-hr period with two consecutive measurements being made at 30-min intervals for each cornea. At the beginning of the 3-hr period, control and experimental corneas typically differed in thickness by less than 10 μm, and if the difference exceeded 30 μm, the pair was rejected based on apparent differences in initial corneal swelling pressure. The PMMA contact lenses (–2 D, 0.225-mm thickness, 7.2–7.5-mm base curve, 11-mm peripheral curve, 10-mm optic zone, and 11-mm diameter) were placed on experimental corneas, and the paired control corneas were bathed without lenses in the same Ringer’s solutions. Corneas were fit 0–0.2-mm flatter than the flat keratometry reading, because it was found that steep fits artificially increased the apparent thickness measured through the microscope. All bathing solutions were used throughout the equilibration and experimental periods, except in experiments involving diltiazem, dichloroacetate, and 2-chloropropionate where agents were added 3–5 min before lens placement. All solutions and agents were placed on the tears and aqueous sides except for dichloroacetate and 2-chloropropionate, which were placed on the tears side only. The thickness of each cornea was measured over a 3-hr period, and the swelling rate for each group of five to seven corneas was determined by least-squares linear-regression analysis as previously described. Corneal swelling rates (± standard deviation [SD]) for controls and experimental and controls were compared by analysis of covariance at the P < 0.05 level of significance, as described by Huff et al. Where inhomogeneous variances prevented comparisons of swelling rates by analysis of covariance, control and experimental thicknesses (± standard errors of the mean) were compared with a pooled t-test at the P < 0.05 level of significance for each time.

Results

The PMMA lenses typically caused 17–26 μm/hr swelling in corneas bathed in Ringer’s solution (Fig. 1) compared with paired controls (–5–5 μm/hr). Neither diltiazem (10⁻⁴ M; Fig. 2), dexamethasone (10⁻⁷ M; Fig. 3), fructose (20 mM; Fig. 4), nor 0.5 mM adenosine and 0.3 mM reduced glutathione (Fig. 5) ameliorated the edema. In each case the lens-induced swelling was statistically significant (P < 0.01), and none of the agents caused apparent swelling or deswelling in paired contralateral controls.

Adjusting the pH to 8.2 or 7.0 did not prevent edema development (Figs. 6, 7). However, it should be noted that pH 7.0 produced less lens-induced edema relative to pH 7.0 controls (Fig. 7), and inhomogeneity of thickness ± SD are given beside each curve.
Fig. 2. Diltiazem (0.1 mM) in the ambient bathing medium did not protect corneas from PMMA-induced edema (n = 6). **P < 0.01 vs. control swelling rate.

 homogeneous variances for the two regression lines prevented slope comparisons. At pH 7.0, corneal thickness was significantly greater (P < 0.05, pooled t-test) under PMMA lenses than in controls at each time between 1.5–3 hr. The inset of Figure 7 illustrates that in the absence of lenses, pH 7.0 Ringer’s caused slight but significant (P < 0.05) edema; corneas bathed at pH 7.0 swelled 4 ± 1 (mean ± SD) μm/hr compared with −1 ± 2 μm/hr in pH 7.4 controls.

The pyruvate dehydrogenase inhibitor dichloroacetate (3.2 mM; Fig. 8) prevented the edema, and its congener 2-chloropropionate (3.2 mM; Fig. 9) was only partially effective. Neither dichloroacetate nor 2-chloropropionate significantly affected corneal thickness in corneas without lenses (Figs. 8, 9, insets).

Conversely, increasing NaCl osmolality to a similar extent (9 mOsm/l) on the tears side significantly des-welled controls and decreased lens-induced swelling. Thus, the pyruvate dehydrogenase stimulants did not appear to act by producing a hypertonic tears bath. Figure 10 demonstrates that there was no lens-induced edema in corneas equilibrated and bathed on both sides with 20 mM lactate Ringer’s.

Discussion

As previously demonstrated with hypoxia or simulated hypoxia (cyanide), contact lens-induced edema is repeatable and easily measured in vitro. This model was previously used to demonstrate that contact lens-induced edema is stromal in origin and is additive with anion transport inhibitors. In the current study, we pharmacologically and metabolically considered the following as potential mechanisms of the edema: (1) accumulation of intracellular calcium, (2) release of autacoids derived from arachidonic acid, (3) accumulation of the phosphofructokinase inhibitor glucose-6-phosphate, (4) loss of glutathione redox capacity or depletion of adenosine, (5) corneal acidosis, (6) corneal lactic acidosis, and (7) corneal lactate elevation.

Diltiazem is often considered a calcium "slow" channel-blocking agent, but like other calcium antagonists, it also inhibits calcium efflux from cellular organelles and inhibits calmodulin and other calcium-binding proteins. Although calcium blockers have been traditionally used to protect hypoxic, ischemic, or reperfused excitable tissue from calcium-
mediated arrhythmias or contracture, these agents are also known to enhance the survival of ischemic kidney and liver by nonvascular mechanisms involving calcium antagonism. In contrast to diltiazem’s usefulness for treating distressed liver and kidney, there was no significant effect on contact lens-induced cornea edema. Higher concentrations of diltiazem were not used because nonspecific effects have been identified in these ranges, as previously described in the frog corneal epithelium.

Dexamethasone is efficacious as an inhibitor of phospholipase A2 and stabilizes cell membranes, thereby inhibiting arachidonic acid release. These effects result in a reduced arachidonic acid flux through the cyclooxygenase, lipoxygenase, and cytochrome P450 monoxygenase pathways. Although dexamethasone and other glucocorticoids in this concentration range protect the liver from ischemia and shock, this agent showed no effect on PMMA-induced corneal edema. A clinical study by Efron et al demonstrated that the nonsteroidal antiinflammatory agent, naproxen, also has no effect on contact lens-induced edema.

Replacement of glucose with fructose has been shown to protect the ischemic liver from cell membrane damage and adenosine triphosphate (ATP) depletion, probably due to its ability to substitute for glucose or glycogen as an energy source during accelerated anaerobic glycolysis, without the formation and accumulation of the glycolytic inhibitor, glucose-6-phosphate. In the presence of fructose, PMMA-induced edema was significant (Fig. 3) and slightly, but not significantly greater than in any other experiment. This dismisses glycolytic inhibition by glucose-6-phosphate accumulation as a metabolic mechanism and supports lactate accumulation as an osmotic mechanism.

Adenosine can be metabolized to release ribose (a substrate for the pentose shunt), and it stimulates corneal epithelial and endothelial ion transport. Reduced glutathione has also been a popular protector for the cornea and other tissues during either oxidative or hypoxic stress. These protective effects are due to its ability to scavenge free radicals and to stabilize membranes, ATPases, and glycolytic enzymes. The contact lens-induced edema was still significant with adenosine and glutathione Ringer’s; we therefore had no reason to examine each agent separately for their potential protective effects.

The intracellular pH of the corneal endothelium and other tissues is typically similar to the ambient extracellular pH within 0.3–0.5 units in CO2/bicarbonate buffer. This forms the basis for systemic bicarbonate therapy during systemic acidosis. Alkaline bathing media cannot prevent proton pro-
Fig. 7. Corneas with PMMA lenses swelled significantly in acidic media (pH 7.0 during both equilibration and experimental periods n = 8). *P < 0.05 vs. control thickness (pooled t test). The inset demonstrates that corneas without lenses bathed at pH 7.0 swelled significantly more than controls bathed at pH 7.4 (n = 6). **P < 0.05 vs. control swelling rate.

**Production** but would expectedly prevent acidosis. On the other hand, phosphofructokinase activity and, subsequently, lactate formation, is often favored in alkaline pH ranges (and is inhibited in acidic pH ranges),42,43 which is an argument against alkali therapy for lactic acidosis. Since equilibration with alkaline (Fig. 5) or acidic (Fig. 6) media did not stop the edema development, it appears that in this tissue, pH has limited control over contact lens-induced edema development between pH 7.0-8.2. Lower and higher pH values are known to cause edema and cytotoxicity,44,45 these were not examined. The inset in Figure 6 demonstrates that pH 7.0 is relatively nontoxic to the cornea over a 3-hr period, but a slight and significant swelling (4 ± 1 um/hr versus -1 ± 2 um/hr in controls; P < 0.05) occurs compared with pH 7.4 controls. A similar finding was apparent in the acidic controls in the body of Figure 6. It could be argued, however, that subacute or chronic acidosis deserves further study for its effect on edema development and other lens-induced symptoms, however.

A previous report10 demonstrated that two lactate

**Fig. 8. Dichloroacetate (3.2 mM) completely prevented PMMA-induced corneal edema. The inset illustrates that in corneas without lenses, dichloroacetate did not significantly deswell corneas over the 3 hr period (n = 6).**
Fig. 9. 2-Chloropropionate slightly decreased edema, but the swelling was still statistically significant. As in Figure 7, the inset shows that 2-chloropropionate did not significantly deswell corneas without contact lenses (n = 6). *P < 0.01.

dehydrogenase (LDH) inhibitors, sodium oxamate and oxalate, were capable of ameliorating contact lens-induced edema in vitro. This finding supported the hypothesis that contact lens-induced edema is largely a result of lactate accumulation. It was suggested that during LDH inhibition, pyruvate might accumulate sufficiently to be used by aerobic pathways (assuming that the Po2 during lens wear is not zero). The pyruvate dehydrogenase complex is the rate-limiting link between glycolysis and the Krebs cycle; phosphorylation of the dehydrogenase by its kinase inhibits its activity. Both dichloroacetate and 2-chloropropionate disinhibit pyruvate dehydrogenase by inhibiting its kinase, and these agents have been useful for treating lactic acidosis caused by experimental diabetes, ischemia, and hypoxia. Since both agents ameliorate contact lens-induced edema (dichloroacetate does so completely), it would appear that sufficient oxygen is available for aerobic metabolism and that these agents are capable of readjusting the Po2 "set point" for the Pasteur effect. Further examination of agents such as these appears warranted for dissociating edema from other effects of contact lens wear and perhaps in the study of other forms of edema where the suspected origin may be metabolic in nature.

Because 20 mM sodium lactate Ringer's prevents lens-induced edema (Fig. 10), lactate gradients between the stroma and the bathing media may largely account for contact lens-induced edema. This is not entirely conclusive, however, since in some tissues, excess lactate can inhibit anaerobic glycolysis and proton generation. It has been suggested that lactic acidosis in tissues may actually fill two protective roles during ischemia or hypoxia by inhibiting both lactate formation and proton generation. In the corneas preequilibrated and bathed with 20 mM lactate, the maintenance of control thickness and the lack of lens-induced edema suggests the tissue can maintain normal homeostasis for the 3-hr period observed in this study, suggesting that lactate is acutely nontoxic to the tissue.

Based on this study, the development of contact lens-induced edema appears to involve hypoxic lactate accumulation rather than cytotoxic mechanisms since it is not ameliorated by either diltiazem, dexamethasone, fructose, adenosine, reduced glutathione,
or alkalotic media. Lens-induced edema is apparently ameliorated in acidic media, but this appears due to the fact that, in corneas without lenses, acidic Ringer’s itself produces a slight but significant swelling. This suggests acute acidosis as a possible, albeit minor, edematous mechanism. There is good evidence that lens wear causes stromal acidosis in vivo and in vitro, whether this may have a chronic effect on endothelial and epithelial physiology deserves further study. The finding that edema can be ameliorated by pyruvate dehydrogenase stimulants is supportive of a hypoxic edema mechanism and suggests that lactate accumulation can be pharmacologically manipulated in the cornea from the tears side. Neither of these agents is suggested for chronic topical use, due to a number of serious systemic side effects. With further study, however, these and other agents may be used as probes to determine and/or dissociate the mechanisms behind other adverse effects on contact lenses and to elucidate the mechanisms of corneal edema due to corneal and systemic diseases.

**Key words:** contact lenses, corneal edema, pH, pyruvate dehydrogenase stimulants, rabbit

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**References**