The Bull’s Eye: Are We Off-Target for Corneal Endothelial Cell Physiology?

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As the primary refractive surface of the eye, clarity of the cornea is essential for optimal visual acuity. Many disease processes can irreversibly disrupt corneal clarity requiring corneal transplantation to restore visual function. The most common indication for corneal transplantation is opacification from corneal edema.

Corneal edema arises from dysregulation of fluid homeostasis in the cornea. The natural tendency of the cornea is to imbibe fluid from the anterior chamber to facilitate the delivery of nutrients to this avascular tissue, however continuous fluid influx would lead to a constant state of corneal edema. The corneal endothelium, a monolayer of cells on the posterior surface of the cornea, regulates corneal hydration by providing a “leaky barrier”. Incomplete tight junction bands allow fluid influx while gradients formed by ion channels and transporters drive fluid efflux.

Loss of corneal endothelial cells is the primary reason for corneal edema. Because of the nonproliferative nature of these cells, corneal transplantation with cadaveric donor tissue is currently the only means of restoring the endothelial monolayer of cells. However, recent research is pushing towards finding nonsurgical treatment options. Three alternatives are being studied. One focuses on enhancing the proliferative potential of corneal endothelial cells, and drug treatments are now emerging. Another approach targets modulation of the endothelial cell barrier function. The final method seeks to enhance fluid efflux from the stroma across the endothelium and is the focus of my studies.

Fluid efflux across the corneal endothelium is governed by ion movement. For several decades, investigators have questioned the roles of various ions, channels and transporters in the endothelium. In 1965, Brown and Hedbys demonstrated the importance of the Na+/K+ ATPase in supporting corneal deturgescence in rabbit eyes. Further studies on rabbit corneal endothelium confirmed the need for ATP as well as Na+ and HCO3−. Initial investigations in the 1970’s on transport in human cornea demonstrated that human (and monkey) as compared to rabbit corneas had different polarities and responses to changes in extracellular pH. In 1981, Wingham and Hodson showed similar responses in human and bovine corneal endothelial short-circuit current response (a measure of the rate of active ion transport) to extracellular HCO3− concentration and suggested that human, bovine and rabbit corneas had similar endothelial transport mechanisms. They published one final manuscript on human corneal endothelial physiology in 1987 and discussed that they could no longer perform human corneal experiments because of the expense of maintaining a protocol that could be used only when donor eyes became available. Such experiments on native human corneal endothelium have not been published since then, despite the fact that data from the 1970’s demonstrated differences among species.

Four factors should prompt us to revisit this situation. First, there is an obvious need for an in vitro human model system to test any future advances in targeting ion transport as a means for treating corneal edema. Second, human corneal tissue not suitable for transplantation...
is more readily available than several decades ago due to improved corneal storage media and eye banking operations. Third, advances in physiologic instrumentation have streamlined the experimental process. And finally, clinical observations tell us that the current model of fluid transport as developed from animal studies does not directly model human corneal endothelial behavior.

The primary models of fluid transport across the corneal endothelium in animal models incorporate carbonic anhydrase as a key enzyme in cellular buffering of $H^+$ and $\text{HCO}_3^-$. In agreement with this model, carbonic anhydrase inhibitor application to rabbit corneas results in corneal swelling. However, carbonic anhydrase inhibitors used commonly for the treatment of glaucoma rarely cause corneal swelling in humans, implicating a different mechanism for corneal endothelial fluid transport than that suggested from animal models.

We are addressing this concern in my lab utilizing bovine and human corneas. Our human corneas are tissues not suitable for transplantation that have been maintained in standard eye banking storage solutions (Optisol GS, Bausch and Lomb, Rochester, NY, USA; Eusol-C, Alchimia, Padova, Italy). With minor modifications to commercially available instrumentation (Physiologic Instruments, San Diego, CA, USA), we can successfully record the short-circuit current as a measure of transepithelial ion transport activities. The beauty of this system lies in the ability to utilize native corneal endothelial tissue that has not been subject to cell dissociation and culture which are known to alter ion channel expression. We anticipate this avenue of research will provide valuable insight into human corneal endothelial physiology and future treatments for corneal edema.

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Conflicts of Interest

None.

REFERENCES

1. Mishima S, Hedbys BO. Physiology of the cornea. *Int Ophthalmol Clin* 1968;8:527-560.
2. Waring GO 3rd, Bourne WM, Edelhauser HF, Kenyon KR. The corneal endothelium. Normal and pathologic structure and function. *Ophthalmology* 1982;89:531-590.
3. Okumura N, Koizumi N, Ueno M, Sakamoto Y, Takahashi H, Hamuro J, et al. The new therapeutic concept of using a rho kinase inhibitor for the treatment of corneal endothelial dysfunction. *Cornea* 2011;30 Suppl 1:S54-59.
4. Srinivas SP. Cell signaling in regulation of the barrier integrity of the corneal endothelium. *Exp Eye Res* 2012;95:8-15.
5. Brown SI, Hedbys BO. The effect of ouabain on the hydration of the cornea. *Invest Ophthalmol* 1965;4:216-221.
6. Dikstein S, Maurice DM. The metabolic basis to the fluid pump in the cornea. *J Physiol* 1972;221:29-41.
7. Hodson S. Evidence for a bicarbonate-dependent sodium pump in corneal endothelium. *Exp Eye Res* 1971;11:20-29.
8. Fischer F, Voigt G, Liegl O, Wiederholt M. Potential difference and short-circuit current in isolated human cornea. *Pflugers Arch* 1972;332:Suppl 332:R91.
9. Fischer F, Voigt G, Liegl O, Wiederholt M. Effect of pH on potential difference and short circuit current in the isolated human cornea. *Pflugers Arch* 1974;349:119-131.
10. Wigham C, Hodson S. Bicarbonate and the trans-
endothelial short circuit current of human cornea. 
*Curr Eye Res* 1981;1:285-290.

11. Wigham C, Hodson S. The effect of bicarbonate ion concentration on trans-endothelial short circuit current in ox corneas. *Curr Eye Res* 1981;1:37-41.

12. Wigham CG, Hodson SA. Physiological changes in the cornea of the ageing eye. *Eye (Lond)* 1987;1:190-196.

13. Bonanno JA. Molecular mechanisms underlying the corneal endothelial pump. *Exp Eye Res* 2012;95:2-7.

14. Fischbarg J, Lim JJ. Role of cations, anions and carbonic anhydrase in fluid transport across rabbit corneal endothelium. *J Physiol* 1974;241:647-675.

15. Liu C, Cheng Q, Nguyen T, Bonanno JA. Knockdown of NBCe1 in vivo compromises the corneal endothelial pump. *Invest Ophthalmol Vis Sci* 2010;51:5190-5197.

16. Egan CA, Hodge DO, McLaren JW, Bourne WM. Effect of dorzolamide on corneal endothelial function in normal human eyes. *Invest Ophthalmol Vis Sci* 1998;39:23-29.

17. Bildin VN, Iserovich P, Fischbarg J, Reinach PS. Differential expression of Na:K:2Cl cotransporter, glucose transporter 1, and aquaporin 1 in freshly isolated and cultured bovine corneal tissues. *Exp Biol Med (Maywood)* 2001;226:919-926.