Electron microscopic study of hereditary corneal edema

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Hereditary corneal edema is a previously undescribed corneal dystrophy thought to be transmitted as a dominant trait. This study presents five members of a family with hereditary corneal edema and describes the uniqueness of their condition from other corneal dystrophies. Emphasis is placed on electron microscopic studies of a corneal button from one member who had penetrating keratoplasty.

Keywords: heredity, corneal dystrophy, corneal edema, collagen fibrils, Descemet's membrane, corneal endothelium, histopathology, light microscopy, electron microscopy.

During the first year of life, diffuse corneal clouding is usually caused by infantile glaucoma or infections and inflammatory processes. Although several of the mucopolysaccharidases may also cause corneal clouding, other signs and symptoms predominate.

Maumenee described a congenital, hereditary corneal dystrophy characterized by diffuse corneal edema during the first year of life. His report is the only one which even partially resembles the hereditary corneal edema we observed in five members of one family. A general discussion of the particular characteristics of this condition and electron microscopic studies of a corneal button from one member who had penetrating keratoplasty comprise our report.

Case histories

This corneal disease first appeared in Patient I (Caucasian woman) and her twin (presumed monozygotic) sister (Patient II). Their parents have normal-appearing corneas with normal corneal thicknesses, and there is no history of corneal disease in either kinship.

Patient I developed corneal clouding and photophobia at the age of three. Her poor vision necessitated several surgical procedures, and at present she has a clear graft in her right eye.

Patient II has a mild asymptomatic case and has not required surgical treatment. Her best corrected vision is 20/25 Oxford unit (O.U.); corneal endothelial changes with overlying stromal edema are most marked in the periphery. However, all of Patient II's children (Patients III, IV, and V) have profound corneal disease. Patient II's husband has normal corneas with normal corneal thicknesses.

Patient III (boy) developed corneal clouding between three and six months of age. By one
Fig. 1. Pedigree of family with hereditary corneal edema. I1 and I1s Are twins.

Fig. 2. Vascularized, edematous cornea of left eye of K. T. The vascularization developed in four months and prompted keratoplasty.

year of age, his corneas were opaque (Corneal diameter normal). Surgery was deferred because of his age, and his corneas have slowly cleared. When last examined (age five years), his visual acuity was 10/30 O.U.; his corneas were thickened and edematous mainly peripherally.

Patient IV (girl) was asymptomatic when first examined at six months of age. However, peripheral corneal edema and thickening were easily distinguished with a hand light (central corneas were clear). Her ocular findings have remained stable for the past six months.

Patient V (girl) was noted to have bilateral diffuse corneal edema when she was 18 months old (Fig. 1). Four months later she developed photophobia and slight circumcorneal injection in both eyes. Examination revealed a roughened epithelium (pigskin appearance) and stromal edema (more marked peripherally). The anterior chambers were deep. Her left eye worsened and developed marked corneal vascularization inferiorly. The vessels were superficial and midstromal (Fig. 2). On July 1, 1969, she underwent a 7.5 mm. penetrating keratoplasty in her left eye. The anterior chamber, iris, and lens appeared normal at the time of the operation. Three weeks postoperatively her graft was clear, and photophobia had disappeared in this eye. At present she can see well enough to grasp small objects.

Materials and methods

The corneal button was obtained at the time of Patient V's surgery (7/1/69). It was fixed in two per cent osmium tetroxide with phosphate buffer at 4° C. for two hours. Then it was sectioned into smaller pieces, dehydrated through a series of ethyl alcohol solutions, and embedded in epon.

Thick (1μ) sections were stained with toluidine blue and studied simultaneously with the light
microscope for orientation in electron microscopy.

The embedded tissue was cut with a Porter-Blum microtome and stained with uranyl acetate and lead acetate. Uranyl acetate combined with phosphotungstic acid was used for the study of collagen fibrils. Electron micrographs were taken with an Hitachi 11-C microscope.

Results

Low-power electron microscopic examination of thick sections revealed a corneal epithelium with six to seven layers of cells (Fig. 3). The specimen showed distention of intercellular spaces, particularly at the level of the middle or basal cell layer. High-power examination of the corneal epithelium revealed desmosomes of normal appearance. Dense granular material and cell projections were seen within the intercellular spaces (Fig. 4).

In the basal cells some mitochondria were swollen, and there was a decreased density of the matrix with distension of the rough endoplasmic reticulum. Free ribosome particles were detached from their membranes, and there were moderate numbers of vesicles adjacent to the cell membrane. Basal cells with low density cytoplasm showed more advanced alterations of organelles. Both the basal cell membrane and the basement membrane appeared undulated with occasional small gaps between them. However, basal cells were well attached to the basement membrane by hemidesmosomes (Fig. 5).

Bowman's membrane was about 10 to 12 μ in thickness, but was absent in some areas and replaced by disorganized collagen fibrils. Extensive abnormalities were present in the corneal stroma, as well as the Descemet's membrane and the endothelium.

The anterior stroma showed irregular alignment of lamellae with vascularization and altered keratocytes (Fig. 6). A cross section of these collagen fibrils ranged between 250 and 350Å in diameter. The middle and posterior portion of the stroma was swollen. It possessed an irregular arrangement of collagen fibrils within the lamellae and disorganization of lamellae with the formation of several stromal "lakes" (Fig. 9). An interesting observation was the two different types of fibrils found in the middle and posterior stroma. The diameter of the smaller fibrils ranged from 100 to 130Å. These were found between the cell membrane and adjacent lamellae or between larger fibrils. Larger fibrils ranged between 250 and 350Å in diameter (Figs. 7 and 8) with a periodicity of 650 to 700Å.

Most keratocytes had swollen mitochondria, distended rough endoplasmic reticulum, and intracellular vacuoles with retracted cytoplasmic processes (Fig. 10).

Descemet's membrane was irregularly undulated and thinned (Fig. 11). In some areas it was a nonbanded homogeneous structure and in others it showed a non-uniform banded pattern. Between Descemet's membrane and the posterior layer there were numerous nonuniform collagen fibrils; fibrillar material approximately 80 to 100Å in diameter (Fig. 13); and several keratocytes (Fig. 12). This posterior fibrous layer was covered by two to three cell layers which replaced the endothelium. Many microvilli were seen on the posterior
Fig. 4. Distended intercellular spaces and many cytoplasmic projections. Fine dense granules (arrows) show in distended intercellular spaces. (×37,500.)

Fig. 5. Flattened basal cell with distended endoplasmic reticulum. Low density cytoplasm of basal cell appears with swollen mitochondria (M). Basement membrane (BM) is present. (St) Stroma; (N) nucleus. (×25,000.)
cell surface with gentle undulations. The intercellular spaces were narrow at all levels of the posterior layers and desmosomes attached to the plasma membrane (Fig. 14). Hemidesmosome-like figures (Fig. 13) were seen on the plasma membrane which faced the stroma. The posterior layer cells contained nucleus, mitochondria, rough endoplasmic reticulum, Golgi complex, several vacuoles, and numerous tonofilaments. These tonofilaments formed bundles and were concentrated near the cell membrane (Fig. 14).

Discussion

There have been several electron microscopic studies of various types of corneal dystrophy. Although hereditary corneal edema (HCE) is similar in many respects to the congenital hereditary corneal dystrophy (CHCD) studied by Maumenee and Kenyon, it seems different in important ways. The presenting signs and symptoms in our patients were variable as they were in CHCD, but in all cases, some degree of corneal edema was present. Patient V had
more corneal edema, especially in the periphery, than described in CHCD. Although she is much younger than Mau- menee's patient, the histologic differences are significant, and it is not known whether a patient's age would alter findings this much.

Ultrastructural examination showed abnormalities in all corneal layers, particularly the posterior. Outstanding morphologic

Fig. 7. Middle stroma of hereditary corneal edema. Two different diameters of fibrils show in cross section. Smaller fibrils range from 100 to 130 Å (arrows), large fibrils between 250 and 350 Å. (×70,000.)

Fig. 8. Posterior stroma of hereditary corneal edema. High dense amorphous substance is shown between the disoriented collagen fibrils. Smaller fibrils (arrow) can be seen between larger fibrils. (×70,000.)
Fig. 9. Low power posterior stroma shows lamellar disorganization and formation of "lakes" (L). (x28,000.)

Fig. 10. Keratocyte contains distended endoplasmic reticulum (ER) and swollen mitochondria (M). (G) Golgi complex. (x27,000.)
Figs. 11 to 13. For legends see opposite page.
Fig. 14. Note the thin Descemet's membrane (DM) at the upper portion. Many microvilli are seen on the posterior cell surface. The intercellular spaces are narrow with desmosomes (d). The numerous tonofilaments form bundles and are concentrated near cell membrane. (AC) Anterior chamber; (M) mitochondria; (N) nucleus; (TB) terminal bar. (Original magnification x18,000.)

Fig. 11. Thick section of posterior portion shows irregularly undulated, thinned Descemet's membrane (DM) and posterior cell layers (PL). (St) Stroma; (AC) anterior chamber. (Light microscope section. Toluidine blue. x250.)

Fig. 12. Low-power electron microphotograph of posterior portion shows thin descemet's membrane (DM) and two posterior cell layers (PL). (St) Stroma. (x4,400.)

Fig. 13. Fine fibrillar material (80 to 100Å in diameter, [arrows]) can be seen between Collagen fibrils and the posterior cell (PL). Hemidesmosome-like figures appear on the posterior cellular membrane (SD). (St) Stroma. (Original magnification x56,000.)
changes of the epithelium were enlargement of intercellular spaces, swollen mitochondria, and distension of the endoplasmic reticulum. In the chronically swollen epithelium, distended intercellular spaces indicate cell separation. However, desmosomes were present in our case and were morphologically similar to those found in normal corneas. 

Dense granules, which are observed in the intercellular spaces of swollen corneas, also appeared in the intercellular spaces of our specimens.

Absence of the basement membrane can often be observed in eyes with chronic epithelial edema, or corneal dystrophy. According to Goldman and Kuwabara, this alteration is secondary to basal cell pathology and the result of long-standing separation from the underlying tissue. Deficiency or thinning of Bowman's membrane was a rare finding in our case, and most of the specimens showed normal Bowman's membrane with a thickness of 10 to 12 μ. In Kenyon and Maumenee's case, Bowman's membrane varied in thickness and there were subepithelial bullae. As their patient was nine years old at the time of the operation, these changes could be due to the presence of edema for several years.

In our case, the middle or posterior stroma was swollen. It showed large fibrill-free areas or "lakes" (probably occupied by intercellular fluid), and irregularity of the interfibrillary spacing.

According to Jakus, normal collagen fibrils measure from 200 to 260A in diameter. Our specimen showed various diameter of collagen fibrils; smaller fibrils with ½ the normal diameter and larger fibrils with almost ¾ times the normal diameter. However, the periodicity was within the normal 650 to 700A. Kenyon and Maumenee's specimens showed fibrils up to 720A in diameter. In order to explain the increased diameter of these collagen fibrils, they suggested either that collagen fibrils of initially normal size swelled (becoming excessively hydrated) or that enlarged fibrils were caused by a defect in fibrillogenesis. In general, it is believed that hydration of corneal stroma is due to water imbibition by the polysaccharide matrix of interfibrillar space, with no increase in diameter of collagen fibrils. However, delicate filaments (90 to 100A in diameter), which appear in lattice dystrophy, may result from fibril breakdown (fibrillary degeneration). It seems most likely that the varied diameter of collagen fibrils in our case resulted from altered collagen fibrillogenesis during fetal development.

In stromal swelling following endothelial damage, keratocytes show contracted processes with rounding of the cell and its nucleus. However, there are little changes in the intracellular organelles even four days after endothelial damage. Although stromal cells in CHCD were normal, CHE cells had retracted processes, showed swollen mitochondria, distended endoplasmic reticulum, and vacuoles. Long periods of persistant stromal swelling (beginning in the embryonic stage) may have been responsible for these changes.

The marked difference between HCE and CHCD was in Descemet's membrane and the endothelium. In CHCD, Descemet's membrane was thinned and lacked the nonbanded homogeneous layer. In HCE, the nonbanded homogeneous material demonstrated undulation, thinning, and small areas showed banded material having no periodicity. Descemet's membrane (on the posterior layer) was invaded by numerous homogeneous processes. The area between Descemet's membrane and the posterior layer was filled with nonparallel-packed collagen fibers, fibril materials, and numbers of keratocytes. In contrast to HCE, Descemet's membrane in Fuch's dystrophy is thicker (caused by increased periodicity) and the endothelium is thinner than normal.

The endothelium was absent in Kenyon and Maumenee's case and may have been lost in operative or postoperative handling of the specimen rather than by endothelial degeneration. These authors
felt that a true endothelium was present during some period of embryonic development and may have been functionally defective. According to Barber, Descemet's membrane and endothelium begin to form soon after the anterior chamber appears and is fully formed by the 30 mm. stage. They increase in thickness and can be recognized at the 65 to 70 mm. stage. After the fifth month, except for an increase in size, very little change occurs in the cornea until after birth. The HCE specimen suggests that the endothelium was damaged during the formation of Descemet's membrane; its posterior layer being secondarily replaced by fibroblast-like cells or new, altered endothelial cells with a different function. This abnormal posterior layer presumably resulted in edema.

Mr. John Valenti provided expert technical assistance.

REFERENCES