endothelial disorders. Indeed, steroid administration seemed to increase endothelial pump function and ameliorate stromal edema in a patient with bullous keratopathy secondary to Sato refractive surgery.[12] In contrast, clinical observations of a higher incidence of persistent corneal edema after vitrectomy and other surgical procedures for patients with diabetes mellitus have suggested that abnormal corneal endothelial function is associated with diabetes mellitus.[13–18] Specular microscopic studies have shown morphological abnormalities, such as lower endothelial cell density and increased endothelial pleomorphism, in patients with types 1 and 2 diabetes mellitus.[18–27] Some clinical studies have shown that diabetic patients tend to have slightly thicker corneas and a reduced recovery rate from hypoxia-induced corneal edema.[28–31] These observations led us to the idea that Na,K-ATPase activity and pump function of the corneal endothelium may be affected by several hormones, such as glucocorticoids or insulin.

In this review, we outline methods for measuring the enzymatic activity and pump function of Na,K-ATPase in cultured mouse corneal endothelial cells. This is followed by a review of the roles of dexamethasone and insulin in controlling the enzymatic activity and pump function of Na,K-ATPase in corneal endothelial cells. Additionally, we present the mechanisms by which dexamethasone or insulin might affect Na,K-ATPase activity.

MEASUREMENT OF Na,K-ATPase ENZYME ACTIVITY

Corneal endothelial cells were cultured with or without dexamethasone, insulin, staurosporine, GF109203X, or okadaic acid. To measure Na,K-ATPase enzyme activity, ouabain (final concentration, 1 mM) or vehicle was added to cell cultures and incubated for 30 minutes at 37°C. After further addition of adenosine triphosphate (final concentration, 10 mM), the adenosine triphosphate hydrolysis reaction catalyzed by Na,K-ATPase released phosphate. The concentration of phosphate was determined by spectrophotometric measurement with ammonium molybdate. The Na,K-ATPase activity was calculated as the difference in phosphate concentration between cells with and without ouabain.

MEASUREMENT OF ELECTRICAL Na,K-ATPase PUMP FUNCTION

To measure pump function, cells cultured on Snapwell inserts were placed in an Ussing chamber. The endothelial cell surface side was in contact with 1 chamber and the Snapwell membrane side was in contact with another chamber. If Na,K-ATPase was active, then a short-circuit current would be evoked by active sodium and potassium flux. After the short-circuit current had reached a steady state, ouabain was added. The pump function attributable to Na,K-ATPase activity was calculated as the difference in short-circuit current measured before and after the addition of ouabain (Fig. 1).

DEXAMETHASONE STIMULATES Na,K-ATPase ACTIVITY

Mouse corneal endothelial cells were exposed to 10 μM dexamethasone for various periods and Na,K-ATPase activity was then measured. Dexamethasone increased Na,K-ATPase activity in a time-dependent manner, with this effect being significant ($P < 0.05$; Student t test) at 6 hours and maximal at 48 hours (Fig. 2). The stimulatory effect of dexamethasone on Na,K-ATPase activity was also concentration dependent (Fig. 2).[32] The stimulatory effect of dexamethasone on pump function was time dependent, being significant at 24 hours and maximal at 48 hours (Fig. 3A),[32] and concentration dependent, which was apparent at 1 or 10 μM (Fig. 3B).[32] These results were similar to those obtained for Na,K-ATPase activity.

To determine whether dexamethasone affects Na,K-ATPase expression in corneal endothelial cells, we exposed the cells to dexamethasone for 48 hours and measured the expression of total Na,K-ATPase α_1-subunit and phospho-Na,K-ATPase α_1-subunit by Western blot analysis. Phospho-Na,K-ATPase α_1-subunit is considered to be the inactive state of the Na,K-ATPase α_1-subunit. Levels of the Na,K-ATPase α_1-subunit and phospho-Na,K-ATPase α_1-subunit were measured and are presented as relative amounts compared with the signal intensities for β-actin (Fig. 4A).[32] Dexamethasone increased the expression of total Na,K-ATPase α_1-subunit in a concentration-dependent manner, whereas it did not alter the expression of the phospho-Na,K-ATPase α_1-subunit (Figs. 4B, C)[32]; therefore, the ratio of phospho-Na,K-ATPase α_1-subunit expression to total Na,K-ATPase α_1-subunit expression was decreased in a concentration-dependent manner (Fig. 4D).[32]

INSULIN TRANSIENTLY ACTIVATES Na,K-ATPase

To determine whether insulin affects Na,K-ATPase activity in corneal endothelial cells, we exposed the cells to 0.1 μM insulin for various periods and measured the

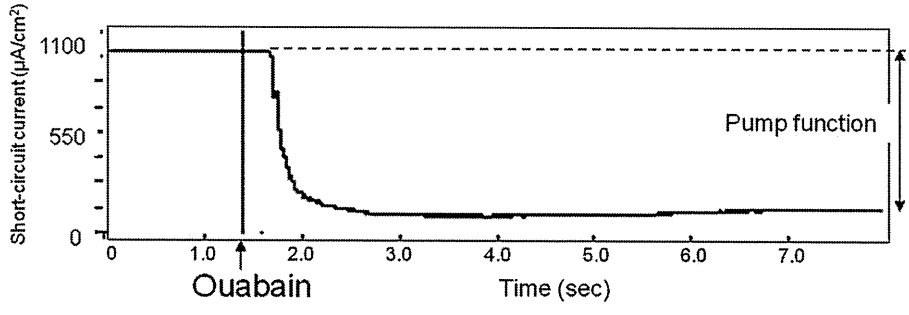

FIGURE 1. Representative trace of short-circuit current (microamperes per well) obtained with cell monolayers in an Ussing chamber. The insert well membrane growth area was 4.67 cm^2. Pump function attributable to Na,K-ATPase activity was calculated as the difference in short-circuit currents obtained before and after the addition of ouabain.

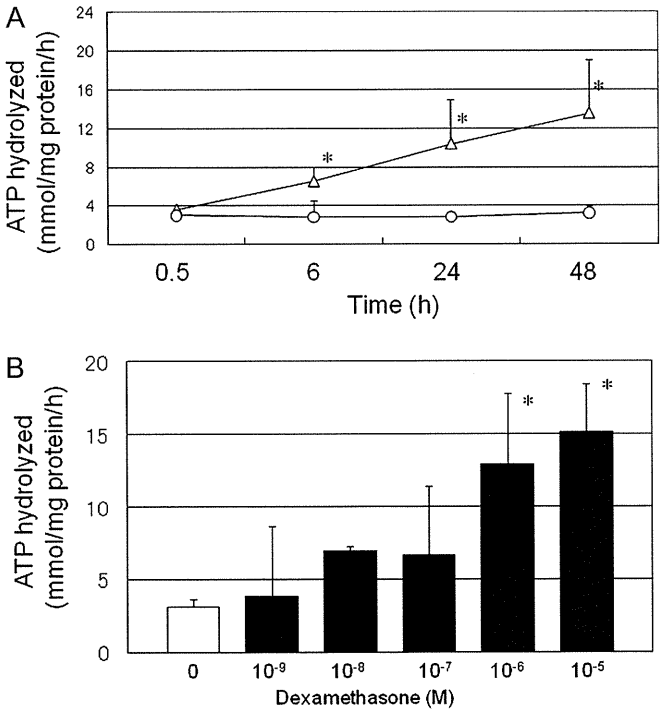

FIGURE 2. Effect of dexamethasone on Na,K-ATPase activity in cultured mouse corneal endothelial cells. A, Cells were incubated in the absence (circles) or presence (triangles) of 10 μM dexamethasone for the indicated times and then assayed for Na,K-ATPase activity. $*P < 0.05$ versus the corresponding value for cells incubated without dexamethasone (Student *t* test). B, Cells were incubated with the indicated concentrations of dexamethasone for 48 hours and then assayed for Na,K-ATPase activity. $*P < 0.01$ for the indicated comparisons (Student *t* test). Modified with permission from Hatou et al.[32]

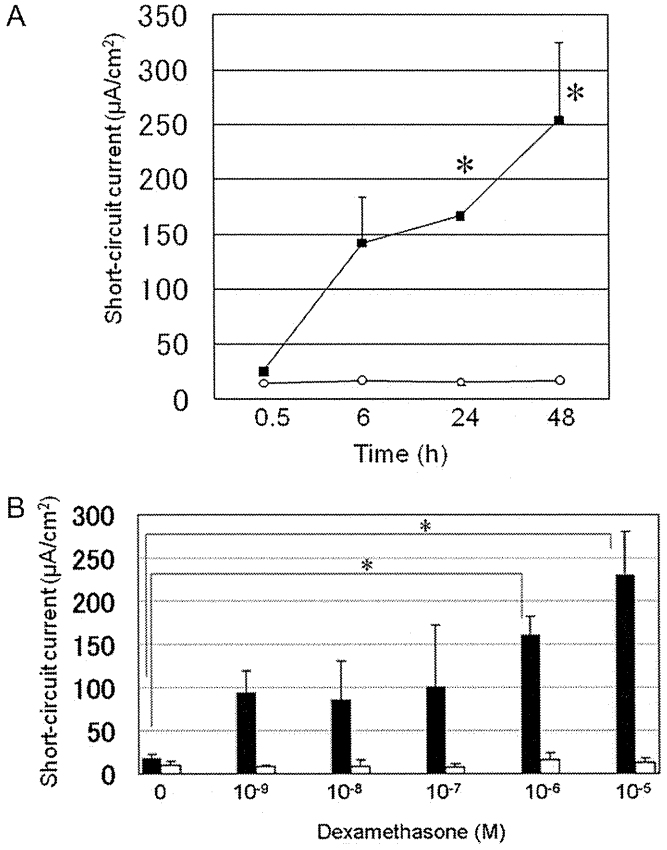

FIGURE 3. Effect of dexamethasone on the pump function of cultured mouse corneal endothelial cells. A, Cells were incubated in the absence (circles) or presence (squares) of 10 μM dexamethasone for the indicated times and then assayed for pump function (microamperes per square centimeter). $*P < 0.05$ versus the corresponding value for cells incubated without dexamethasone (Student *t* test). (B) Pump function (microamperes per square centimeter) attributable to Na,K-ATPase activity was determined 48 hours after incubation of cells in the presence of the indicated concentrations of dexamethasone (black). Ouabain-independent short-circuit current (white) is also presented, which did not change significantly with the indicated concentrations of dexamethasone. Data are means ± SDs of values from 4 replicate experiments. $*P < 0.05$ for the indicated comparisons (Student *t* test). Modified with permission from Hatou et al.[32]

Na,K-ATPase activity. Insulin had a transient stimulatory effect on Na,K-ATPase activity, with this effect being significant at 6 and 12 hours. After 12 hours, Na,K-ATPase activity returned to baseline levels. The stimulatory effect of insulin on Na,K-ATPase activity was also concentration dependent (Fig. 5).[33]

We next examined whether insulin affects the pump function of corneal endothelial cells. Insulin at 0.1 μM increased the ouabain-sensitive pump function of the corneal endothelial cells compared with control cells. This effect of insulin was statistically significant ($P < 0.05$) at 6 hours. The stimulatory effect of insulin on pump function was concentration dependent, and these results were similar to the results obtained for Na,K-ATPase activity (Fig. 6).[33]

PROTEIN KINASE C MEDIATION OF INSULIN-INDUCED Na,K-ATPase ACTIVATION

To test whether the stimulatory effect of insulin on Na,K-ATPase activity was mediated by protein kinase C (PKC), we examined the effects of staurosporine and GF109203X. The increase in Na,K-ATPase activity induced by insulin was significantly inhibited ($P < 0.01$) by staurosporine and GF109203X (Fig. 7).[33] We next examined whether okadaic acid affected the Na,K-ATPase activation induced by insulin. The activity of Na,K-ATPase in conjunction with 0.1 μM insulin was significantly reduced ($P < 0.01$) in the presence of 1 μM okadaic acid (Fig. 7).[33]

We exposed cells to 0.1 μM insulin for 6 hours and then measured the expression levels of total Na,K-ATPase α_1-subunit and phospho-Na,K-ATPase α_1-subunit by Western blotting to determine whether insulin affected Na,K-ATPase expression in corneal endothelial cells. Although there was no statistically significant difference in the expression of total Na,K-ATPase α_1-subunit (Fig. 8A, B),[33] insulin significantly decreased ($P < 0.05$) the ratio of phospho-Na,K-ATPase α_1-subunit expression to total Na,K-ATPase α_1-subunit (Fig. 8C).[33]

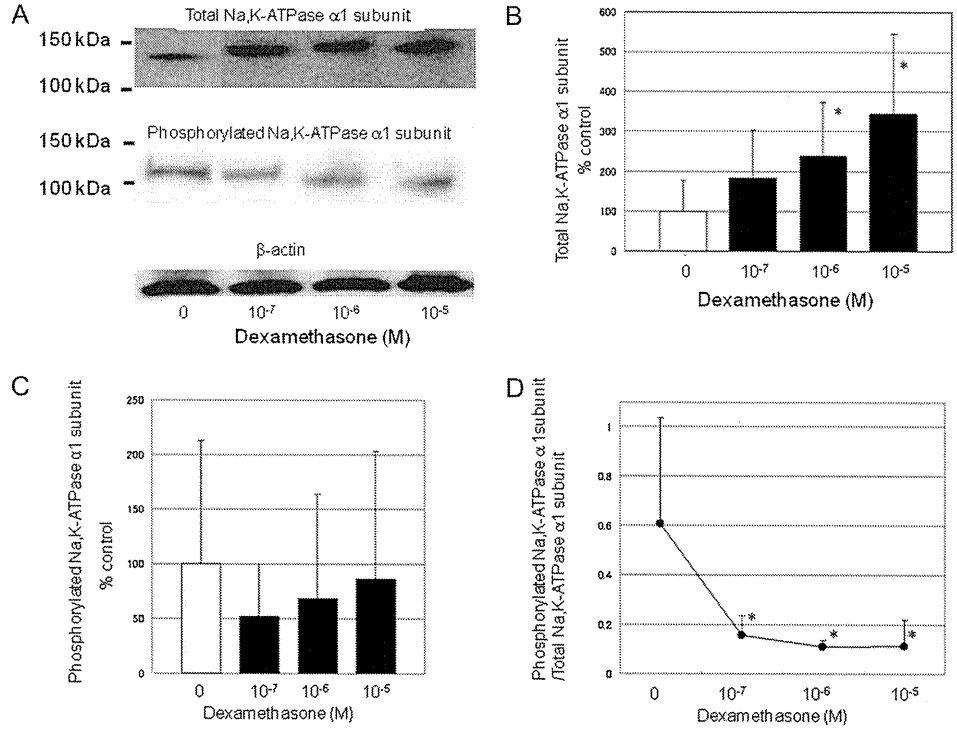

FIGURE 4. Western blot analysis of Na,K-ATPase α_1-subunit and phospho-Na,K-ATPase α_1-subunit expression. A, Representative signals of expression. Top: Na,K-ATPase α_1-subunit. Middle: phospho-Na,K-ATPase α_1-subunit. Bottom: β-actin. The relative intensity of each band with respect to β-actin was measured and expressed as a ratio. B, Cells were incubated with the indicated concentrations of dexamethasone for 48 hours and then assayed for expression of the Na,K-ATPase α_1-subunit. Data are means ± SDs from 5 experiments, expressed as a percentage of control. $^*P < 0.05$ for the indicated comparisons (Student *t* test). C, Cells were incubated with the indicated concentrations of dexamethasone for 48 hours and then assayed for expression of the phospho-Na,K-ATPase α_1-subunit. Data are means ± SDs from 5 experiments, expressed as a percentage of control. D, The rate of the inactive state of Na,K-ATPase α_1-subunit with the indicated concentrations of dexamethasone. The values represent the ratio of phospho-Na,K-ATPase α_1-subunit expression to Na,K-ATPase α_1-subunit expression. Data are means ± SDs of values from 5 experiments. $^*P < 0.05$ for the indicated comparisons (Student *t* test). Modified with permission from Hatou et al.[32]

In the presence of staurosporine, GF109203X, and okadaic acid, the expression of total Na,K-ATPase α_1-subunit was unchanged; however, insulin-induced dephosphorylation of the Na,K-ATPase α_1-subunit was diminished.

Immunocytochemistry was employed to determine whether the effects of insulin changed cell surface expression of the Na,K-ATPase α_1-subunit. Insulin-treated corneal endothelial cells expressed more Na,K-ATPase α_1-subunit at their lateral cell membranes compared with control cells. In the presence of inhibitors, such as GF109203X, Na,K-ATPase α_1-subunit expression at the lateral cell membrane was observed to be decreased (Fig. 9A–C).[33]

DISCUSSION

We have shown that dexamethasone increases Na,K-ATPase activity and pump function in cultured corneal endothelial cells. Changes in Na,K-ATPase activity and pump function were strongly correlated under various experimental conditions. Our results support the stimulatory effect of dexamethasone on Na,K-ATPase activity in corneal endothelial cells.

Our results further suggest that the regulation of Na,K-ATPase activity by dexamethasone in corneal endothelial cells was mediated by Na,K-ATPase subunit synthesis. Na,K-ATPase is the largest protein complex in the family of P-type cation pumps, and its minimum functional unit is a heterodimer of the α- and β-subunits.[34] Ewart and Klip[8] reported that the activation of Na,K-ATPase by steroid hormones seemed to be mediated by the synthesis of new α- and β-subunits. In our study, dexamethasone increased the proportion of active-state Na,K-ATPase α_1-subunits and the total number of Na,K-ATPase α_1-subunits. The antiphospho-Na/K-ATPase α_1 antibody we used recognizes the Na,K-ATPase α_1-subunit only when phosphorylated at Ser-18. Phosphorylation at Ser-18 triggers endocytosis of Na,K-ATPase α_1-subunits and results in inhibition of Na,K-ATPase activity.[35,36] Dexamethasone may prevent Na,K-ATPase α_1-subunits from Ser-18 phosphorylation and thereby increase the proportion of active-state Na,K-ATPase α_1-subunits.

Our results showed that insulin increased Na,K-ATPase activity and pump function in cultured corneal endothelial cells, but the observed effect of insulin on Na,K-ATPase activity was transient. A lack of insulin in type 1 diabetes mellitus or a chronic reduced level of insulin signaling because of insulin resistance in type 2 diabetes mellitus is essential for the pathogenesis of corneal abnormalities in diabetes.

Insulin has been shown to stimulate electrogenic sodium transport in a variety of cells.[8,37–46] In most cases, the increase in Na^+ transport is thought to be a result of the stimulation of Na,K-ATPase. There have been various advocated mechanisms of insulin action, including changes in the kinetic properties of the enzyme,[37,38] an increase in the intracellular Na^+ concentration that leads to subsequent pump stimulation,[39–43] and an increase in the pump concentration at the cell surface by serum- and glucocorticoid-dependent kinase.[44–46] Regardless of whether insulin stimulates pump activity by a previous increase in cytosolic Na^+, its affinity for Na^+, or in-pump availability at the cell surface, the insulin receptor

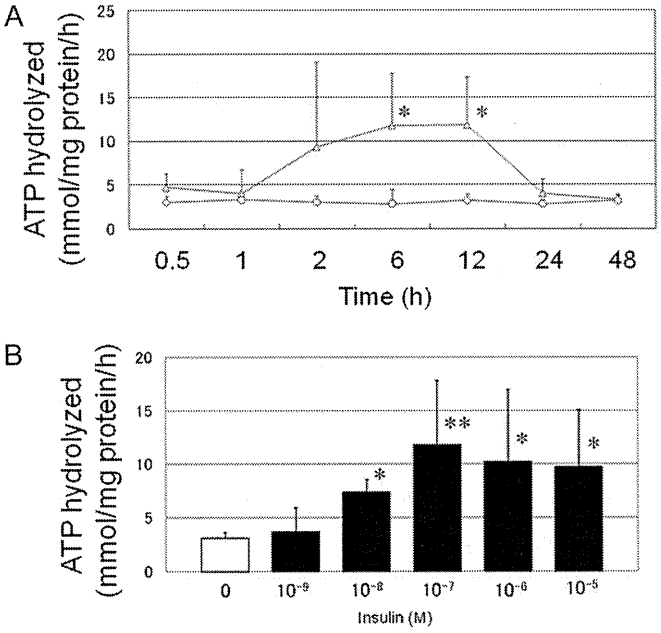

FIGURE 5. Effect of insulin on Na,K-ATPase activity in cultured mouse corneal endothelial cells. A, Cells were incubated in the absence (circles) or presence (triangles) of 0.1 μM insulin for the indicated times and then assayed for Na,K-ATPase activity. Data are means ± SDs of values from 4 replicate experiments. $*P < 0.05$ versus the corresponding value for cells incubated without insulin (Student *t* test). B, Cells were incubated with the indicated concentrations of insulin for 6 hours and then assayed for Na,K-ATPase activity. Data are means ± SDs of values from 4 replicates of 4 representative experiments. $*P < 0.05$, $**P < 0.01$ for the indicated comparisons (Student *t* test). Modified with permission from Hatou et al.[33]

signaling cascades must be involved.[8] These signaling cascades include those mediated by protein kinases, such as PKC. PKC is thought to trigger the rapid action of insulin on Na,K-ATPase and to be involved in the stimulation of Na,K-ATPase by insulin in muscle cells.[8] In our study, Western blot analysis suggested that the stimulation of Na,K-ATPase activity by insulin in corneal endothelial cells was associated with a decrease in the levels of the inactive state of the Na,K-ATPase α_1-subunit. Na,K-ATPase activation by insulin seemed to be mediated by PKC, protein phosphatase 1 (PP1), and/or PP2A. The immunocytochemistry results indicated that insulin increased cell surface expression of the Na,K-ATPase α_1-subunit, and the presence of inhibitors, such as GF109203X, decreased its expression.

In conclusion, we have shown that dexamethasone and insulin increase Na,K-ATPase activity and pump function in corneal endothelial cells. Furthermore, our results support a model in which Na,K-ATPase activation by dexamethasone is mediated by Na,K-ATPase subunit synthesis and an increase in the proportion of Na,K-ATPase α_1-subunits in the active state. In contrast, the observed effect of insulin on Na,K-ATPase activity was transient. This may be because Na,K-ATPase activation by insulin in corneal endothelial cells was mediated by an increase in the active state of the Na,K-ATPase α_1-subunits via PKC, PP1, and/or PP2A pathways, but not by Na,K-ATPase subunit synthesis. A lack of insulin in type 1 diabetes mellitus or a reduced level of insulin signaling caused by insulin resistance in type 2 diabetes mellitus may play a role in the pathogenesis of corneal abnormalities in diabetes. Pharmacological manipulation of dexamethasone or insulin in corneal endothelial cells is a potential therapeutic approach for increasing the pump function in corneal endothelial cells.

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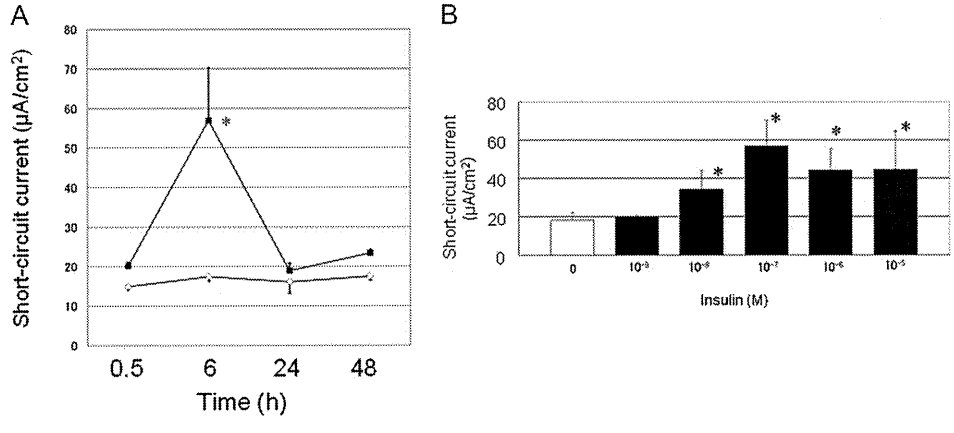

FIGURE 6. Effect of insulin on the pump function of cultured mouse corneal endothelial cells. A, Pump function (microamperes per square centimeter) attributable to Na,K-ATPase activity was determined in the absence (circles) or presence (squares) of 0.1 μM insulin for the indicated times. Data are means ± SDs of values from 4 replicates of a representative experiment. $*P < 0.05$ versus the corresponding value for cells incubated without insulin (Student *t* test). B, Pump function (microamperes per square centimeter) attributable to Na,K-ATPase activity was determined 6 hours after incubation of cells in the presence of the indicated concentrations of insulin. Data are means ± SDs of values from 4 replicates of 4 representative experiments. $*P < 0.05$ for the indicated comparisons (Student *t* test). Modified with permission from Hatou et al.[33]

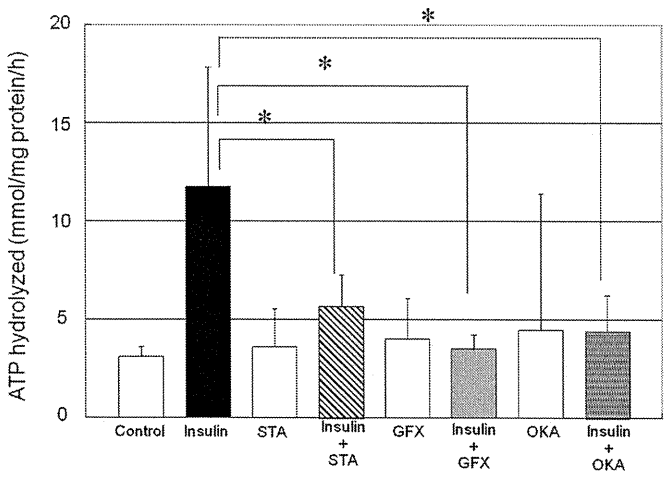

FIGURE 7. Effect of staurosporine (STA), GF109203X (GFX), and okadaic acid (OKA) on insulin-induced Na,K-ATPase activity in cultured mouse corneal endothelial cells. Cells were incubated for 30 minutes in the absence or presence of 1 μM staurosporine, 0.1 μM GF109203X, or 1 μM okadaic acid and then for an additional 6 hours in the presence of 0.1 μM insulin before measurement of Na,K-ATPase activity. Data are means ± SDs of values from 4 replicates of 4 representative experiments. $*P < 0.01$ versus the value for cells incubated with insulin alone (Student *t* test). Na,K-ATPase activity did not significantly increase in the presence of staurosporine + insulin, GF109203X + insulin, or okadaic acid + insulin compared with controls. Modified with permission from Hatou et al.[33]

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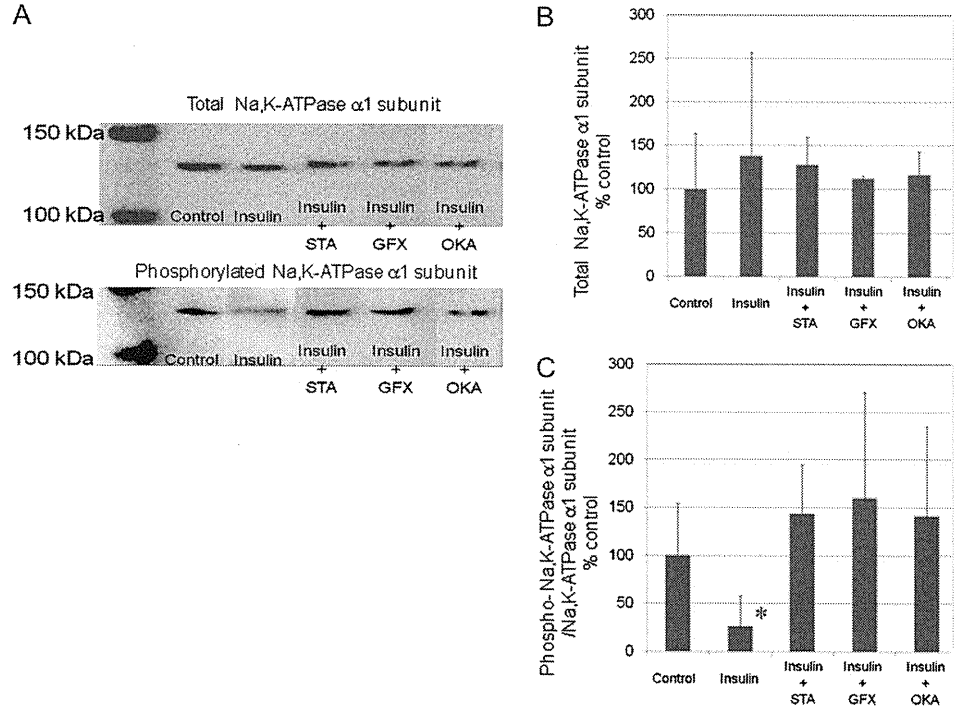

FIGURE 8. Western blot analysis of Na,K-ATPase α_1-subunit and phospho-Na,K-ATPase α_1-subunit expression. A, Representative signals of expression. Top: Na,K-ATPase α_1-subunit. Bottom: phospho-Na,K-ATPase α_1-subunit. The relative intensity of each band compared with β-actin was measured by a densitometer and expressed as a ratio. B, Cells were incubated in the absence (control) or presence of 0.1 μM insulin for 6 hours, 0.1 μM insulin for 6 hours with a 30-minute preincubation with 1 μM staurosporine (insulin + STA), 0.1 μM GF109203X (insulin + GFX), or 1 μM okadaic acid (insulin + OKA) and then assayed for the expression of Na,K-ATPase α_1-subunit. Data are means ± SDs from 5 experiments, expressed as a percentage of control. C, The rate of inactive state of Na,K-ATPase α_1-subunit with insulin, insulin + STA, insulin + GFX, and insulin + OKA. The values represent the ratio of phospho-Na,K-ATPase α_1-subunit expression to Na,K-ATPase α_1-subunit expression. Data are means ± SDs of values from 5 experiments. $*P < 0.05$ versus the value for cells incubated without insulin (Student *t* test). Modified with permission from Hatou et al.[33]

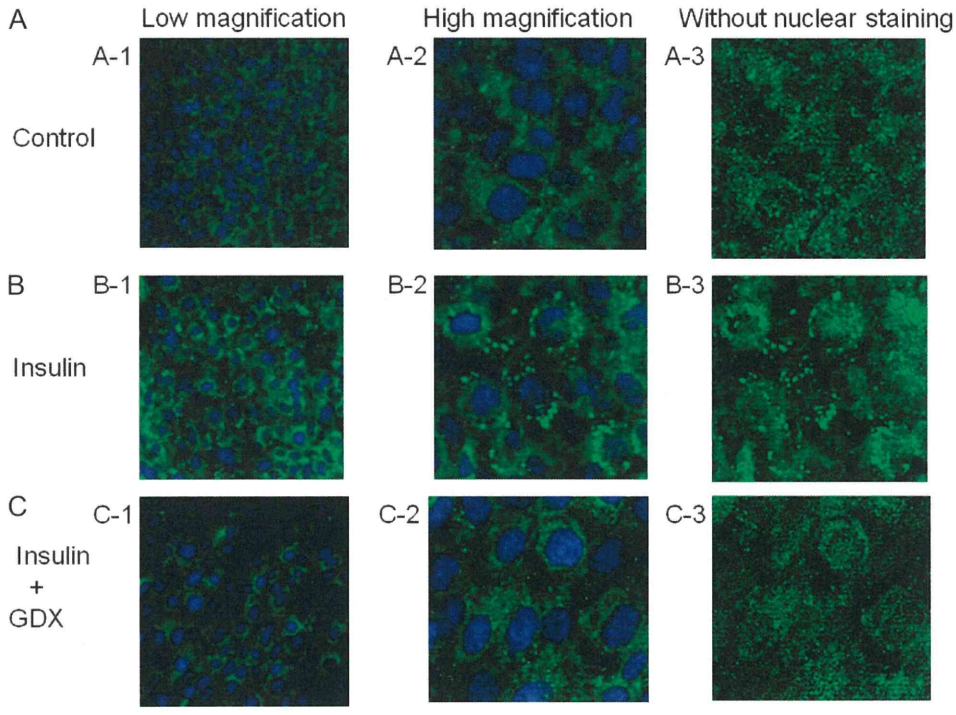

FIGURE 9. Effect of insulin on Na,K-ATPase α_1-subunit cell surface expression. Cells were incubated in the absence of insulin (A) or in the presence of 0.1 μM insulin for 6 hours (B) or 0.1 μM insulin for 6 hours with a 30-minute preincubation with 1 μM GF109203X (C) and then assayed for cell surface expression of the Na,K-ATPase α_1-subunit by immunocytochemistry. A-1, B-1, and C-1: low magnification; A-2, B-2, and C-2: high magnification; and A-3, B-3, and C-3: without nuclear staining. Modified with permission from Hatou et al.[33]

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In Vivo Confocal Microscopic Evidence of Keratopathy in Patients with Pseudoexfoliation Syndrome

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Purpose. To measure the density of cells in different layers of the cornea and to determine whether morphologic changes of the subbasal corneal nerve plexus are present in eyes with the pseudoexfoliation (PEX) syndrome.

Methods. Twenty-seven patients with unilateral PEX syndrome and 27 normal controls were investigated. All eyes underwent corneal sensitivity measurements with an esthesiometer and in vivo confocal microscopic study. Densities of the epithelial, stromal, and endothelial cells were measured. The density and tortuosity of the subbasal corneal nerve plexus were also evaluated.

Results. Eyes with PEX syndrome had significantly lower cell densities in the basal epithelium ($P = 0.003$), anterior stroma ($P = 0.007$), intermediate stroma ($P = 0.009$), posterior stroma ($P = 0.012$), and endothelium ($P < 0.0001$) than in the corresponding layers of normal eyes. PEX eyes also had lower subbasal nerve densities and greater tortuosity of the nerves than normal eyes. Fellow eyes of patients with PEX also had significantly lower densities of the basal epithelial and endothelial cells than the normal eyes. Corneal sensitivity was significantly decreased in PEX eyes, and this was significantly correlated with the decrease of basal epithelial cell and subbasal nerve densities.

Conclusions. These results have shed light on understanding of the pathogenesis of decreased corneal sensitivity in eyes with PEX syndrome. PEX syndrome is probably a binocular condition for which keratopathy of the fellow eye also requires observation. (*Invest Ophthalmol Vis Sci.* 2011;52:1755–1761) DOI:10.1167/iovs.10-6098

The pseudoexfoliation (PEX) syndrome is a common age-related disorder of the extracellular matrix and is frequently associated with severe chronic secondary open angle glaucoma and cataract.[1–3] The prevalence of PEX syndrome varies widely in different racial and ethnic populations. In addition, the prevalence of PEX is dependent on the age and sex distribution of the population examined, the clinical criteria used to diagnose PEX, and the ability of the examiner to detect early stages and more subtle signs of PEX. For example, the highest rates in studies of persons older than 60 years of age have been reported to be approximately 25% in Iceland and more than 20% in Finland.[3,4] The ocular manifestation of PEX syndrome is the production and progressive accumulation of abnormal extracellular fibrillar material in almost all the inner wall tissues of the anterior segment of the eye. This characteristic alteration predisposes the eye to a broad spectrum of intraocular complications including phacodonesis and lens subluxation, angle closure glaucoma, melanin dispersion, poor mydriasis, blood-aqueous barrier dysfunction, posterior synechiae, and other related complications.[1–3]

The PEX syndrome is associated with corneal endotheliopathy, and this has been suggested to be the cause of the so-called atypical non-guttata Fuchs endothelial dystrophy.[5,6] PEX endotheliopathy, a slowly progressing disease of the corneal endothelium, is usually bilateral but is often asymmetrical. It can lead to early corneal endothelial cell decompensation, which can then induce severe bullous keratopathy, a vision-threatening disorder.

Clinical signs of PEX syndrome include decreased corneal sensitivity, thinning of the central corneal thickness, and impaired tear film stability.[7–9] However, the underlying cause of these clinical findings has not been well investigated, possibly because objective and accurate in vivo examination techniques are not available.

Recent advances in imaging technology have improved the ability of these instruments to diagnose different ocular diseases. The Rostock Cornea Module (Heidelberg Engineering, Heidelberg, Germany), consisting of a contact lens system attached to the Heidelberg Retina Tomograph II (Heidelberg Engineering), is such an instrument. It uses laser scanning technology to investigate the cornea at a cellular level, and structures such as the subbasal nerve plexus, which cannot be seen by slit-lamp microscopy, can be clearly seen.[10,11]

In vivo confocal microscopy (IVCM) was used by Martone et al.[12] to examine one eye with PEX syndrome, and noncontact IVCM was used by Sbeity et al.[13] to study PEX, PEX-suspect, and normal eyes. However, there has not been a detailed and quantitative study of the morphologic changes in the corneas of eyes with PEX syndrome.

Thus, the purpose of this study was to examine the underlying pathogenesis of PEX keratopathy and to obtain evidence to explain clinical findings such as the decreased corneal sensitivities observed in patients with PEX syndrome. To accomplish this, we used IVCM to determine cell densities in different corneal layers of eyes with PEX syndrome and their clinically unaffected fellow eyes. These findings were compared with those in normal control eyes. The nerve densities in the subbasal layer were also analyzed, and their relationship with the alterations of clinical corneal sensitivity were analyzed.

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Subjects and Methods

Subjects

We studied 27 patients (16 men, 11 women; mean age, 74.4 ± 6.3 years; age range, 65–90 years) with diagnoses of unilateral PEX syndrome. In all eyes, exfoliation material (XFM) was seen by slit-lamp microscopy at the pupillary border or on the anterior lens capsule. Eyes with PEX syndrome were placed in the PEX group, and clinically normal fellow eyes were placed in the PEX fellow eye group. Age- and sex-matched normal subjects (16 men, 11 women; mean age, 72.7 ± 6.5 years; age range, 61–92 years) were also studied. One eye from the normal control group was randomly selected and used in the statistical analyses. Exclusion criteria included Stevens-Johnson syndrome, lymphoma, sarcoidosis, corneal dystrophy, injury, inflammation, systemic therapy with drugs with known corneal toxicity; treatment with topical anti-glaucoma drugs, steroids, or NSAIDs; contact lens wear; previous ocular surgery; and other ophthalmic diseases.

The procedures used conformed to the tenets of the Declaration of Helsinki. Informed consent was obtained from all subjects after an explanation of the nature and possible consequences of the procedures. The protocol used was approved by the Ethics Committee of Ehime University School of Medicine.

Corneal Sensitivity Measurements

Measurement of the corneal sensitivity was performed with a Cochet-Bonnet nylon thread esthesiometer, as described.[14] The examination was begun with a 60-mm length of nylon filament applied perpendicularly to the central cornea, and the tests were continued by shortening the filament by 5 mm each time until the subject felt the contact of the filament. Each subject was measured twice with a between-test interval of at least 5 minutes, and the average of two measurements was used for the statistical analyses.

In Vivo Confocal Microscopy

IVCM was performed on all subjects with the Rostock Corneal Module of the Heidelberg Retina Tomograph II (HRTII-RCM; Heidelberg Engineering). After topical anesthesia with 0.4% oxybuprocaine (Santen Pharmaceuticals, Osaka, Japan), the subject was positioned in the chin and forehead holder and instructed to look straight ahead at a target to make sure that the central cornea was scanned. The objective of the microscope was an immersion lens (magnification ×63; Zeiss, Chester, VA) covered by a polymethylmethacrylate cap (TomoCap; Heidelberg Engineering). Comfort gel (Bausch & Lomb, Berlin, Germany) was used to couple the applanating lens cap to the cornea. The polymethylmethacrylate cap was applanated onto the center of the cornea by adjusting the controller, and in vivo digital images of the cornea were seen on the monitor screen. When the first layer of superficial epithelial cells was seen, the digital micrometer gauge was set to zero, and then a sequence of images was recorded as the focal plane was gradually moved toward the endothelium. Each subject underwent scanning three times at intervals of at least 15 minutes.

The laser source of the HRT-II RCM is a diode laser with a wavelength of 670 nm. Two-dimensional images consisting of 384 × 384 pixels covering an area of 400 × 400 μm were recorded. The digital resolution was 1.04 μm/pixel transversally and 2 μm/pixel longitudinally, as stated by the manufacturer.

Image Analyses

Central corneal images of all subjects were taken, and the three best-focused images from the superficial epithelium, basal epithelium, subbasal nerve plexus, anterior stroma, intermediate stroma, posterior stroma, and endothelium were selected for analyses. The selected images were randomly presented to two masked observers (XZ, SO) for evaluation. All data are presented as averages of three images.

Cell Density Analyses

Morphologic characteristics and densities in the different layers of the cornea in the PEX and PEX fellow eyes were assessed and compared with those of normal controls. Superficial epithelial cells were identified as polygonal cells with clearly visible cell borders, bright cytoplasm, and dark nuclei. Basal epithelial cells were identified as the layer just above the amorphous-appearing Bowman membrane. Basal cells had bright borders, a uniform shape, and nonhomogeneous cytoplasm. The anterior stroma was identified as the first layer immediately beneath the Bowman membrane, and the posterior stroma was identified as the layer just anterior to the Descemet membrane and the endothelium. The intermediate stroma was defined as the layer halfway between the anterior and posterior stroma.[15] The corneal endothelium consisted of a monolayer of regularly arranged hexagonal cells with dark borders and bright reflecting cytoplasm.

After selecting a frame of the image and manually marking the cells inside the frame (>50 cells), cell densities were calculated automatically by the software installed in the instrument. Cells partially contained in the area analyzed were counted only along the upper and right margins. The results are expressed in cells per square millimeter.

Analyses of Subbasal Nerve Plexus

The subbasal nerve plexus layer is located between the Bowman membrane and the basal epithelial layer through which numerous nerve fibers pass. The density and tortuosity of the subbasal nerve plexus were analyzed as described.[14,16] Two parameters were analyzed: the long nerve fiber density (LNFD) was determined by dividing the number of long nerves by the image area (0.16 mm^2), and the nerve branch density (NBD) was determined by dividing the total number of long nerves and their branches by the image area. Nerve tortuosity was classified into 4 gradings: grade 1 = approximately straight nerves; grade 4 = very tortuous nerves with significant convolutions throughout their course.[16]

Statistical Analyses

Data were analyzed with statistical software (JMP, version 8.0 for Windows; SAS Japan Inc., Tokyo, Japan). All data are expressed as the mean ± SD. The differences of cell densities between PEX eyes and normal controls or between PEX fellow eyes and normal controls were evaluated with two-tailed Student's *t*-tests. The differences of cell densities between PEX eyes and their fellow eyes were evaluated by paired *t*-tests. The Wilcoxon rank sum test was used to compare the values of corneal sensitivity, LNFD, NBD, and the nerve tortuosity between PEX patients and normal controls. Spearman's correlation was used to determine the correlation among the parameters of basal epithelial cell density, subbasal nerve density, and corneal sensitivity. $P < 0.05$ was considered statistically significant.

Results

The mean age was not significantly different between patients with PEX and normal controls (two-tailed Student's *t*-tests, $P = 0.725$). Eyes with PEX showed typical whitish exfoliation material on the pupillary border or on the anterior lens capsule on slit-lamp examination. Pigmented keratoprecipitates and slight folding of Descemet membrane were also detected in some patients. Fellow eyes of PEX eyes and normal control eyes appeared normal by slit-lamp microscopy.

Corneal Sensitivity

The mean corneal sensitivity was 47.8 ± 5.6 mm for PEX eyes and 53.7 ± 4.9 mm for PEX fellow eyes. This difference was significant ($P = 0.005$; Wilcoxon rank sum test). Mean corneal

sensitivity was 55.6 ± 4.7 mm for the normal control subjects, and the corneas of eyes with PEX were significantly less sensitive than those of normal control eyes ($P < 0.0001$). The difference in corneal sensitivity between PEX fellow eyes and normal controls was not significant ($P = 0.378$).

Cell Densities

The density of the corneal superficial epithelial cells was 872.6 ± 95.3 cells/mm^2, and that for the basal epithelial cells was 4829.7 ± 462.1 cells/mm^2 in PEX eyes. Densities for the corresponding layers in PEX fellow eyes were 910.4 ± 80.8 cells/mm^2 and 4996.7 ± 438.7 cells/mm^2, and densities for the normal control eyes were 886.4 ± 101.7 cells/mm^2 and 5446.4 ± 639.9 cells/mm^2. The density of the basal epithelial cells was significantly lower for PEX eyes and PEX fellow eyes than for the control eyes ($P = 0.003$ and $P = 0.015$, respectively; two-tailed Student's *t*-tests; Fig. 1). The difference in the density of the basal epithelial cells between PEX eyes and PEX fellow eyes was not significant ($P = 0.589$; paired *t*-test). Differences in the densities of the superficial epithelial cells among the three experimental groups also were not significant (Fig. 1).

Densities of the cells in the three stromal layers of PEX eyes, PEX fellow eyes, and normal control eyes are shown in Figure 2. Compared with normal controls, the cell densities of PEX eyes were significantly lower in all three layers of the stroma (anterior stroma, $P = 0.007$; intermediate stroma, $P = 0.009$; posterior stroma, $P = 0.012$; two-tailed Student's *t*-tests). The densities in these three stromal layers in PEX fellow eyes were also lower, but the decrease was not significant ($P = 0.196$; $P = 0.261$; $P = 0.08$; respectively; Fig. 2).

Endothelial cell densities were 2240.7 ± 236.6 cells/mm^2, 2386.6 ± 200.8 cells/mm^2, and 2738.7 ± 233.2 cells/mm^2 for PEX eyes, PEX fellow eyes, and normal eyes, respectively. Differences between PEX eyes and normal controls ($P < 0.0001$; two-tailed Student's *t*-test; Fig. 1) and between PEX fellow eyes and normal controls were significant ($P = 0.001$). The difference in endothelial cell density between PEX and PEX fellow eyes was not significant ($P = 0.754$; paired *t*-test).

There was a higher degree of pleomorphism and polymegethism in PEX eyes than in control eyes. The coefficient of variation (CV) of the cell area was 45.2% ± 8.7%, and the percentage of hexagonal cells (HEX) in PEX eyes was 30.5% ± 10.3%. Both values are significantly different from those of normal control eyes (CV, 30.6% ± 5.6%, $P = 0.016$; HEX, 50.3 ± 6.8%, $P = 0.008$; two-tailed Student's *t*-test). PEX fellow eyes also showed a similar tendency of increased pleomorphism and polymegethism, but the differences were not statistically significant.

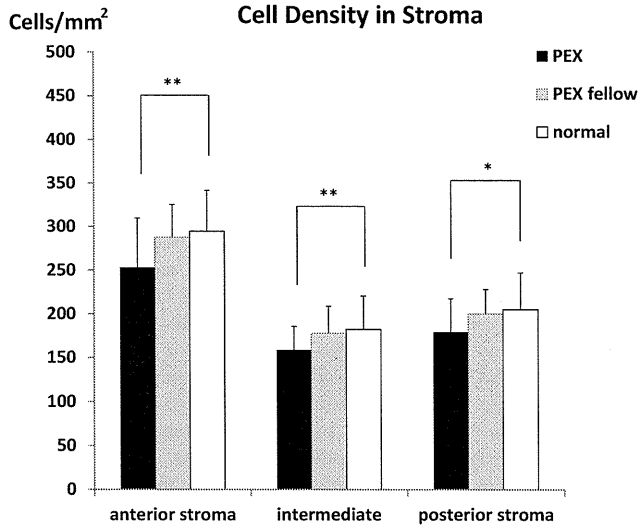

FIGURE 2. Cellular densities of anterior, intermediate and posterior stroma of eyes with PEX syndrome, their clinically unaffected fellow eyes, and eyes of normal control subjects. $^{**}P < 0.01$; $^{*}P < 0.05$.

Subbasal Nerve Plexus

The LNFD and NBD were significantly decreased in PEX eyes (17.4 ± 6.3 and 32.2 ± 8.3 nerves/mm^2, respectively) compared with those in normal controls (35.9 ± 8.2 and 72.2 ± 8.8 nerves/mm^2; $P < 0.0001$ and $P < 0.0001$, respectively; Wilcoxon rank sum test; Fig. 3). PEX fellow eyes also had decreased LNFD and NBD, but these changes were not

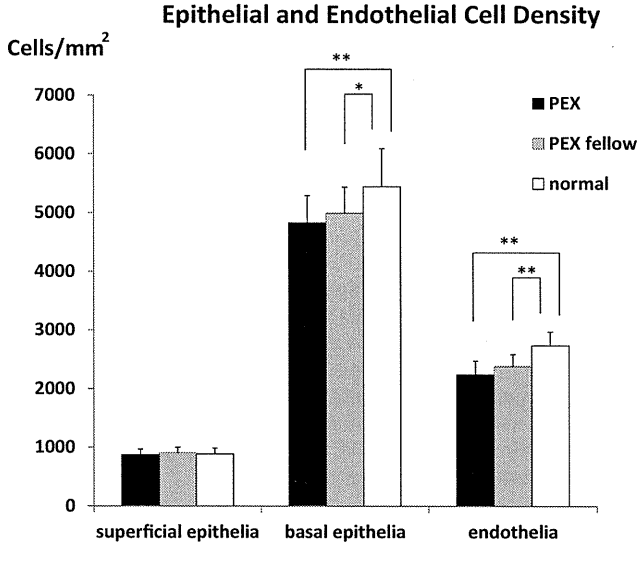

FIGURE 1. Corneal epithelial and endothelial cell densities of eyes with PEX syndrome, their clinically unaffected fellow eyes, and eyes of normal control subjects. $^{**}P < 0.01$; $^{*}P < 0.05$.

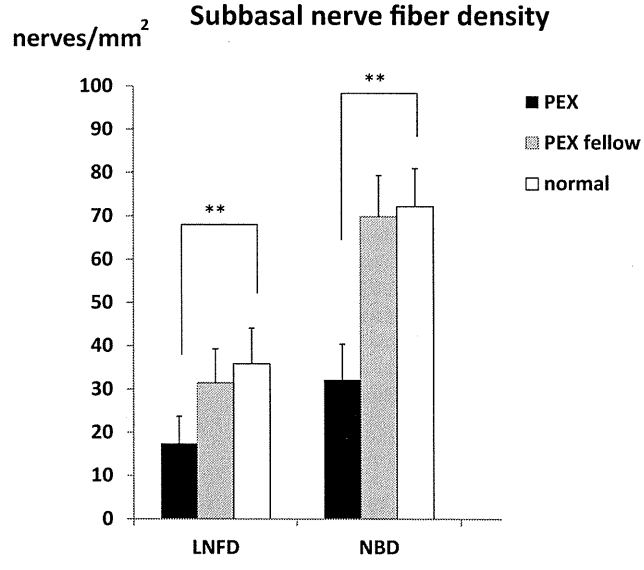

FIGURE 3. Subbasal LNFD and NBD in eyes with PEX syndrome, their clinically unaffected fellow eyes, and eyes of normal control subjects. $^{**}P < 0.01$.

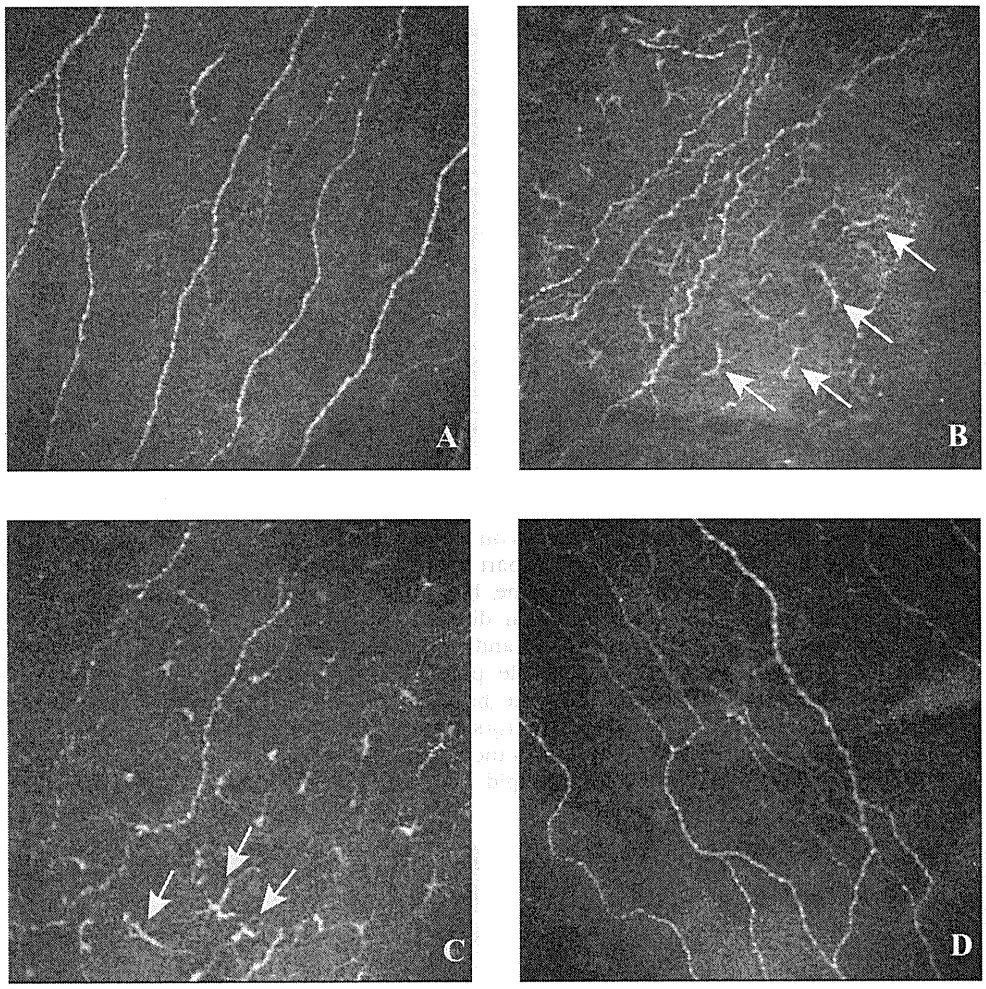

FIGURE 4. In vivo confocal microscopic images of the subbasal nerve plexus in patients with PEX syndrome and a normal control subject. (**A**) Representative image from a normal control subject showing subbasal nerve plexus with long nerve fibers running parallel to the Bowman layer. The nerve fibers appeared to be straight with minimal tortuosity. The subbasal LNFD was 31.3 nerves/mm^2, and the nerve tortuosity was grade 1. (**B**) Representative image from a PEX syndrome eye showing very tortuous nerves with significant convolutions throughout their course. The tortuosity grade was 4. Note the intensive infiltration of dendritic cells (*arrows*) in close vicinity of the nerve fibers. (**C**) Confocal image of the subbasal nerve plexus of another PEX eye showing the thinning of the nerves, short nerve sprouts, fewer branches from the main nerve trunk, and significantly decreased nerve density. The LNFD was 6.3 nerves/mm^2. *Arrows*: dendritic cell infiltration. (**D**) Confocal image of a PEX fellow eye showing moderately tortuous subbasal nerve plexus with a tortuosity grade of 3 and an LNFD of 18.8.

significantly different from those of the controls (31.5 ± 7.8 and 69.9±9.4 nerves/mm^2; $P = 0.093$ and $P = 0.301$).

Confocal images of PEX eyes showed extremely tortuous nerve fibers, thinning of nerves, short nerve sprouts, fewer branches from the main nerve trunk, and highly reflective inflammatory infiltrates in close vicinity of the subbasal nerves. Representative confocal images of the three groups are shown in Figure 4. In PEX eyes, 85.2% (23 of 27 eyes) had grade ≥3 subbasal nerve tortuosity, and the degree of tortuosity in PEX eyes was significantly higher than that of the controls (3.2 ± 0.7 vs. 1.6 ± 0.6; $P < 0.0001$; Wilcoxon rank sum test). The degree of tortuosity in PEX fellow eyes was also greater than that of normal controls, although the difference was not significant (2.1 ± 0.9 vs. 1.6 ± 0.6; $P = 0.054$).

It was our impression that PEX eyes had more inflammatory cells, including dendritic cells, infiltrating the subbasal cell layer and anterior stroma, and these changes were more severe in eyes with decreased subbasal nerve densities and lower corneal sensitivities (Fig. 4).

Correlation between Corneal Sensitivity and Subbasal Nerve Density and Basal Epithelial Cell Density

Spearman's correlation analyses showed that there was a significant positive correlation between corneal sensitivity and the subbasal nerve densities (LNFD, $r = 0.764$, $P < 0.0001$; NBD, $r = 0.634$, $P < 0.0001$; Spearman correlation coefficient). Corneal sensitivity was also significantly and positively correlated with basal epithelial cell density and significantly and negatively correlated with subbasal nerve tortuosity (Table 1).

Confocal Microscopic Detection of Hyperreflective Material

IVCM showed hyperreflective material, probably XFM, in the subbasal epithelial layer or the anterior stroma of 22 of the 27 PEX eyes (81.5%). The hyperreflective material was also observed abundantly in the endothelia of all PEX eyes. Five of 27 (18.5%) PEX fellow eyes showed hyperreflective deposits in the subbasal epithelial layer or anterior stroma, and 14 of 27 (51.9%) had endothelial surface deposits of hyperreflective material. In sharp contrast, none of the normal eyes showed hyperreflective material in the subbasal epithelial or anterior

TABLE 1. Correlation among Corneal Sensitivity, Subbasal Nerve Fiber Density, Tortuosity, and Basal Epithelial Cell Density

	Corneal Sensitivity	
	Spearman Correlation Coefficient	*P*
Long nerve fiber density	0.7640	<0.0001*
Nerve branch density	0.6341	<0.0001*
Subbasal nerve fiber tortuosity	−0.8250	<0.0001*
Basal epithelial cell density	0.6971	<0.0001*

* Statistically significant.

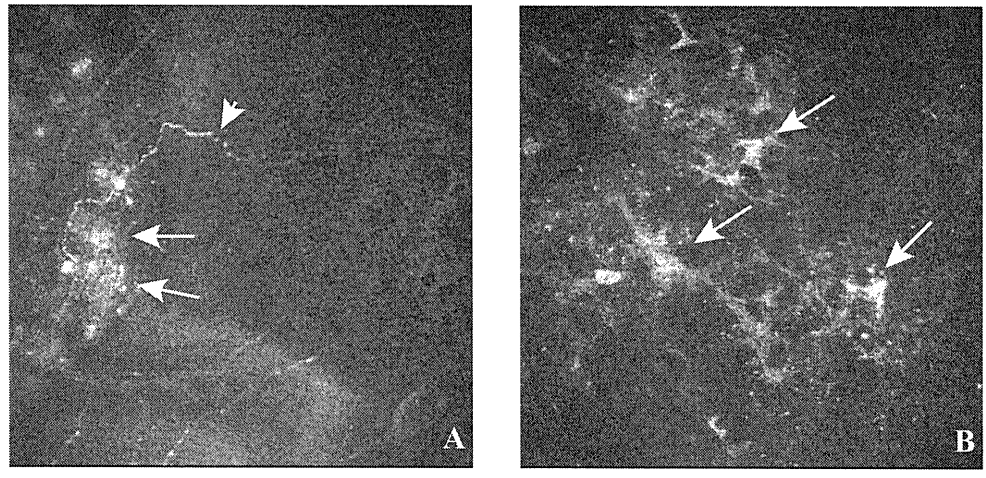

FIGURE 5. Confocal microscopic images showing XFM in the subbasal nerve plexus layer of a patient with PEX syndrome. (**A**) Nerve fiber thinning with tortuous morphology can be seen (*arrowhead*), and XFM (*arrows*) is seen in close vicinity of the pathogenic nerve fibers. (**B**) Hyperreflective deposits (*arrows*) indicative of XFM can be seen in the subbasal amorphous layer of the cornea of another patient in the PEX eye group.

stromal layers, and only two (7.4%) had a small amount of hyperreflective material on the endothelial surface (Figs. 5, 6).

DISCUSSION

The manifestations of PEX syndrome in the anterior segment are widely known to affect intraocular surgery with poor mydriasis and intensive postoperative inflammation. The fact that aggregates of XFM can be identified in autopsy specimens of the heart, lung, liver, kidney, and other organs in patients with ocular PEX suggests that the ocular PEX syndrome is part of a general systemic disorder.[1-3,17] In fact, PEX syndrome has been reported to be associated with cardiovascular diseases, chronic cerebral disorders, Alzheimer disease, and acute cerebrovascular events.[1-3] Two single nucleotide polymorphisms in the lysyl oxidase-like 1 (*LOXL1*) gene have been recently identified as strong genetic risk factors for PEX syndrome and PEX glaucoma.[18]

IVCM with the HRTII-RCM provides a new imaging method that allows rapid, noninvasive, high-resolution, and microstruc-

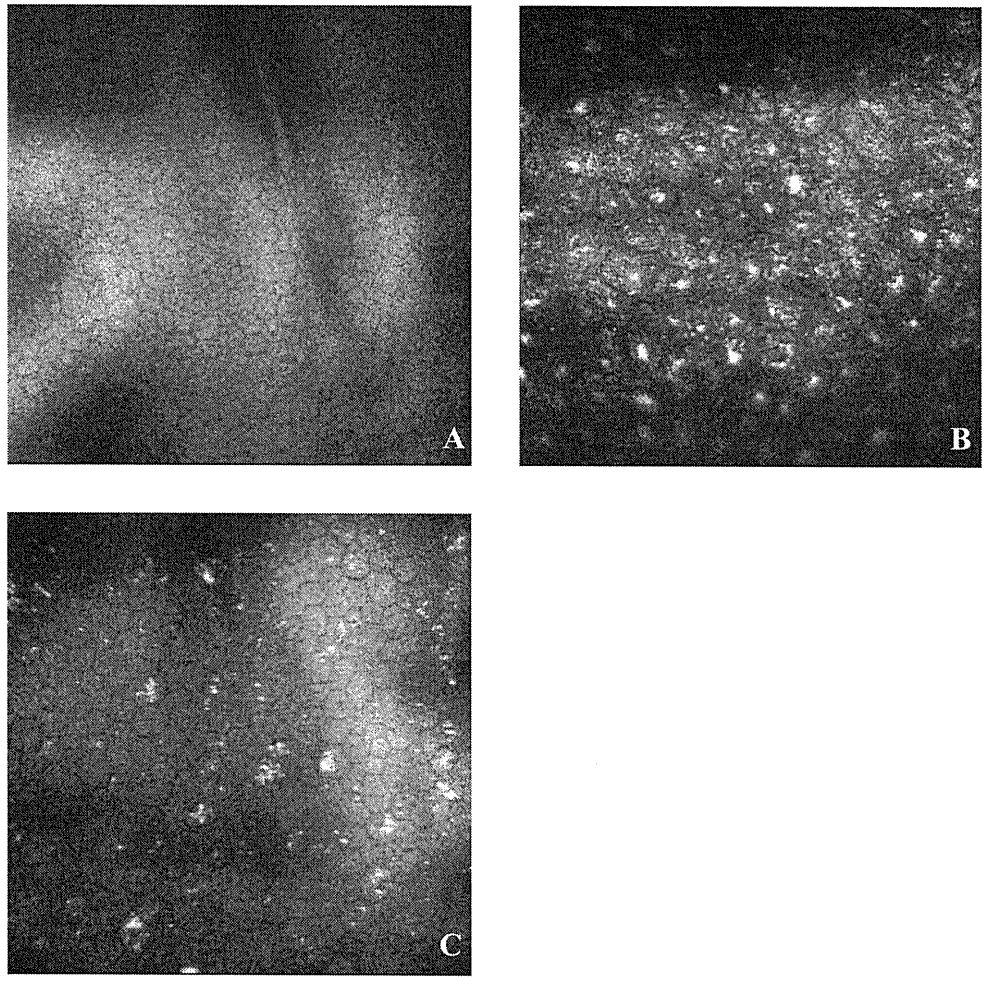

FIGURE 6. Representative confocal microscopic images of the endothelial layers of PEX syndrome eye, PEX fellow eye, and normal control eye group. (**A**) Normal subject with regularly arranged hexagonal endothelial cells. (**B**) PEX eye showing increases in pleomorphism and polymegethism and decrease in cell density. Intense hyperreflective materials indicative of XFM can be seen. (**C**) PEX fellow eye showing similar changes of endothelial cells and deposition of XFM.

tural examination of the cornea.[10,11] Only two studies have used IVCM to study the corneas of patients with PEX syndrome. Martone et al.[12] reported the findings in one case, and they reported that ICVM can detect hyperreflective deposits and dendritic cells infiltrating the basal epithelial cell layer. Fibrillar subepithelial structures were found, and the endothelial layer showed cellular anomalies. In a prospective observational case series, Sbeity et al.[13] used noncontact IVCM to detect XFM on the lens surfaces and corneal endothelia of PEX eyes and their fellow eyes.

Our study was the first to use IVCM to investigate cell densities in different layers of the cornea and to determine alterations of subbasal nerve density and tortuosity in PEX and PEX fellow eyes. Our results showed a significant decrease in the densities of the corneal endothelial cells in PEX eyes and their fellow eyes, which is in agreement with earlier observations by specular microscopy.[8,19,20] In addition, the clear confocal images allowed us to detect pleomorphisms and polymegethisms of the endothelial cells. All PEX eyes and 51.9% of PEX fellow eyes showed deposits of hyperreflective material in the endothelium, indicative of either pigment granules or XFM. In agreement with Sbeity et al.,[13] we believe that the pleomorphic and irregular deposits found on the corneal endothelium most likely represent XFM rather than pigment granules, which are round and uniform in size.[13] In addition, a number of patients who had no visible pigment keratoprecipitates on slit-lamp microscopy were found to have abundant large and irregular hyperreflective deposits on the endothelium in the confocal images.

PEX syndrome–associated corneal endotheliopathy has been suggested to be caused by one or a combination of the following alterations: hypoxic changes in the anterior chamber, accumulation of extracellular matrix, fibroblastic changes of the endothelium, and increased concentration of TGF-β.[1–3] Our confocal microscopic findings suggest that the XFM, possibly at different stages of the normal course of PEX, may be deposited on the endothelium or may migrate from the endothelial cells that undergo fibroblastic changes. Our findings also showed that hyperreflective materials are found not only on the endothelium of PEX eyes but also in their fellow eyes, indicating that the fellow eyes might be at a preclinical stage of PEX syndrome. A bilateral decrease in the endothelial cell counts and morphologic alterations of endothelium support the idea that PEX is a binocular and systemic abnormality. Patients with unilateral PEX syndrome may have asymmetric manifestation of this slowly progressing disease.

Of clinical significance was our finding that the decreased stromal cell densities observed by IVCM could possibly explain the report that the central corneas of PEX eyes were thinner than those of normal subjects.[8] The pathogenesis of the decrease of stromal cell density in PEX eyes warrants future study. Because XFM deposits were simultaneously observed in the anterior stroma of PEX eyes, we suggest that the XFM may be somehow causative for this alteration, perhaps by inducing apoptosis of the keratocytes. Other pathogenic factors, such as altered levels of cytokines or chemokines in the cornea, could also be responsible, and this definitely warrants future investigation. In addition, PEX fellow eyes also had lower cell counts in the stroma, although the difference was not statistically significant. We suggest that the cause of the binocular differences in our study might have been because the two eyes were at different stages of the PEX process, and PEX fellow eyes may still be at a preclinical stage of PEX syndrome.

Other important findings were found in the subbasal nerve plexus. Our results showed that the subbasal nerve density was significantly lower and the nerves were mostly tortuous, with beading and thinning in PEX eyes. Interestingly, PEX fellow eyes also had similar alterations, though the changes were not significant. These findings support the idea that PEX syndrome is a binocular abnormality that is expressed in both eyes but to different degrees. The important clinical significance of our study is that our correlation analyses showed that the decreased subbasal nerve density and increased tortuosity were significantly correlated with decreased corneal sensitivity. These results provide evidence, for the first time, that the cause of the decreased corneal sensitivity in eyes with PEX syndrome is the decreased subbasal nerve density. For patients with PEX syndrome, it would be practical and feasible to examine corneal sensitivity to assess the severity of PEX keratopathy and perhaps to predict the progression of PEX syndrome. In addition, detection of the morphologic changes in cell densities and subbasal nerve abnormalities by IVCM in the fellow eyes indicates that it is a sensitive tool for the diagnosis of preclinical stage of PEX syndrome. Our findings showed that PEX keratopathy may develop before any clinically visible XFM deposits are detected on the lens capsule or iris. If these findings are confirmed, then keratopathy may be the first event of the ocular complications of PEX syndrome. These findings also indicate that clinically unaffected fellow eyes of patients with PEX syndrome are probably at risk for PEX syndrome, and more frequent ophthalmologic examinations are necessary.

This study has increased our understanding of the keratopathy of this most likely systemic abnormality. Whether the alternations of the subbasal corneal nerves are primary or secondary changes of the disease must be determined. Because of the increase in the elastic microfibril components and imbalances in the matrix metalloproteinases (MMPs) and tissue inhibitors of MMP in eyes with PEX syndrome, PEX fibrils accumulate in the tissues.[1–3] Our findings that XFM deposits were frequently observed close to the subbasal epithelial layer or anterior stroma support the idea that besides an abnormal aggregation of elastic microfibrils into exfoliation fibers (the elastic microfibril hypothesis),[1–3,21] other extracellular matrix components, such as basement membrane components, may possibly interact and become incorporated into the composite XFM (the basement membrane hypothesis).[2,3] In addition, our observation of an infiltration of dendritic cells in close vicinity of the subbasal nerve plexus layer indicates the possibility that accumulation of extracellular XFM may induce inflammatory responses, which then recruit antigen-presenting cells such as immunocompetent dendritic cells. This excessive deposition of XFM and infiltration of dendritic cells may play a role in the neuropathy of the subbasal nerve plexus, resulting in decreased corneal sensitivity in patients with PEX syndrome.

Some limitations were present this study. First, the IVCM scans a very small area of the cornea, which may generate biases among different portions of scanning of different groups. As mentioned, efforts were taken to scan the center of the cornea of each subject. In addition, we also confirmed our findings by scanning the midperipheral and peripheral portions of the cornea (data not shown).

Second, IVCM images may not represent the true histologic changes of the cornea. By applying the same criteria for image evaluation, we can conclude that the differences between the studied groups were still detected. Furthermore, it was our impression that fewer keratocytes were seen in the stromas of corneal specimens obtained from PEX syndrome patients with penetrating keratoplasty.

Future investigations, including a thorough and quantitative analysis of the exfoliation material by confocal imaging, are needed. In addition, the correlations between IVCM findings with endothelial barrier function should be determined. If the confocal findings can provide clues for preclinical stages of endothelial barrier dysfunction of the cornea in PEX syndrome, their clinical significance can be used in designing an early treatment protocol.

In summary, our study demonstrated that eyes with PEX syndrome have decreased cell densities in the cornea. The subbasal nerve density was also significantly decreased, and this was significantly correlated with clinically decreased corneal sensitivity. Our study sheds light on understanding the cause of impaired corneal sensitivity in patients with PEX syndrome. The PEX syndrome is probably a bilateral event in which the keratopathy of the fellow eye also must be observed.

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ARTICLE

Outcomes of cataract surgery in eyes with a low corneal endothelial cell density

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PURPOSE: To evaluate the surgical outcomes of cataract surgery in eyes with a low preoperative corneal endothelial cell density (ECD) and analyze factors affecting the prognosis.

SETTING: Tokyo Dental College, Ichikawa General Hospital, Chiba, Japan.

DESIGN: Noncomparative case series.

METHODS: Eyes with a preoperative ECD of less than 1000 cells/mm^2 that had cataract surgery between 2006 and 2010 were identified. Standard phacoemulsification with intraocular lenses was performed using the soft-shell technique. The rate of endothelial cell loss, incidence of bullous keratopathy, and risk factors were retrospectively assessed.

RESULTS: Sixty-one eyes (53 patients) with a low preoperative ECD were identified. Preoperative diagnoses or factors regarded as causing endothelial cell loss included Fuchs dystrophy (20 eyes), laser iridotomy (16 eyes), keratoplasty (10 eyes), traumatic injury (3 eyes), trabeculectomy (3 eyes), corneal endotheliitis (2 eyes), and other (7 eyes). The corrected distance visual acuity improved from 0.59 ± 0.49 logMAR preoperatively to 0.32 ± 0.48 logMAR postoperatively (P<.001). The mean ECD was 693 ± 172 cells/mm^2 and 611 ± 203 cells/mm^2, respectively (P=.001). The mean rate of endothelial cell loss was 11.5% ± 23.4%. Greater ECD loss was associated with a shorter axial length (AL) (<23.0 mm) and diabetes mellitus. Bullous keratopathy developed in 9 eyes (14.8%) and was associated with posterior capsule rupture.

CONCLUSIONS: The results suggest that modern techniques for cataract surgery provide excellent visual rehabilitation in many patients with a low preoperative ECD. Shorter AL, diabetes mellitus, and posterior capsule rupture were risk factors for greater ECD loss and bullous keratopathy.

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Corneal endothelial cell loss, a major complication that sometimes occurs after cataract surgery, can lead to corneal edema and decompensation if cell density falls to 500 cells/mm^2 or below. Causes of this complication are thought to include corneal distortion, ricocheting of nuclear fragments, intraocular lens (IOL) contact, and release of free radicals.[1–3] Bullous keratopathy is a vision-threatening complication, and pseudophakic bullous keratopathy is a leading indication for corneal graft surgery.[4] Although many procedures can reduce corneal endothelial damage, including the phaco-chop and soft-shell techniques,[5–8] there is often a reluctance to risk cataract surgery in cases in which the preoperative corneal endothelial cell density (ECD) is low, especially if it is below 1000 cells/mm^2.

To our knowledge, no large-scale studies have evaluated the outcomes of cataract surgery in eyes with a low preoperative corneal ECD. The aim of this study was to determine the outcomes of cataract surgery and which factors affect the prognosis in cases with a low preoperative corneal ECD.

PATIENTS AND METHODS

Patients with a clear cornea and an ECD less than 1000 cells/mm^2 preoperatively were identified from those who had cataract surgery at Tokyo Dental College between January 2006 and May 2010 and were included in this retrospective study. All patients provided written informed consent. This study was performed in accordance with the tenets of the Declaration of Helsinki. Approval was obtained from the ethics committee at the institution.

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Patients had slitlamp microscopy and Landolt corrected distance visual acuity (CDVA), fundus, intraocular pressure (IOP), and ECD testing before and after cataract surgery. Exclusion criteria included preoperative bullous keratopathy with obvious corneal edema, cataract surgery by a technique other than phacoemulsification, combined surgical procedures including penetrating keratoplasty and Descemet-stripping automated endothelial keratoplasty (DSAEK), and a follow-up of less than 6 months. Bullous keratopathy was defined as persistent corneal edema resulting from a decreased ECD.

The central corneal ECD was measured using the EM-3000 device (Tomey Corp.) before surgery and at each follow-up visit. The ECD at the final visit was taken as representing the patient's postoperative ECD. Cataracts were evaluated according to the Emery-Little classification.[9]

Surgical Technique

Phacoemulsification and aspiration were performed by 1 of 4 experienced surgeons (J.S., S.D., Y.S., T.Y.) using topical or sub-Tenon anesthesia of lidocaine 2%. A 2.75 mm clear corneal incision was placed superiorly or temporally. After instillation of sodium hyaluronate 1.0% (Opegan-Hi) and sodium hyaluronate 3.0%–chondroitin sulfate 4.0% (Viscoat) into the anterior chamber to stabilize the anterior chamber and protect endothelial cells (soft-shell technique), a continuous curvilinear capsulorhexis was created with a bent 27-gauge needle. After hydrodissection and standard endocapsular phacoemulsification of the nucleus was performed using the phaco-chop technique, the residual cortex was aspirated with a balanced salt solution using an Infiniti or Legacy phaco device (Alcon Laboratories, Inc.). The phaco device settings for the Infiniti device were bottle height, 60 to 65 cm; vacuum pressure, 250 mm Hg; flow rate, 25 mL/min; and longitudinal ultrasound power, 60%. The phaco device settings for the Legacy device were bottle height, 75 to 85 cm; vacuum pressure, 230 mm Hg; flow rate, 23 mL/min; and longitudinal ultrasound power, 60%. Viscoat was again placed in the anterior chamber to protect endothelial cells if remaining ophthalmic viscosurgical devices (OVD) were removed during the phacoemulsification. The lens capsule was inflated with a cohesive OVD, after which a foldable acrylic IOL was placed in the capsular bag. After IOL insertion, the OVD was thoroughly evacuated. The surgical protocols for each technique were standardized to reflect best practice by each surgeon.

Postoperative medications included levofloxacin (Cravit), diclofenac sodium 0.1% (Diclod), and betamethasone sodium phosphate 0.01% (Sanbetazon) 3 times a day for 1 to 2 months.

Main Outcome Measures

Two outcome measures were used to determine and analyze the change in the endothelial cell count after surgery. First was the proportional loss of cells, defined as the percentage reduction in cell count at the patient's final visit. The mean percentage cell loss was calculated in various subgroups. The second outcome measure was the incidence of bullous keratopathy, which was diagnosed by slitlamp examination. Based on the preoperative evaluation, the patients were divided into several groups according to ECD, the cause of the low ECD, and other factors, including age, sex, cataract grade, visual acuity, axial length (AL), systemic hypertension, and diabetes mellitus. The 2 outcome measures were compared between the groups.

Statistical Analysis

Differences in the percentage of endothelial cell loss were compared between the groups at every evaluation using the Mann-Whitney test, single-factor analysis of variance, or the Kruskal-Wallis test. The incidence of bullous keratopathy between the groups was evaluated using the Fisher exact test, chi-square for independence test, or Cochran-Armitage test. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using SSRI software (SSRI Co. Ltd.).

RESULTS

Demographics and Clinical Features

During the study period, 3558 patients had cataract surgery; in this group, 61 eyes of 52 patients had a preoperative ECD less than 1000 cells/mm^2. Table 1 shows the patients' demographics. The proportion of women was greater than that of men. The mean follow-up was 14.6 months ± 11.0 (SD) (range 6 to 43 months). The most frequent preoperative diagnosis or factor regarded as causing endothelial cell loss was Fuchs dystrophy followed by laser iridotomy and keratoplasty (Table 1).

Table 2 shows surgical outcomes in the 61 eyes. The improvement in CDVA and the decrease in IOP from before surgery to after surgery were statistically significant (both $P<.001$). The postoperative ECD was unobtainable in 15 eyes, mainly because of the presence of corneal edema, including bullous keratopathy. There was a statistically significant decrease in ECD postoperatively ($P=.001$). The mean rate of endothelial cell loss was 11.5% ± 23.4%, and 9 eyes (14.8%) developed bullous keratopathy. Figure 1 shows the survival curve for all patients.

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Table 1. Patient demographics.

Parameter	Value
Age (y)	
Mean ± SD	
Range	72.3 ± 9.8
Sex, n (%)	32 to 95
Male	12 (22.6)
Female	41 (77.4)
Mean preop AL (mm) ± SD	23.2 ± 2.0
Mean preop ACD (mm)	3.7 ± 1.5
Mean cataract grade* ± SD	2.8 ± 0.9
Cause of low ECD, n (%)	
Fuchs dystrophy	20 (32.8)
Laser iridotomy	16 (26.2)
Keratoplasty	10 (16.4)
Trabeculectomy	3 (4.9)
Trauma	3 (4.9)
Corneal endotheliitis	2 (3.3)
Other	10 (16.4)
Diabetes mellitus, n (%)	
Present	9 (14.8)
Absent	52 (85.2)
Hypertension, n (%)	
Present	23 (37.7)
Absent	38 (62.3)
Complication, n (%)	
Capsule rupture/vitreous loss	2 (3.3)
None	59 (96.7)

ACD = anterior chamber depth; AL = axial length; ECD = endothelial cell density
*Emery-Little classification

Table 2. Surgical results in all cases.

Parameter	Preop	Postop	*P* value
CDVA			
Mean LogMAR ± SD	0.59 ± 0.49	0.32 ± 0.48	<.001
Better than 20/20, n (%)	3 (4.9)	19 (31.1)	—
20/40–20/20, n (%)	17 (27.9)	27 (44.2)	—
Worse than 20/40, n (%)	41 (67.2)	15 (24.6)	<.001
Mean IOP (mm Hg) ± SD	13.1 ± 3.9	11.3 ± 3.8	<.001
Mean ECD (cells/mm^2) ± SD	693 ± 173	611 ± 203	.001

CDVA = corrected distance visual acuity; ECD = endothelial cell density; IOP = intraocular pressure

Clinical Characteristics and Surgical Outcomes

In Relation to Preoperative Epithelial Cell Density There were no statistically significant differences in clinical or surgical parameters between patients with a preoperative ECD less than 600 cells/mm^2, patients with a preoperative ECD between 600 cells/mm^2 and 800 cells/mm^2, and patients with a preoperative ECD between 800 cells/mm^2 and 1000 cells/mm^2 (Table 3). Although the rate of ECD loss was not significantly different between the 3 groups, the incidence of bullous keratopathy tended to be higher in the lower ECD groups (P=.066).

In Relation to Cause of Low Endothelial Cell Density There were no statistically significant differences in age, preoperative CDVA, IOP, prevalence of diabetes mellitus, or intraoperative complications between patients with Fuchs dystrophy, patients with laser iridotomy, and patients with keratoplasty (Table 4). The AL was significantly shorter in the laser iridotomy group (P=.008). The cataract grade was significantly lower in the keratoplasty group (P=.002). Hypertension was more common in the Fuchs dystrophy group (P=.007). The postoperative visual acuity was significantly better in the Fuchs dystrophy group than in the other groups (P=.043). Although the rate of ECD loss was not significantly different between the groups, the incidence of bullous keratopathy tended to be lower in the Fuchs dystrophy group. There were no cases of capsule rupture or vitreous loss in any of the 3 groups.

Other Risk Factors

Table 5 shows the rate of ECD loss and the incidence of bullous keratopathy in the various groups. The rate of ECD loss was significantly higher in the shorter AL group (P=.019) and in patients with diabetes mellitus (P=.049). All cases with posterior capsule rupture developed bullous keratopathy, and a statistically significant difference was observed between eyes with posterior capsule rupture and eyes without posterior capsule rupture (P=.020). No differences were observed with regard to other factors.

Keratoplasty for Bullous Keratopathy

Keratoplasty was performed in 4 eyes with bullous keratopathy (Table 6). Penetrating keratoplasty was

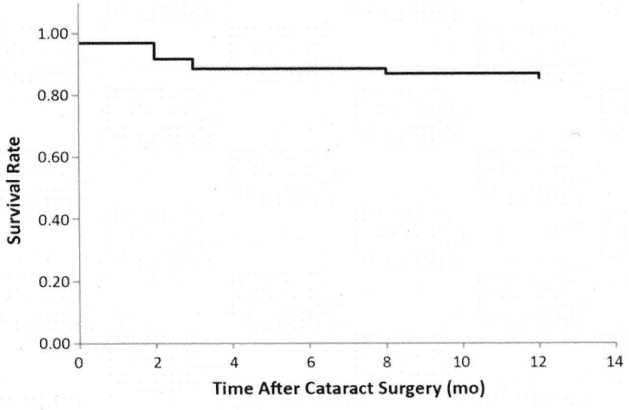

Figure 1. Survival curve of all patients having cataract surgery with low preoperative ECD.

Table 3. Characteristics and surgical results according to preoperative ECD.

Parameter	Preoperative ECD (Cells/mm^2)			*P* Value
	<600	600 to <800	800 to ≤1000	
Eyes (n)	18	26	17	
Mean age (y) ± SD	70.3 ± 5.4	71.9 ± 12.6	75.0 ± 8.5	.289
M/F sex (n)	3/15	3/22	6/11	.180
Mean preop CDVA (logMAR) ± SD	0.52 ± 0.36	0.64 ± 0.51	0.59 ± 0.58	.725
Mean preop IOP (mm Hg) ± SD	11.8 ± 3.8	13.5 ± 4.4	14.2 ± 2.8	.170
Mean preop ECD (cells/mm^2) ± SD	486 ± 91	700 ± 55	901 ± 66	<.001*
Mean AL (mm) ± SD	22.7 ± 1.3	23.8 ± 2.3	22.8 ± 2.1	.168
Mean cataract grade ± SD	2.94 ± 0.87	2.85 ± 0.92	2.53 ± 0.87	.361
Diabetes mellitus present (n)	4	3	2	.375
Hypertension present (n)	4	11	8	.120
Capsule rupture/vitreous loss (n)	1	1	0	.359
Mean postop CDVA (logMAR) ± SD	0.34 ± 0.59	0.32 ± 0.41	0.28 ± 0.48	.935
Mean postop IOP (mm Hg) ± SD	10.2 ± 2.9	11.8 ± 4.0	11.9 ± 4.1	.333
Mean endothelial cell loss (%) ± SD	4.4 ± 31.0	13.0 ± 17.8	16.2 ± 24.9	.449
Patients with bullous keratopathy (n)	5	3	1	.066

AL = axial length; CDVA = corrected distance visual acuity; ECD = endothelial cell density; IOP = intraocular pressure
*Statistically significant

performed in 2 eyes and DSAEK in 2 eyes. The CDVA improved in all cases after keratoplasty, and the grafts survived at the patients' last visits. The mean observation period after keratoplasty in the 4 eyes was 28.8 ± 6.3 months.

DISCUSSION

We evaluated the outcomes of cataract surgery in cases with a low preoperative ECD. Although outcomes and factors affecting endothelial cell loss after cataract surgery have been reported, little information is available on cases with a low preoperative ECD. This makes an accurate postoperative prognosis for cataract surgery difficult. Therefore, we believe that the results in this study will provide surgeons and patients with much needed information about endothelial cell loss, the incidence of bullous keratopathy, and prognostic factors—all of which may be useful in deciding the indications for and timing of surgery.

Endothelial cell density decreases after ophthalmic procedures such as cataract surgery,[10,11] vitreoretinal surgery in aphakic eyes[12,13] and glaucoma,[14,15]

Table 4. Characteristics and surgical results according to cause of low ECD.

Parameter	Fuchs Dystrophy	Laser Iridotomy	Keratoplasty	*P* Value
Eyes (n)	20	16	10	
Mean age (y) ± SD	72.3 ± 8.1	73.3 ± 6.3	66.8 ± 13.2	.416
M/F sex (n)	1/19	1/15	6/4	<.001*
Mean preop CDVA (logMAR) ± SD	0.37 ± 0.33	0.47 ± 0.26	0.66 ± 0.46	.105
Mean preop IOP (mm Hg) ± SD	12.1 ± 3.0	13.6 ± 4.1	13.0 ± 4.8	.492
Mean AL (mm) ± SD	23.1 ± 2.0	21.9 ± 0.7	24.1 ± 2.0	.008*
Mean preop ECD (cells/mm^2) ± SD	725 ± 143	608 ± 146	746 ± 243	.072
Mean cataract grade ± SD	2.9 ± 0.8	3.0 ± 0.8	1.9 ± 0.6	.002*
Diabetes mellitus present (n)	0	3	2	.115
Hypertension present (n)	12	2	2	.007*
Mean postop CDVA (logMAR) ± SD	0.10 ± 0.16	0.22 ± 0.32	0.51 ± 0.69	.043*
Mean postop IOP (mm Hg) ± SD	10.4 ± 2.3	11.3 ± 2.9	11.5 ± 5.9	.581
Mean endothelial cell loss (%) ± SD	13.5 ± 14.3	13.4 ± 33.2	3.1 ± 25.0	.310
Patients with bullous keratopathy (n)	0	3	2	.115

AL = axial length; CDVA = corrected distance visual acuity; ECD = endothelial cell density; IOP = intraocular pressure
*Statistically significant

Table 5. Risk factors for corneal endothelial cell loss and bullous keratopathy.

Subgroup	Eyes (n)	Mean Cell Loss (%)	*P* Value	Patients with BK	*P* Value
Age (y)			.748		1.000
≤69	19	11.0		3	
70–79	30	9.6		4	
80–90	12	16.8		2	
Sex			.414		.361
Male	12	19.6		3	
Female	49	9.6		6	
Preop CDVA (decimal)			.379		.475
≥20/50	30	14.2		3	
<20/50	32	8.6		6	
Axial length			.019*		.488
≤23.0 mm	33	19.4		6	
>23.0 mm	28	3.6		3	
Cataract grade			.228		.076
NS1–NS2	26	2.5		2	
NS3	22	15.7		3	
NS4–NS5	13	10.7		4	
Diabetes mellitus			.049*		.120
Absent	52	9.8		6	
Present	9	19.6		3	
Hypertension			.406		.462
Absent	38	10.2		7	
Present	23	14.0		2	
Capsule rupture/ vitreous loss					.020*
Present	2	—		2	
Absent	59	11.5		7	

BK = bullous keratopathy; CDVA = corrected distance visual acuity; NS = nuclear sclerosis
*Statistically significant

pterygium surgery,[16] and keratorefractive surgery with the use of mitomycin-C.[17] In cataract surgery, several preoperative and intraoperative parameters can influence the risk for ECD loss. A high nucleus grade, long phaco time, high ultrasound energy, exfoliation syndrome, diabetes mellitus, and a short AL are all associated with an increased risk for endothelial cell damage.[10,18–23] In this study, cases with a low preoperative ECD showed an association between greater ECD loss and shorter AL (<23.0 mm) or diabetes mellitus. In eyes with diabetes mellitus, morphologic abnormalities and a delay in the resolution of postoperative corneal edema have been reported.[24–26] In a study by Lee et al.,[27] patients with diabetes had an increased central corneal thickness and coefficient of variation and a decreased ECD and percentage of hexagonal cells than healthy controls after cataract surgery. The results in the present study suggest that in diabetic patients, corneal endothelial cells are susceptible to damage and careful attention must be paid in deciding the timing of the surgery and during cataract surgery, especially in cases with a low ECD. Our study also found posterior capsule rupture to be a risk factor for bullous keratopathy. Challenging conditions, such as a hard nucleus, a shallow anterior chamber, and intraoperative corneal edema due to a low preoperative ECD, can cause intraoperative complications. Attention must be given to posterior capsule rupture during surgery to avoid a further decrease in ECD and the development of postoperative bullous keratopathy.

In this study, the mean ECD loss was approximately 80 cells/mm^2 (11.5%). Recent studies evaluating eyes with a normal preoperative ECD[7,20,28,29] report an endothelial cell loss of 1.8% to 15.0% after phacoemulsification. Previous studies[20,29] also found that the preoperative cell count was not predictive of corneal endothelial cell loss in eyes with a normal ECD. The rate of ECD loss in our study was almost the same as that in earlier studies, although the endothelial cells in the eyes in our study may have been under greater stress due to preoperative conditions. This suggests that the relatively low ECD loss in our study was because the soft-shell and phaco-chop techniques were used to reduce intraoperative endothelial cell damage.

Table 6. Prognosis in patients with subsequent bullous keratopathy who had keratoplasty.

		Before Cataract Surgery				Before Keratoplasty		After Keratoplasty	
Age	Sex	Primary Disease or Procedure	VA	ECD	Type of Cataract Surgery	CDVA	Type of Keratoplasty	CDVA	FU (Mo)
71	M	Trabec; uveitis	0.40	352	Phaco + IOL	1.00	DSAEK	0.40	31
73	M	LI	0.82	574	Phaco + IOL	1.00	DSAEK	0.15	27
63	M	PKP	0.52	342	Phaco + IOL	2.30	PKP	0.52	36
72	F	Unknown	0.82	590	Phaco + anterior vitrectomy	0.40	PKP	0.10	21

CDVA = corrected distance visual acuity (logMAR); DSAEK = Descemet stripping automated endothelial keratoplasty; ECD = endothelial cell density; FU = follow-up; IOL = intraocular lens implantation; LI = laser iridotomy; PKP = penetrating keratoplasty; Phaco = phacoemulsification; Trabec = trabeculectomy

In this study, no eye in the Fuchs dystrophy group developed bullous keratopathy and the postoperative visual acuity in this group was significantly better than in eyes with laser iridotomy and eyes with keratoplasty. However, cataract surgery in eyes with Fuchs dystrophy and a low preoperative ECD remains a challenge. Seitzman[30] and Seitzman et al.[31] evaluated the clinical outcomes of cataract surgery in 136 Fuchs dystrophy cases; 5 cases had progression to keratoplasty within the first year postoperatively. Because Fuchs dystrophy is a progressive disorder, more comprehensive long-term evaluation is required in future studies.

With the recent advent of a new endothelial keratoplasty procedure, DSAEK,[32–34] the guidelines for the treatment of bullous keratopathy or cataract with a low preoperative ECD may have changed. However, it is still not known whether cataract surgery alone or a DSAEK triple procedure yields a better visual prognosis in patients with a low ECD. In our study, 75% of patients achieved a CDVA of better than 20/40. In earlier studies,[33–36] 55% to 80% of cases achieved that level of CDVA after DSAEK. Postoperative endothelial cell loss, which was 38% at 1 year in 1 study,[37] is a problem with DSAEK. Assuming no significant difference in visual outcome between cataract surgery and DSAEK, the results in our study suggest that cataract surgery with careful attention to preventing complications (eg, posterior capsule rupture) provides good visual rehabilitation in cases with a low preoperative ECD.

A limitation of this study was that we calculated the rate of ECD loss without taking into consideration the cases in which ECD was unobtainable due to bullous keratopathy. Therefore, we may have underestimated the loss of ECD. However, if the postoperative ECD in cases that developed bullous keratopathy was 400 cells/mm^2, the mean rate of ECD loss was 13.5% $\pm$ 23.6%, which is comparable to that in cases with a normal ECD. Other limitations were the follow-up period and that we did not evaluate intraoperative phaco time and the length of the corneal tunnel. Bullous keratopathy can occur several years after cataract surgery. Although we believe that the mean follow-up period in our study was sufficient to evaluate postoperative ECD loss and incidence of bullous keratopathy, we included patients with a follow-up of less than 12 months. In eyes with a shorter AL, IOLs with higher power might damage the corneal endothelial cells around the incision by direct contact with the cartridge or IOL. A longer observational prospective evaluation, including intraoperative ultrasound time or complications and postoperative ECD loss, would provide clinicians and patients with valuable information on ECD loss and the incidence of bullous keratopathy.

In conclusion, cataract surgery alone provided a safe and favorable surgical outcome in more than 85% of patients with a low preoperative ECD, although some of the patients required subsequent keratoplasty. A shorter AL, diabetes mellitus, and posterior capsule rupture were risk factors for greater ECD loss or incidence of bullous keratopathy. Although cataract surgery in eyes with a low preoperative ECD is a challenge, the rate of ECD loss in this study was almost the same as that in previous studies in eyes with a normal ECD.

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