Corneal thickness in nephropathic cystinosis

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SUMMARY Cystinosis is a rare autosomal recessive metabolic disorder in which non-protein cystine accumulates within cellular lysosomes owing to a defect in lysosomal cystine transport. The pathognomonic ocular manifestation of cystinosis is the deposition of distinctive iridescent crystals in the cornea, not associated with any inflammatory response or recognised change in corneal function. We measured corneal thickness in nine patients with infantile nephropathic cystinosis. We also studied a corneal button from one of these patients who underwent corneal transplantation. All nine patients had increased corneal thickness in comparison with an age matched control population. Electron microscopy analysis of the cystinotic button revealed structural changes of both epithelium and endothelium layers. Increased corneal thickness in patients with nephropathic cystinosis may reflect subclinical corneal oedema.

Cystinosis is an autosomal recessive metabolic disorder characterised by the accumulation of non-protein cystine within lysosomes of cells owing to a defect in lysosomal cystine transport.¹ It occurs in three phenotypical forms: a nephropathic childhood form, a benign adult form, and an intermediate adolescent form. The childhood form is the most common and the most devastating. Systemic complications include the renal Fanconi syndrome with rickets and growth retardation, and renal failure that necessitates transplantation by an average age of 10 years.² The adolescent and adult forms are associated with milder disease but share the pathognomonic ocular manifestation of cystinosis: distinctive iridescent crystals in the cornea and conjunctiva.³ The crystals are birefringent, intracellular, and of varying form. Needle shaped or fusiform crystals are seen in the cornea, whereas polymorphous, rectangular, or rhomboidal crystals infiltrate the conjunctiva.^{4,5} Ultrastructural analysis has shown these crystals to be intralysosomal.^{6,7} Crystallographic analysis has shown the rectangular forms to be composed of L-cystine.⁸ Patients with cystinosis commonly complain of photophobia and occasion-

ally develop corneal erosions.⁹ A pigmentary retinopathy affecting the periphery has been described in the nephropathic form of the disease¹⁰ but not in the benign form.¹¹ Crystals are also seen in the iris, ciliary body, choroid, retinal pigment epithelium, sclera, episclera, extraocular muscles, and optic nerve sheath.^{3-5,12}

The formation of corneal cystine crystals has a recognisable spatial as well as temporal sequence.¹³ Anterior deposition begins early in life and proceeds posteriorly as the patient ages; deposition advances more rapidly at the periphery.¹³ We measured corneal thickness in a group of patients with infantile-onset nephropathic cystinosis to determine whether the accumulation of corneal intralysosomal cystine is associated with recognisable changes. We also examined a corneal button from one of our patients undergoing corneal transplantation and correlated its ultrastructure with our clinical observations.

Subjects and methods

We studied nine patients between the ages of 3 and 28 (mean age=12.7 years) with infantile onset nephropathic cystinosis. Three were male and six female. Three were taking oral cysteamine (a free thiol with cystine-depleting capacity). Five of the nine had had successful renal transplantation and three of these

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were taking steroids. The patients had a complete neuro-ophthalmological examination by one of us (BK). All were essentially emmetropic and had abundant crystal deposition in their conjunctiva and cornea. Pupillary responses were normal, without evidence of afferent pupillary defect. Ophthalmoscopic examination revealed mild depigmentation of the peripheral retina in all the patients. The fundus in all of them was patchy and mottled from the mid equator to the ora serrata. The optic nerves were of normal colour, contour, and capillarity. No abnormality of nerve fibre layer could be detected.

A control group consisted of nine normal youngsters without corneal abnormalities or history of ocular disease. Their ages ranged from 3 to 16 years (mean 10.4 years). Four were male and five female. None of the subjects in either group were taking oral contraceptives or wore contact lenses—factors which may cause an increase in corneal thickness.¹⁴

We used a commercially available ultrasonic pachymeter (DGH-2000 Ultrasonic Pachymeter, DGH Technology Inc., Pennsylvania) to determine corneal thickness.¹⁵ The pachymeter has a 1.5 mm diameter solid probe tip and a transducer frequency of 20 mega hertz. The instrument has a measurement range of 0.20 to 1.3 mm, a resolution of 1 μ m, and an accuracy of ± 5 μ m.

All measurements were made by one of us (BK). The pachymeter probe was applied to the centre of the cornea after topical application of 0.5% proxymetacaine (proparacaine). Three measurements were recorded for each eye; the average of the three measurements was calculated and recorded.

We examined a corneal button obtained from patient 8, who (after our study) underwent corneal transplantation at age 20. The button was taken from the operating room and immediately fixed in half-strength Karnovsky's solution (2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2). The tissue was processed for transmission electron microscopy by post-fixation in 2.0% osmium tetroxide, and dehydrated through a series of graded concentrations of ethyl alcohol and in propylene oxide. The tissue was embedded in Polybed resin (Polysciences, Warrington, PA). Selected areas were examined by transmission electron microscopy. Sections 1 μ m thick were prepared for light microscopy by toluidine blue staining.

Results

The mean corneal thickness in the patients with cystinosis was 611.2 μ m ($n=18$, $SD=42.4$, $SE=10.0$) (Table 1). The mean corneal thickness of the normal subjects was 548.4 μ m ($n=18$, $SD=28.4$, $SE=6.7$)

Table 1 Corneal thickness in patients with nephropathic cystinosis

Patient no.	Age (years)	Central corneal thickness (μ m)	
		Right eye	Left eye
1	3	623	627
2	4	647	599
3	7	605	573
4	9	628	648
5	9	572	558
6	15	644	647
7	19	562	562
8	20	665	675
9	28	590	549
Mean (SD)	12.7 (8.4)	611.2 (42.4)	

Table 2 Corneal thickness in control group

Patient no.	Age (years)	Central corneal thickness (μ m)	
		Right eye	Left eye
1	3	555	589
2	6	576	575
3	8	552	557
4	9	547	556
5	11	517	504
6	13	559	544
7	13	563	571
8	15	564	558
9	16	494	491
Mean (SD)	10.4 (4.3)	548.4 (28.4)	

(Table 2). Corneal thickness was significantly higher in the cystinosis patients (Student's t test, $p<0.001$). Simple linear regression analysis showed poor correlation of the corneal thickness in cystinosis with patient age ($r=0.2$).

This increase in corneal thickness was not accompanied by obvious abnormality of epithelium or endothelium, nor was it reflected in decreased acuity or clinically recognisable oedema. It was not accompanied by raised intraocular pressure. No abnormality recognisable at the slit-lamp (other than stromal infiltration with crystalline material) could be seen.

Histological examination of the corneal button revealed an intact epithelium with normal Bowman's layer and Descemet's membrane. Basal cells of the epithelium showed intracellular oedema. Superficial keratocytes showed vacuolated cytoplasm. There was degeneration of the endothelium with focal cell loss. Ultrastructural examination of the cornea showed the presence of rectangular intracytoplasmic crystalline inclusions throughout the entire corneal stroma, within keratocytes. Deposition of crystalline inclusions was not associated with any recognisable inflammatory reaction or change of extracellular

Fig. 1 Electron microscopy of endothelial cell (E) reveals swollen mitochondria with disarray of cytoplasmic organelles. Legend: E=endothelial cell. B (arrow)=banded basement membrane. D=Descemet's membrane. N=nucleus of endothelial cell ($\times 4800$).

matrix. The endothelial cells showed disorganised organelles within their cytoplasm. Mitochondria were swollen and cristae were indistinct (Fig. 1).

Discussion

Corneal thickness varies little either between patients or between eyes of the same patient.¹⁶ The ultrasonic pachymetric determination of corneal thickness used in this study has been shown to be a highly reliable method which eliminates errors due to observer interpretation inherent in the optical measurement of corneal thickness.¹⁷ The mean corneal thickness of our control patients is similar to that found by previous investigators,^{18,19} who reported the average thickness of the normal human cornea (measured centrally) to be between 0.50 and 0.57 mm.

Corneal thickness depends on the water content of the cornea; there is a linear relationship between corneal thickness and hydration.¹⁹ For the human cornea Mishima found the relation between the two measurements to be defined by the equation: $H = 7.0q - 0.64$, where H is the hydration expressed in water weight per dry weight of tissue and q is the

corneal thickness in millimetres.¹⁹ The thickened cystinotic cornea may therefore reflect increased water content.

Absorption of water into the polysaccharide matrix of the cornea leads to increased corneal thickness. Yet the cornea swells only in a direction perpendicular to its plane of curvature, a result of the unique arrangement of collagen fibrils parallel to the plane of the corneal curvature.¹⁹ The expansion of the stroma along this corneal plane is restricted by the planar arrangement of the collagen fibrils; there are few fibrils running in the anterior to posterior direction to restrain swelling.

The maintenance of corneal hydration is dependent on metabolic activity within the corneal endothelium. Ouabain, an inhibitor of $\text{Na}^+ - \text{K}^+ - \text{ATPase}$ (an enzyme necessary for active transport), causes corneal swelling when applied directly to endothelium. Oxygen is necessary for the maintenance of corneal hydration,¹⁹ which indicates that the aerobic phase of the carbohydrate metabolism provides energy for the maintenance of the corneal thickness. Langham and Taylor found an inhibitory effect of 2,4 dinitrophenol on the fluid excretion of the cornea²⁰ and concluded that the dehydration of the cornea is

linked to oxidative phosphorylation. Since the corneal endothelium is rich in mitochondria, the site of the electron transport system, it is assumed that the active trichloroacetic acid cycle in the corneal endothelium supplies the energy necessary for the maintenance of normal corneal thickness.

The cornea swells when damaged. Increased corneal thickness may be an early sign of corneal dystrophy, keratitis, or trauma, when each interferes with corneal endothelial function; the degree of swelling generally parallels the severity of the underlying disease.¹⁹ Epithelial damage also results in swelling of the cornea, though it is less pronounced.

Patients with nephropathic cystinosis have excessive amounts of cystine in cellular lysosomes. Cystine is not known to be increased in collagen fibrils of the cornea, but it accumulates within keratocytes and epithelial cells.²¹ An osmotic gradient might result from increased cystine within the corneal stroma and account for some element of increased stromal hydration. The intralysosomal site of cystine deposition, however, would argue against the crystals themselves generating any change in tissue oncotic pressure. We think it more likely that the altered corneal thickness observed in our cystinotic patients is a reflection of subclinical dysfunction of epithelial and/or endothelial cells leading to the occurrence of clinically unrecognised stromal oedema. This hypothesis is supported by the ultrastructural changes we observed in the epithelial and endothelial layers of the cornea in our cystinosis patients. We speculate that increased amounts of cystine interfere with fluid transporting mechanisms of the corneal endothelium and epithelium, leading to altered water content of the cystinotic cornea. There is experimental support for striking similarities between transport related characteristics of the corneal endothelium and the kidney proximal tubule epithelium.²² The alterations of corneal physiology which we believe are caused by epithelial and endothelial dysfunction may be analogous to the disturbances in the proximal tubule epithelium that culminate in the Fanconi syndrome. Interference with active transport across epithelial surfaces may be the mechanism whereby accumulation of cystine causes both the renal Fanconi syndrome and increased water content of the cornea manifest as increased corneal thickness. Whether this shared epithelial anomaly and ensuing increase in corneal thickness occur in association with non-cystinotic causes of the Fanconi syndrome require further study; such studies are now in progress.

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