Phagocytosis of Polystyrene Spheres in the Rabbit Corneal Endothelium: Contribution of Lysosomal Enzymes to the Endothelial Degeneration

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The rabbit corneal endothelium phagocytized polystyrene spheres 0.5 μm in diameter. After phagocytizing spheres, the endothelium degenerated, and lost from the Descemet's membrane. Lysosomal enzyme activities of the endothelium-Descemet's membrane complex, such as acid phosphatase, β-glucuronidase and N-acetyl-β-D-glucosaminidase, were assayed and the total activities per microgram DNA were almost constant. The unsedimentable activities in the complex, however, increased by phagocytosis of polystyrene spheres, which indicated an extralysosomal release of lysosomal enzymes. Released lysosomal enzymes probably would have accounted for the degeneration of the corneal endothelium. Invest Ophthalmol Vis Sci 26:1631-1634, 1985

Although the cultured corneal endothelium has been reported to phagocytized polystyrene beads and take up low density lipoprotein, few reports describe endocytosis of the corneal endothelium. Saccharated iron and colloidal particles have been taken up by the endothelium of living rabbit corneas after the irrigation of those substances into the anterior chamber. Pigment granules have been degraded in the human corneal endothelium, and copper has been found in the corneal endothelium in Wilson's disease.

The corneal endothelium sometimes is degenerated by the uptake of saccharated iron or copper. Lyso-somal enzymes, which have very high activities in the corneal endothelium (unpublished data), digest phagocytized substance. It is, therefore, important to understand the changes of lysosomal enzyme activities and the intracellular distribution of those enzymes during phagocytosis to clarify the pathogenesis of corneal endothelial degeneration. The purpose of this paper is to describe phagocytosis of polystyrene beads by the rabbit corneal endothelium by electron microscopy and changes in the intracellular distribution of the lysosomal enzymes biochemically.

Materials and Methods. Polystyrene spheres were washed in three changes of the sterile balanced salt solution (BSS), pH 7.6, according to the method of Hollyfield and Ward. The concentration of polystyrene was adjusted to 10^11 particles per milliliter. Injection of polystyrene spheres: New Zealand white rabbits, weighing 2 to 3 kg each, were anesthetized with an intramuscular injection of ketamine hydrochloride. A 27-gauge needle inserted at the limbus was used to aspirate 200 μl of aqueous humor; the same amount of polystyrene sphere suspension was injected into the anterior chamber of the left eye at the point. The anterior chamber of the right eye received 200 μl of BSS as a control. This investigation adhered to the ARVO Resolution on the Use of Animals in Research.

Tissue preparation. At 20 min, 1 hr, 4 hr, and 24 hr after the injection of polystyrene spheres, the animals were killed by an injection of ketamine into the marginal ear vein, and the eyes were enucleated. All of the following procedures were carried out at about 4°C. For electron microscopic study, the excised cornea was fixed in 2.5% glutaraldehyde containing 0.1 M phosphate buffer, pH 7.4, for 2 hr. For biochemical study, the Descemet's membrane-endothelium complex of 8 mm in diameter was peeled from the stroma with a sharpened jeweler's forceps. The complex was put in 0.5 ml of 0.25 M sucrose solution and the homogenate was made using the same Potter-Elvehjem homogenizer for each experiment. A 0.1 ml aliquot was used to determine the DNA content. The remaining 0.4 ml was centrifuged at 20,000 g for 20 min; the supernatant was the unsedimentable fraction. The pellet was resuspended in 0.5 ml of 0.25 M sucrose solution, and that was the sedimented fraction, which contained most of the intact lysosomes. These unsedimentable and sedimented fractions were subjected to the lysosomal enzyme assays after being frozen and thawed six times. Total lysosomal enzyme activities of the homogenate were, therefore, obtained by adding the activities in the sedimented fraction and in the unsedimentable fraction.
Fig. I. Transmission electron micrograph of the corneal endothelium at 20 min after the injection of polystyrene sphere suspension into the anterior chamber. Polystyrene spheres (arrows) were phagocytized and surrounded by a membrane, indicating a phagosome. Vacuoles and swollen mitochondria were seen in the cytoplasm (x27,000).

Fig. 2. DNA content in the 8-mm diameter endothelium-Descemet's membrane complex. At each time period after injection of polystyrene sphere suspension into the anterior chamber, one eye was enucleated, and the 8-mm diameter endothelium-Descemet's complex was peeled and prepared for biochemical analysis as described in the text. DNA content was measured by the method of Kissane and Robins. Means and standard deviations of four separate experiments are shown.

Lysosomal enzyme assay: Acid phosphatase [E.C. 3.1.3.2] β-glucuronidase [E.C. 3.2.1.31] and N-acetyl-β-D-glucosaminidase [E.C. 3.2.1.30] were assayed in the manner described by Hayasaka and Shiono, using p-nitrophenyl derivatives as substrate.

Determination of DNA content: The DNA of Descemet's membrane-endothelium complex was fluorometrically assayed by the method of Kissane and Robins using diaminobenzoic acid. Calf thymus DNA was used as the standard. Specific lysosomal enzyme activities of the corneal endothelium-Descemet's membrane complex were expressed as total activities of the complex per microgram of DNA.

Histologic examination: The excised cornea was also examined with transmission electron microscopy (TEM). The tissue was postfixed in 1% osmium tetroxide and dehydrated in a series of graded ethanol. The tissue was embedded in epoxy resin (EPON 812), thin sectioned, stained with lead citrate-uranyl acetate, and examined by a JEOL-100c transmission electron microscope.
**Measurement of the intraocular pressure:** At 20, 40, 60, 120, 240 min and one day after the injection of spheres, intraocular tension was measured in the rabbits using the Perkins hand-held applanation tonometer, following local anesthesia with a drop of 4% oxybuprocaine hydrochloride. No restraint was used on the rabbits during the measurement.

**Results.** All values were determined as the means plus standard deviations of four different experiments. The rabbit corneal endothelium showed no remarkable changes after the injection of BSS. After 20-min exposure to polystyrene sphere suspension, the rabbit corneal endothelium phagocytized the spheres (Fig. 1). The sphere was surrounded by a membrane, which disclosed a phagosome. A few endothelial cells were degenerated and contained numerous vacuoles and swollen mitochondria. Cell membranes were disrupted and a large number of spheres penetrated into the cell after 60-min exposure to polystyrene sphere suspension. As the corneal endothelium was exposed to the spheres for a longer period, the extent of degenerated cells increased, resulting in direct exposure of Descemet’s membrane to the anterior chamber, caused by the large number of cells lost. Figure 2 shows the DNA content in the 8-mm diameter corneal endothelium-Descemet’s complex. The decrease of DNA indicated a loss of endothelium from the complex.

Specific activities of acid phosphatase, β-glucuronidase, and N-acetyl-β-D-glucosaminidase in the complex did not change during experiments (Fig. 3). These three lysosomal enzyme activities in the unsementable fraction, however, increased significantly (Fig. 4). The intraocular pressure, which rose to 13 Perkins tonometer readings 20 min after the injection of spheres, decreased to 10 Perkins tonometer readings 60 min after the injection.

**Discussion.** Under certain clinical situations the corneal endothelium has been induced to take pigment granules and copper. It is, therefore, clear that endocytosis occurs in the corneal endothelium in pathologic conditions, although the role of endocytosis in this tissue is not known in physiologic situation.

The corneal endothelial cells that take up copper or iron sometimes show vacuolation of their cytoplasm. In this study, the corneal endothelium also degenerated after the phagocytosis of polystyrene spheres. Although the intraocular pressure temporarily increased to 13 during the experiments, the pressure rise could not have caused the endothelial degeneration, because no changes were noticeable in the control corneal endothelium irrigated with BSS after maintaining the ocular pressure at 15 for 20 min.

Possibly, the degeneration of the corneal endothelium under the present conditions occurred as a result of changes in the intracellular distribution of the lysosomal enzyme though the mechanism of the extra-lysosomal release of lysosomal enzymes is still unclear. Unsementable activities of lysosomal enzymes in-
creased after the injection of polystyrene spheres, which indicated an extralysosomal release of lysosomal enzymes. Released lysosomal enzymes probably would have accounted for the degeneration of the corneal endothelium. Disturbances of the phagolysosomal system might account for the degeneration of the endothelial cells in some pathologic conditions.

Key words: cornea, endothelium, phagocytosis, polystyrene spheres, lysosomal enzymes

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References

Hydration Stability of Intracorneal Hydrogel Implants
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A hydrogel intracorneal lens for refractive keratoplasty must have predictable and stable optics when implanted in the corneal stroma. A series of experiments was performed to evaluate the hydrogel hydration stability when in the corneal stromal environment. Hydrogel ICLs of 54%, 63%, 66% and 71% water content showed no loss of hydration (by weight) after one week in the rabbit corneal stroma. In vitro experiments with hydrogel discs of 56%, 65%, 69.5% and 75% water content were subjected to swelling pressures ranging from 55 to 150 mmHg in a suction chamber. Only the hydrogel of 75% water content showed a significant loss of hydration at the physiologic swelling pressure of 55 mmHg. This study shows that hydrogel materials with up to 69.5% water content can be expected to be dimensionally stable when used in keratorefractive surgery. Invest Ophthalmol Vis Sci 26:1634–1636, 1985

Refractive keratoplasty with hydrogel lenticules has the potential advantage of utilizing predictable preoperative designed lenticules. Yet, McCarey and Andrews,1 observed that Permalens lenticules, when measured by pachymetry, were 28 ± 2% (n = 13) thinner in vivo than the preoperative measurement. This was further confirmed in preliminary investigations in monkeys. The thinning was considerable and thought to possibly influence the amount of refractive change by the hydrogel intracorneal lens (ICL). Klyce et al2 observed high water content hydrogel materials (93% and 95% water content) to have, respectively, a 26% and 29% thinning of their original thickness when exposed to the physiologic stromal swelling pressure. In this study we examined changes in the hydration of hydrogel ICLs of various water contents (55%–75% water) when subjected to normal stromal swelling pressure. The objective of this investigation was to establish if there is dimensional stability of the hydrogel lenticules used in experimental alloplastic keratorefractive surgery.

Materials and Methods. Water content and diameter of hydrogel intracorneal implants in rabbits: Hydrogel ICLs (Vistamarc®; Jacksonville, FL) with water contents of approximately 55%, 63%, 66%, and 70% were individually measured preoperatively for their wet weights and diameters. The wet weight was determined after the lenses were dehydrated at 60°C in a vacuum desiccator for 48 hr.

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