Moderate Fuchs' Endothelial Dystrophy
ATPase Pump Site Density

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The Na⁺, K⁺-ATPase pump site density on corneal endothelial cells from Fuchs' endothelial dystrophy corneas has been shown to be drastically decreased in end-stage disease (McCartney et al, Invest Ophthalmol Vis Sci 28:1955, 1987) and significantly increased in the early stages (Geroski et al, Ophthalmology 92:759, 1985) as compared to normal endothelium. In order to provide values for corneas between these two extremes, eye bank corneas from donors with no evidence of corneal edema but with guttata across the extent of the cornea were processed for autoradiography as well as immunohistochemistry. Pump site density was increased compared to end-stage disease but was less than values reported for either functional tissue or early stage disease. Similarly, immunohistochemistry results showed the amount of Na⁺, K⁺-ATPase antibody localization to be increased in respect to end-stage disease, but reduced as compared to functional tissue. These results suggest that pump site density on endothelial cells affected with Fuchs' endothelial dystrophy follows a gradual decline towards end-stage disease values as opposed to a sudden sharp deterioration after an initial increase. Invest Ophthalmol Vis Sci 30:1560-1564, 1989

Fuchs' endothelial dystrophy is a primary disorder of the corneal endothelium that accounts for approximately 10% of the penetrating keratoplasty performed annually.¹ Fuchs' is a progressive disease that is inherited as an autosomal dominant trait and generally affects women more severely.² Characteristic guttata form posterior to Descemet's membrane and the endothelial cells get progressively thinner with irregular borders as the disease advances. Concomitant with these morphological changes corneal edema and thickness increase.

Previous studies on early Fuchs' endothelial dystrophy have reported increased permeability³-⁵ and increased Na⁺, K⁺-ATPase pump site density as compared to normal.⁶ In end-stage disease, where there is edema from central to peripheral regions of the cornea, the pump site density is significantly reduced in comparison to functional corneal endothelium.⁷ In order to obtain values between these two extremes, the current study has used autoradiography and immunohistochemistry to examine eye bank corneas with no history of edema, but with guttata across the width of the cornea.

Materials and Methods

Two eye bank corneas (69-year-old female and 79-year-old female), obtained within 1 hr postmortem, were judged to have "moderate" Fuchs' endothelial dystrophy. Corneal guttata were present across the width of the cornea, but no case history of corneal edema was noted. The corneas were excised from the globes and the cornea placed in McKarey-Kaufman media.⁸ The corneas were then divided in half with one-half being processed for autoradiography and the other for immunohistochemistry (see below).

Two keratoconus corneas were obtained at the time of transplant surgery from keratoconus eyes that were examined preoperatively to ensure that there were no endothelial abnormalities or previous history of hydrops. Corneal buttons were placed in McKarey–Kaufman media⁸ immediately after surgical dissection and transported to the laboratory where processing for immunohistochemical localization of Na⁺, K⁺-ATPase began within the same time period as for the Fuchs' corneas.
Autoradiography

The half of the cornea designated for autoradiography was processed according to the methodology previously described. Briefly, the cornea was cut in half with the half serving as a control being preincubated in 10^{-6} M nonradioactive (cold) ouabain (ICN Pharmaceutical, Plainview, NY). The preincubation of the tissue with nonradioactive ouabain provided competitive inhibition for the noncovalent binding steroid nucleus of the radioactive ouabain with the receptor. Both halves were then incubated in 7 × 10^{-7} M ^3H-ouabain (New England Nuclear, Boston, MA: specific activity 20 Ci/mMol) for 1 hr. Following extensive rinsing in 0.1 M sodium cacodylate buffer (Polysciences, Warrington, PA), the halves were frozen in liquid nitrogen, freeze-dried, osmicated under vacuum and embedded in Spurr resin. Semithin sections (0.5 μm) were placed on glass slides, covered with Kodak NTB-2 emulsion (Eastman Kodak, Rochester, NY), exposed and photographically developed.

In order to calculate the number of silver grains per 100 μm^2 of cell, bright and darkfield light micrographs were taken of the same section. Cell area measurements were calculated from morphometric analysis of the brightfield photographs which corresponded to those areas photographed in darkfield for silver grain counts. Silver grains were counted only over those areas shown to contain endothelial cells in brightfield micrographs. Established formulas were used to determine which silver grains should be considered arising from radiation bound to the tissue. Background silver grain density was determined using the alternating grid square method over areas containing no tissue. Comparisons were subsequently made between the experimental and control tissue for each cornea. Silver grain development in the basa epithelium served to confirm the viability of the technique for each cornea.

Immunohistochemistry

The half of the Fuchs' corneas designated for immunohistochemistry as well as the keratoconus surgical buttons removed at the time of transplant surgery were processed according to the methodology previously described. Briefly, the tissue was fixed for 1 hr in an aldehyde fixative (3% paraformaldehyde, 0.1% glutaraldehyde), rinsed and cryo-sectioned. Incubation of the tissue in the primary antisera obtained from whole serum goat anti-Na^+, K^+-ATPase (Accurate Chemical and Scientific Corporation, Westburg, NY) using a modification of the (NH₄)₂SO₄ precipitation technique of Jonak and secondary antisera, swine anti-goat horseradish peroxidase (HRP) conjugate (Boehringer Mannheim Biochemicals, Indianapolis, IN), was for 12 hr each at 4°C with three washes with 0.05 M tris buffered saline (TBS) of 20 min each before and between antisera. Following a final 20 min wash with TBS, the location of the HRP-antibody complex was visualized using the chromagen 3,3'-diaminobenzidine tetrahydrochloride (DAB) (Sigma, St. Louis, MO).

Control tissue was incubated in either: (1) nonimmune serum followed by secondary antibody; (2) primary antibody that had been preabsorbed with excess antigen (rabbit kidney Na^+, K^+-ATPase) followed by secondary antibody; or (3) secondary antibody alone. The tissue was then processed in the identical manner to experimental tissue.

Results

Autoradiography

The endothelium (Fig. 1) and the basa epithelium of the moderate Fuchs' corneas showed silver grain development that was different from the values calculated for control tissue (Experimental: 41.35; Control: 5.11 per 100 μm^2). Qualitatively, this silver grain development appeared to vary from one region to another in these corneas (Figs. 1, 2). Silver grain density in the control endothelium was greatly reduced (Fig. 3). When the silver grain density over the endothelial cells was quantified on the moderate Fuchs' corneas, there was an average of 41.35 silver grains per 100 μm^2.
Immunohistochemistry

En face sections of the endothelium from the keratoconus buttons showed an even distribution of antibody labeling along the hexagonally arranged lateral membranes of these functional cells (Fig. 4). Sections of the moderate Fuchs' endothelial dystrophy corneas showed a patchy antibody labeling (Fig. 5) along the lateral borders of these pleomorphic cells. No substantial differences could be detected in the amount of antibody labeling between central and peripheral endothelial cells. Control tissue incubated with non-immune serum or preabsorbed primary antibody showed little or no antibody labeling.

Discussion

Previous studies on Na⁺, K⁺-ATPase pump site density in end-stage Fuchs' endothelial dystrophy, a stage characterized by pan-corneal edema, have indicated that the pump site density is significantly reduced in comparison to functional tissue. In contrast, the pump site density on early Fuchs' dystrophy is significantly increased as compared to normal corneal endothelium. It appears that as the disease progresses there is a transition from increased pump density to decreased pump site density during end-stage disease. It is not known as yet when the transition from increased to decreased pump site density occurs and whether this transition is abrupt or gradual. The current study has examined corneas with "moderate" Fuchs' endothelial dystrophy using autoradiography and immunohistochemistry in an effort to provide data for a disease stage between these two extremes.

Corneas used in this study had guttata across the width of the cornea but no reported history of corneal edema. Accordingly, these corneas were judged to be at a stage of disease between early and end-stage. Quantitative assessment of the endothelial pump site density on these moderate Fuchs' corneas showed that pump site density appeared to be increased in comparison to end-stage disease but less than values reported for functional corneal endothelium and early Fuchs' dystrophy. These data suggest that the transition from increased levels in early Fuchs' to the low levels in end-stage disease is a gradual rather than an abrupt decline. The starting point of this decline or what the triggering mechanism is remains to be elucidated by obtaining even earlier stages than were available in the current study.

The Na⁺, K⁺-ATPase antibody aliquots used in this study had previously been used to label functional and dysfunctional human corneal endothelium as well as the positive control tissue, rabbit kidney. Antibody labeling on the Fuchs' corneas in this study appeared to be increased from the amount of labeling previously shown in end-stage disease, but still was qualitatively less than functional tissue. Na⁺, K⁺-ATPase has previously been shown to be localized to lateral membranes of corneal endothelial cells. The antibody labeling of the lateral mem-

Fig. 2. Darkfield light micrograph showing silver grain deposition over the endothelium from the central region of a "moderate" Fuchs' endothelial dystrophy cornea. The silver grain (arrows) densities varied between central and peripheral (Fig. 1) regions. S = stroma; bar = 20 μm.

Fig. 3. Darkfield light micrograph showing silver grain development in the control half of the cornea illustrated in Figure 1. Silver grain (arrows) density in the endothelium of the control tissue was drastically reduced in comparison to experimental tissue. A fold in the tissue section (open arrows) appears as a white line in this darkfield micrograph. S = stroma; bar = 20 μm.

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Fig. 4. Light micrograph of cryosectioned functional corneal endothelium (keratoconus) incubated in the primary antibody (goat anti-Na\textsuperscript{+}, K\textsuperscript{+}-ATPase) followed by incubation in the secondary antibody (swine anti-goat HRP). This en face section shows dense antibody labeling (arrows) along the lateral borders of the endothelial cells. Bar = 20 \textmu m.

Fig. 5. Light micrograph of a cryosectioned "moderate" Fuchs' endothelial dystrophy cornea incubated in goat anti-Na\textsuperscript{+}, K\textsuperscript{+}-ATPase antibody followed by swine anti-goat HRP. The antibody labeling (arrows) appeared patchy along the lateral borders of these pleomorphic cells. Bar = 20 \textmu m.

progression has been visualized using a cytochemical stain, cytochrome oxidase,\textsuperscript{19} which is an indicator of the functional activity of the cells.\textsuperscript{26} It appears that there is a spectrum of disease severity contained within one cornea and that this spectrum may not be accounted for in any of the disease staging paradigms. Krachmer et al\textsuperscript{27} and Wilson and Bourne\textsuperscript{28} have suggested stages that are a reflection of the severity of guttata present during slit-lamp examination. Although these stages can be used for relative divisions of the overall severity of the disease, the inherent problem of variation amongst the cells, which has been noted using invasive techniques,\textsuperscript{17,19} remains. These difficulties were evident in the current study where the overall stage of the disease was classified as "moderate" but silver grain densities were increased in peripheral cells compared to central cells. In studies on Fuchs' endothelial dystrophy the overall disease severity may be classified in broad terms, but a potential problem exists whenever cellular parame-
ters from various studies are compared. Future studies may require comparisons based on the overall severity of the disease as well as the measurement of individual parameters in discrete areas of the cornea.

In conclusion, this study has provided data suggesting that the endothelial cell pump site density on "moderate" Fuchs' endothelial dystrophy corneas is midway between functional and end-stage disease values. We suggest that there is a gradual transition from the increase Na⁺, K⁺-ATPase pump site values in early Fuchs'6 to the decreased end-stage values shown for these cells,7 rather than an abrupt deterioration after an initial increase.

Key words: ATPase, autoradiography, immunohistochemistry, moderate Fuchs' endothelial dystrophy, human

References