Effect of phenylephrine on normal and regenerated endothelial cells in cat cornea

Kenneth L. Cohen, Diane L. Van Horn, Henry F. Edelhauser, and Richard O. Schultz

Topical commercial phenylephrine HCl (Neo-Synephrine 10%) has been shown to cause an increase in corneal thickness and reversible vacuolization of corneal endothelial cells in rabbits. Using an in vivo model of regenerated corneal endothelial cells in the cat, we compared the cytotoxicity of phenylephrine-HCl 10% to regenerated and to normal, nonregenerated cells. Following removal of the epithelium, topical application of the drug causes the appearance of anterior and posterior bands of stromal edema and reversible vacuolization in both normal and regenerated endothelial cells. Phenylephrine was not more damaging to the regenerated cells. Polymorphonuclear leukocytes infiltrated between the regenerating cells 24 hr after treatment but did not appear to destroy them. Phenylephrine may therefore be implicated as a causative factor of corneal edema and postoperative inflammation.

Key words: phenylephrine, cornea, corneal edema, corneal endothelium, electron microscopy, endothelial regeneration, inflammation

New techniques in retina and vitreous surgery require maximal pupillary dilatation for prolonged periods of time. This may require repeated administration of mydriatic drugs to corneas with damaged or denuded epithelium. Clouding of the corneal stroma may occur, and Machemer has suggested that phenylephrine-HCl should not be used for vitreous because it causes endothelial haze.

Commercial phenylephrine HCl (Neo-Synephrine 10%) when applied topically to normal rabbit corneas (with the epithelium removed) causes corneal edema and reversible vacuolization of the corneal endothelium. The purpose of this study was to determine whether such vacuolization would occur in the endothelium of cat corneas, and if so, would it be more damaging in large, flat, regenerated cells. We have previously shown that regeneration of the corneal endothelium in cat (following destruction of the central endothelial cells by freezing) occurs by enlargement and migration of cells at the margin of the wound. The cells covering the wound are therefore similar to large cells seen in aged humans or in eyes that have undergone phacoemulsification or combined cataract extraction and intraocular lens insertion.

Materials and methods

Corneal freezing. Brass probes were machined to closely adapt to the corneal curvature of adult cats. Using a calculation of corneal surface area,
we selected an 8 mm diameter probe which would freeze circles 10 mm in diameter and would destroy 50% of the corneal endothelial cells. After an intramuscular injection of ketamine HCl (10 to 15 mg/kg) for sedation, several drops of benzoxinate hydrochloride 0.4% (Dorsacaine) were applied topically to the corneas. The probes were cooled in liquid nitrogen and placed centrally on the corneal surface for 15 sec. The corneal endothelial cells were then allowed to regenerate for 7 to 10 days.

Controls. Two drops of benzoxinate hydrochloride 0.4% were applied to each cornea, and the epithelium was removed by scraping with a Paufique knife. The corneas had been frozen 7 to 10 days previously in four eyes and had not been frozen in three eyes. The animals were sacrificed with an overdose of sodium pentobarbital, their eyes were enucleated, and corneas were fixed for electron microscopy at 1 hr after removal of the epithelium.

Experimental groups. Commercial phenylephrine HCl (Neo-Synephrine 10% in a sterile aqueous buffered vehicle, containing sodium phosphate, sodium biphosphate, and benzalkonium Cl 1:10,000, Winthrop Laboratories, New York, N. Y.) was administered topically (1 drop every 5 min, three times) to the following four experimental groups: eyes with nonfrozen corneas (three eyes with the corneal epithelium present and six eyes with the corneal epithelium removed) and eyes with previously frozen corneas (four eyes with the epithelium present and 24 eyes with the epithelium removed). In all groups except the last the animals were sacrificed, their eyes were enucleated, and corneas were fixed for electron microscopy at 1 hr after administration of the drops. In the last group, 16 eyes were obtained and fixed at 1 hr, two eyes at 6 hr, two eyes at 12 hr, and four eyes at 24 hr after treatment in order to determine whether any alterations produced were reversible. The normal cells in the periphery were compared to the regenerated cells in the center of each cornea.

Electron microscopy. The animals were sacrificed at the designated times with an overdose of sodium pentobarbital. The eyes were enucleated immediately, and the corneas were excised with a 1 mm scleral ring and placed in 2.7% phosphate-buffered glutaraldehyde. The specimens were postfixed in phosphate-buffered osmium tetroxide. For scanning electron microscopy (SEM), one half of each cornea was passed through a graded series of water-ethanol and ethanol-Freon 113 prior to critical-point drying from Freon 13 in a Denton DCP-1 critical-point dryer. The specimens were mounted on stubs with conductive paint, sputter-coated with carbon, gold, and palladium, and examined at 20 kv in an AMR 1000 scanning electron microscope. For transmission electron microscopy (TEM), the other half of each cornea was cut in wedge-shaped pieces which were embedded in a low-viscosity epoxy resin as previously described.10

Pachometry and biomicroscopy. Corneal thickness was measured with a pachometer on a Haag-Streit slit lamp before scraping the epithelium and at 1 hr after application of the first drop of phenylephrine or buffer in at least four eyes in each control and experimental group. In another
Fig. 3. Scanning electron micrograph of regenerated endothelium 10 days after transcorneal freezing. The epithelium was removed 1 hr before biopsy. The cells are larger than normal (compare to Fig. 1) and irregular in shape and size. The cell surfaces are usually smooth but some have blebs and microvilli-like structures. (x1000.)

Fig. 4. Transmission electron micrograph of same cornea. The regenerateed cells contain normal organelles and are thin and overlap each other. Collagen-like fibrils have been laid down posterior to Descemet's membrane. (x5400.)

Fig. 5. Scanning electron micrograph of endothelium of cornea with intact epithelium treated with neosynephrine. The endothelial cells do not appear to be altered by the drug. (x1400.)

Fig. 6. Transmission electron micrograph of same cornea. The endothelial cells appear normal except for some swelling of mitochondria and rough endoplasmic reticulum. (x7300.)

Results
Controls. Topical anesthesia and removal of the corneal epithelium from nonfrozen, adult cat corneas caused no electron microscopic alterations in the corneal endothelial cells. SEM of the endothelium showed regular, normal-sized polygonal cells with a few microvilli and serrated cell borders (Fig. 1). TEM revealed endothelial cells with normal intracellular organelles, overlapping cell borders, and intact apical junctional complexes (Fig. 2).

Corneas which were previously frozen had enlarged and irregularly shaped central regenerated endothelial cells when examined by SEM (Fig. 3). Gaps were present between cell borders, and numerous microvilli covered the posterior surfaces of some cells. TEM showed an abnormal fibrous layer between Descemet's membrane and the thin
regenerated cells (Fig. 4). The cell borders did not overlap normally, and apical junctional complexes were sometimes lacking.

**Experimental groups**

The effect of phenylephrine HCl 10% on nonregenerated endothelial cells. Topical administration of phenylephrine HCl 10% to nonfrozen cat corneas with epithelium resulted in minimal alterations of the corneal endothelial cells (Figs. 5 and 6). Some cells had large mitochondria and swollen-appearing profiles of rough endoplasmic reticulum, but this was true in controls as well. In contrast, after removal of the epithelium, phenylephrine HCl 10% produced vacuoles in the endothelial cells in all corneas in this group. SEM of the endothelial cells showed swelling and pitting of the cell surfaces.
(Fig. 7). TEM revealed that the pitting was due to the presence of extensive intracytoplasmic membrane and nonmembrane-bound vacuoles (Fig. 8). Except for occasional mitochondrial swelling, the intracellular organelles were usually normal in appearance. Similar vacuoles were present in the stromal cells.

The effect of phenylephrine HCl 10% on regenerated endothelial cells. No pitting or vacuolization of the regenerated endothelial cells was found in the corneas with the epithelium present. The regenerated cells were similar in appearance to those in untreated but previously frozen corneas. In contrast, extensive pitting of the posterior surfaces of the endothelial cells was seen by SEM in corneas which had had the epithelium removed (Fig. 9). TEM revealed the presence of membrane- and nonmembrane-bound intracytoplasmic vacuoles in the attenuated cytoplasm of the regenerated cells (Fig. 10). Except for occasional mitochondrial swelling, the intracellular organelles did not appear to be altered.

Reversibility. Pitting and vacuoles were
Fig. 13. Transmission electron micrograph of cytoplasmic vacuole containing electron-dense material and vesicles 12 hr after epithelial removal and drug treatment. Residual bodies are also seen in cytoplasm (arrow). (x14,000.)

Fig. 15. Transmission electron micrograph of regenerated cells in same cornea. Vacuoles are no longer present, but a PMN is seen beneath the endothelium. (x3700.)

Fig. 14. Scanning electron micrograph of regenerated endothelial cells 24 hr after epithelial removal and treatment. No pitting is seen. (x1000.)

Fig. 16. Scanning electron micrograph of regenerated endothelium in another cornea 24 hr after Neosynephrine treatment. The cornea had been frozen 7 days prior to treatment. Numerous inflammatory cells can be seen on and between the endothelial cells. (x700.)

still present in the normal, peripheral, and the central, regenerated cells 6 hr after epithelial removal and drug treatment (Figs. 11 and 12). Some of the vacuoles were beginning to accumulate electron-dense and vesicular material (Fig. 12). At 12 hr one cornea showed extensive pitting in both cell types, whereas the other had pitting only in regenerated cells. TEM revealed that vacuoles were present in some of the regenerated cells. Electron-dense and vesicular material was frequently present in the vacuoles, and residual-like bodies were present in the cytoplasm (Fig. 13). In contrast, cytoplasmic vacuoles were no longer present at 24 hr (Figs. 14 and 15). However, SEM and TEM showed polymorphonuclear leukocytes (PMNs) and possibly lymphocytes infiltrating but not destroying the regenerated endothelial cells after 24 hr (Figs. 15 and 16). No
PMNs were seen infiltrating the normal endothelium in the periphery of these corneas. Leukocytes were not observed infiltrating either normal or regenerated cells at 6 or 12 hr.

**Biomicroscopy and pachometry.** No biomicroscopic or corneal thickness changes occurred in three normal eyes in which 10% Neo-Synephrine was applied topically without removing the epithelium. Biomicroscopy of normal corneas treated with Neo-Synephrine after removal of the epithelium revealed the appearance of a narrow band of subepithelial edema and a marked band of edema in the posterior third of the stroma for the first 2 hr. After 4 hr the stroma appeared uniformly edematous. No distinct bands appeared in buffer-treated corneas, but generalized stromal edema was apparent by 4 hr.

Corneal thickness (mean ± S.E.M.) increased from 0.63 ± 0.02 mm before scraping the epithelium to 0.66 ± 0.06, 0.66 ± 0.04, 0.78 ± 0.04, and 0.88 ± 0.02 mm after 1, 2, 4, and 24 hours, respectively, in the four normal eyes which received buffer after scraping the epithelium. Corneal thickness increased from 0.63 ± 0.04 before scraping and 0.60 ± 0.06 mm 1 hr after scraping to 0.69 ± 0.02, 0.76 ± 0.02, and 0.84 ± 0.04 mm after 2, 4, and 24 hr, respectively, in the four normal eyes which received 10% Neo-Synephrine after scraping. There was no significant difference between the groups at any of the times.

In four eyes with previously frozen corneas, corneal thickness decreased from 1.05 ± 0.10 mm before scraping the epithelium to 0.79 ± 0.08 mm 1 hr after buffer application. Similarly, corneal thickness decreased from 1.05 ± 0.03 to 0.85 ± 0.05 mm in nine eyes 1 hr after treatment with Neo-Synephrine.

**Discussion**

Commercial phenylephrine HCl (Neo-Synephrine 10%), when topically applied to adult cat corneas with the epithelium removed, caused transient cytoplasmic vacuolization in the corneal endothelial cells within 1 hr after administration of the drug. These vacuoles formed equally in normal and in newly regenerated endothelial cells. The pitting seen with SEM was the result of the collapse of the cytoplasmic bridges over these vacuoles during processing. This vacuolization, however, did not disrupt the cell and did not appear to cause any permanent ultrastructural damage. Because vacuolization did not occur when the epithelium was present, one can conclude that the intact epithelium acted as a barrier to the drug.

Six hours after administration of the drug, the vacuoles began to accumulate electron-dense material, and multivesicular bodies and residual bodies were present at later times. The similarity in appearance of the vacuoles in the endothelial cells to multivesicular bodies and the presence of residual bodies may suggest that phenylephrine HCl and/or its breakdown products are phagocytosed by the corneal endothelial cells and incorporated into secondary lysosomes. Trump and Janigan have suggested that lysosomal enlargement may also be a part of the reaction of cells to injury. Little is known about the metabolism or cellular uptake of phenylephrine, but accumulation of norepinephrine has been demonstrated histochemically in smooth muscle cells by measuring fluorescence brightness of tissues incubated with norepinephrine. We speculate that phenylephrine is taken up by the stromal and endothelial cells, thus producing the transient vacuolization and possibly lysosomal activity.

After intravenous infusion of epinephrine in rabbit, similar vacuoles were also found in the endothelial cells of femoral mesenteric veins. These authors speculated that the vacuoles may be related to the release of plasminogen activator or antihemophile globulin. Thus, an alternative possibility is that the phenylephrine HCl may stimulate the release of a mediator. If so, this mediator may be responsible for the PMN response that was found in the 12 and 24 hr specimens.

The formation of the vacuoles does not appear to be related to the drug vehicle. Vac-
Vacuoles were found in rabbit corneal endothelial cells after topical application of phenylephrine HCl 2.7% in distilled water. In a previous study in rabbits, there was a significantly greater increase in corneal thickness during the first 2 hr in eyes in which phenylephrine (2.5% or 10%) was applied topically after scraping the epithelium than in eyes which received no treatment (or NaCl) after the epithelium was scraped. The cats in the present study were sedated with ketamine HCl and their lids remained open for about 2½ hr until the sedative wore off. This may explain why there was no difference between the rates of corneal swelling in treated and untreated normal eyes and a decrease in corneal thickness occurred in previously frozen eyes. In addition, the osmolarity of the phosphate buffer is less than that of the drug, and this may have contributed to the lack of a difference. Biomicroscopy, however, revealed the rapid appearance of localized anterior and posterior bands of stromal edema in the drug-treated cat eyes. These bands were not observed in the eyes which received buffer.

New techniques in anterior segment surgery cause a reduction in corneal endothelial cell density, and phenylephrine HCl has been noted to cause endothelial haze during vitreous surgery. It was the purpose of this investigation to determine whether enlarged, regenerated endothelial cells were more susceptible to the toxic effects of this drug. There was no difference in the response of the regenerated and the nonregenerated cells. Vacuolization did not destroy the cells and was reversible in both cell types. However, there was an inflammatory reaction in the 24 hr specimens in cat (48 hr specimens in rabbit). Therefore, phenylephrine HCl may be implicated not only as a causative factor of corneal edema but also as a contributing factor to postoperative inflammation and should be used with caution in eyes with compromised endothelium. Also, alternative mydriatic solutions should be used during surgical procedures in which perhaps accidental or intentional removal of the corneal epithelium may be anticipated.

REFERENCES