Wound Healing, Haze, and Urokinase Plasminogen Activator After Laser Vision Correction Surgery

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*P*hotorefractive surgery corrects the refractive error of the eye by directly changing the curvature of the front surface of the cornea. Some fraction of the patients who have laser vision correction surgery develop haze (cloudy cornea) after a few weeks to a few months during the wound healing period after surgery. Once haze develops, it can last from weeks to months, with some occurrences lasting more than a year. The condition can be mild to severe, with or without affecting visual acuity. We review our observations and experiments demonstrating that urokinase plasminogen activator ameliorates the occurrence of haze during the corneal wound healing process.

INTRODUCTION

The cornea is the eye's optical window through which humans see. It is a transparent, dome-shaped structure comprising the front of the eye (Fig. 1). The cornea, covered with its tear film, acts as the major refractive component of the eye, contributing approximately 75% of the eye's focusing power toward the convergence of an image onto the retina, with the remainder of the focusing coming mainly from the lens. The refractive power of the cornea derives from its index of refraction, curvature, and thickness.

The relationship between the axial length of the eye and the refractive power of the cornea and lens is balanced in patients with normal sight, a condition called emetropia (Fig. 2a). In this case, parallel light rays that enter the eye meet at a focal point on the retina and provide a clear image. If the axial length of the eye and the refractive power of the cornea and lens are mismatched, parallel incident light rays converge at a focal point in front of (anterior) or behind (posterior) the retina, corresponding to myopia (nearsightedness) or hyperopia (farsightedness) (Figs. 2b and 2c, respectively). Each eye could have one or another of these conditions. In addition, a curvature anomaly of the refractive media could exist (called astigmatism), where the cornea is more curved in one direction than in another. Astigmatism is characterized by parallel incident light rays that do not converge at a point but are drawn apart along a line, causing the appearance of a distorted image on the retina (Fig. 2d).

Refractive problems can be corrected by wearing eyeglasses or contact lenses. The basic optical lensing
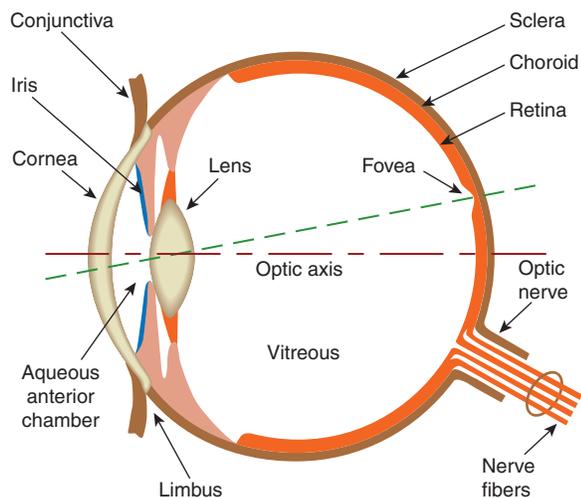
Figure 1. Anatomy of the eye.

correction concepts use negative and positive lenses for myopia and hyperopia, respectively. Correcting astigmatism can involve a combination of positive and negative lensing at appropriate angles to satisfy the individual need. External corrective lenses, either eyeglasses or contact lenses, are generally considered safe and effective.

As an alternative to corrective lenses, refractive surgery[1-4] has become a popular option. Refractive surgery modifies the curvature of the cornea in an attempt to achieve the proper focus of incident light rays on the retina. However, following refractive surgery, the cornea undergoes wound healing, occasionally with the occurrence of unwanted visual problems. An example is a transient loss of corneal transparency, termed haze[5-8] (e.g., see Fig. 3). Haze can occur weeks to months after surgery and can last for weeks, months, or even more than a year. Although haze is a temporary inconvenience, it can be bothersome. Generally, laser refractive surgery is a safe, effective, and predictable procedure, but if a complication occurs, it affects a previously completely healthy eye. Therefore adverse surgical effects are less acceptable than in other types of surgery.

This article describes our efforts at ameliorating the occurrence of haze. We have studied the activity of an enzyme, urokinase plasminogen activator (uPA), which is a normal component of tears.[9-11] During the wound healing process following refractive surgery, the activity of uPA in tears varies with time. After describing laser refractive surgery, we review some aspects of the wound healing process through our observations[12-14] of variations of uPA in tears and the correlation with post-surgical corneal haze.

LASER REFRACTIVE SURGERY

The cornea is organized in five basic layers (Fig. 4). The epithelium is the outermost region, making up
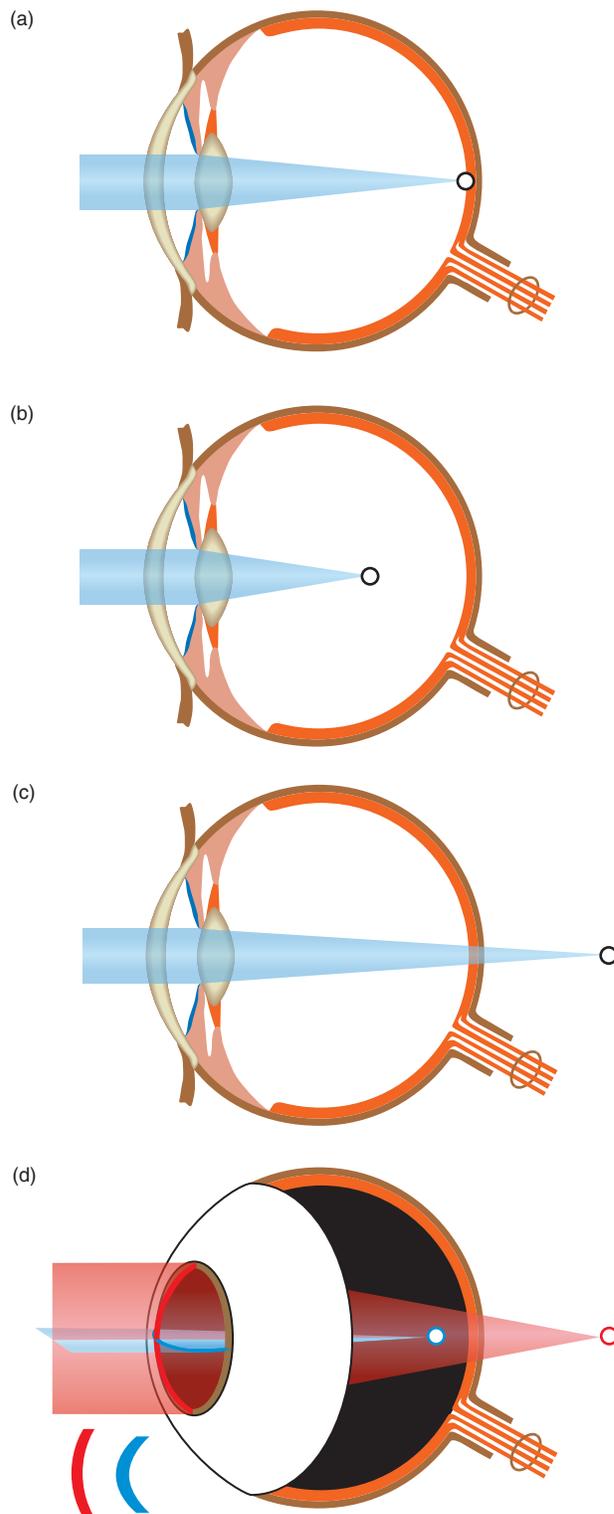
Figure 2. The focusing power of the eye in relation to ocular structure creates several standard conditions: (a) normal sight (emetropia), when parallel light rays entering the eye converge at a point on the retina; (b) nearsightedness (myopia), when convergence is in front of the retina; (c) farsightedness (hyperopia), when convergence is beyond the retina; and (d) astigmatism, when the cornea is not spherical and therefore convergence is not uniform from all incident planes.
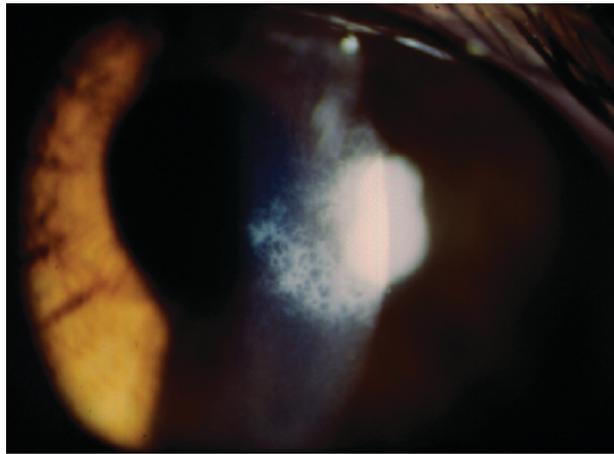
Figure 3. Photo of a patient's eye 3 months after photorefractive keratectomy (PRK) using slit-lamp illumination. The circular-like white central area is due to relections of light from the surface of the cornea. The white fuzzy or cloudy area in the circular band is haze or backscatter from the stromal layers of the cornea. Visual acuity was decreased by the haze in this case.

about 10% of the corneal thickness. Epithelial cells are regenerative and regulate the flow of oxygen and nutrients from the tears to the rest of the cornea. Bowman's layer lies below the epithelium and is composed of a thin layer of collagen fibers. The stroma is the next layer, comprising about 90% of the corneal thickness and giving the cornea its strength, elasticity, and form. The stroma consists of layers of parallel collagen fibrils that are surrounded by an optically homogeneous ground substance consisting of glycosaminoglycans, other proteins, salts, and water. Cells called keratocytes are interspersed between the layers of collagen fibrils. The next layer is Descemet's membrane and it is composed of a thin layer of collagen fibers (different from those of the stroma).
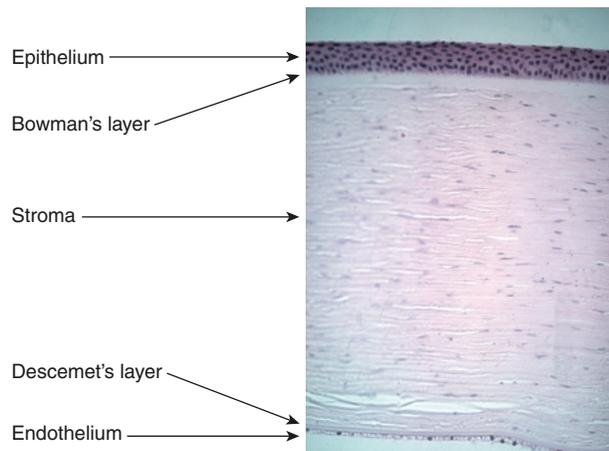
The innermost layer is the endothelium, a single layer of nonregenerative cells responsible for maintaining fluid balance in the cornea.

Laser refractive surgery uses incident argon fluoride excimer laser (UV wavelength = 193 nm) photon energy to break molecular bonds in corneal tissue and ablate the material. The ablated tissue consists mostly of collagen fibrils in the stromal layer of the cornea. By calculated ablative removal of stromal tissue, the laser energy reshapes the corneal surface (Fig. 5). When nearsightedness (myopia) is treated, the laser profile decreases the cornea's relative curvature, thus reducing its refractive power and causing it to focus farther back and onto the retina. When farsightedness (hyperopia) is treated, the laser profile increases the cornea's relative curvature and therefore its refractive power. Based on the new corneal profile, parallel light rays that enter the eye are expected to meet at a focal point on the retina and give a clear image.

Patients over age 40 commonly experience a hardening of the lens that creates a physiologic loss of accommodation (or focusing ability) called presbyopia. As the lens hardens, the accompanying gradual loss of accommodation causes the nearest point at which the eye can focus to recede, a condition sometimes described as the patient's arm becoming "too short for reading." Since presbyopia occurs from lens hardening, accommodation cannot be restored by laser surgery. However, the near point of focus can be brought closer to the eye by increasing the curvature of the cornea, but not without an effect on far distance visual acuity. Therefore a common ablating technique for ametropia (refractive error) combined with presbyopia is to correct one eye's corneal curvature for far distance and the other eye's corneal curvature for near distance. This combined technique is called monovision since it reduces the three-dimensionality of vision. Not everyone is comfortable with having monovision, and this needs to be checked by ophthalmic examination before surgery.

Figure 4. Structure of the cornea: laser vision correction surgery ablates a portion of the stroma posterior to the epithelium and Bowman's layer.
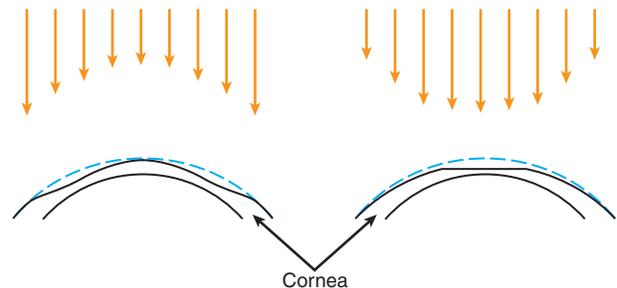
Figure 5. Laser correction concept. The laser beam reshapes the cornea by changing the curvature of the anterior surface. Left: The profile for hyperopia, which increases the cornea's relative curvature. Right: The profile for myopia, which decreases the cornea's relative curvature.

Here we describe three laser refractive surgical procedures that are currently being practiced, i.e., photorefractive keratectomy (PRK), laser *in situ* keratomileusis (LASIK), and laser-assisted subepithelial keratectomy (LASEK), and compare their outcomes.

Photorefractive Keratectomy

PRK is an excellent option for mild to moderate refractive corrections (myopic, hyperopic, and astigmatic), particularly for cases associated with thin corneas and recurrent erosions, or in people with a predisposition for trauma (military, etc.). In Europe, PRK remains the dominant choice in refractive surgery. The first step in the PRK procedure is to remove the epithelial layer of the cornea mechanically, chemically, or by laser (Fig. 6). Then photoablation with the excimer laser removes the Bowman membrane (Fig. 4) and a calculated amount of the anterior stromal layers. For instance, a central ablation depth of approximately 10 μm corrects 1 diopter of myopia with a 5.5-mm-dia. treatment area.[15] (A diopter is a unit of measure of the optical power of the eye.) Removal of the epithelial layer usually causes some discomfort within the first 48 h following the procedure. Wearing therapeutic contact lenses during the first few days sometimes solves this problem. Visual recovery follows re-epithelialization,[16] which takes 3 to 5 days. After surgery, vision improves over the next few days to weeks depending on the individual healing process. Possible complications include regression of the refractive correction (that is, a return toward the presurgical refractive condition) and visual problems such as haze and night vision disturbances.[5-8,17,18] Notwithstanding the possibility of complications, the long-term prognosis for PRK is generally considered excellent.[19,20]
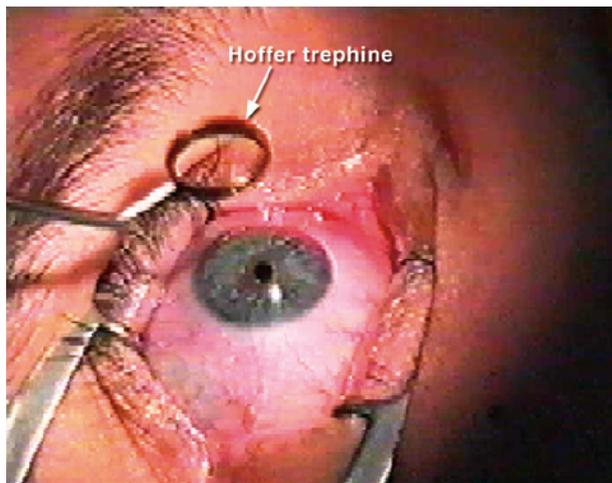
Figure 6. PRK surgery is performed under local anesthesia. A blunt keratome blade knife is used for de-epithelialization after epithelial marking with a Hoffer trephine.

LASIK

LASIK is currently the dominant procedure in refractive surgery in the United States.[21] The procedure involves a surgical cut through the stromal layer of the cornea using a microkeratome or a femtosecond laser (Fig. 7). This creates a hinged flap, typically 160 μm thick (a human hair is about 100 μm thick). The flap is lifted from the surface of the eye while remaining attached by its hinge, the excimer laser ablates a measured amount of stromal tissue, and the flap is subsequently replaced over the ablated surface. The main advantage of LASIK over PRK is increased comfort during the early post-operative period and rapid visual recovery. LASIK complications include a number of different flap-related issues.[2,22–24] Reported refractive complications are undercorrection, regression, astigmatism, and decentration, and visual problems include glare, halos, and haze.[25] LASIK recovery involves a long period of sensory denervation,[26] often leading to the complication of dry eye. The positive side is that visual recovery after LASIK is usually fast (a few days) and painless, although there can be some visual fluctuation during the early (few weeks) post-operative period.

LASEK

This procedure is a more recently introduced modification of PRK.[3,27,28] In the LASEK procedure, an epithelial flap is created using alcohol debridement, the flap is lifted from the eye surface, the excimer laser ablates the outer surface of the eye by a measured amount, and the flap is subsequently replaced after the ablation. The benefits, if any, of the creation of an epithelial flap compared to traditional PRK or LASIK are not fully understood. Post-surgical complications are similar to those found with PRK.[29] Advocates of LASEK claim that there is less discomfort in the early post-operative period compared to PRK and faster visual recovery. LASEK is suggested for patients who have thin corneas or corneal disease, are at risk of occupational damage to the eye, or are reluctant to have a LASIK flap.[3]

Refractive and Visual Outcomes

Refractive and visual outcomes are almost identical in patients having PRK-based and flap-based (LASIK and LASEK) procedures, especially in eyes with mild-to-moderate myopia.[4,20,23,29–32] In the majority of cases, the refractive outcome is within ±0.5 diopters of that intended.[23] With respect to post-surgical haze, there is wide variability in the reported prevalence.[2,4,33–35] In our own study, we found a 7.8% occurrence of mild haze following PRK.[12] Generally, haze after LASEK is thought to be similar to that found after PRK.[4,18–20,27–29] The incidence of haze after LASIK is considered to be less than with PRK, although similar levels of incidence are reported in the literature.[2,25,34,35] Although haze
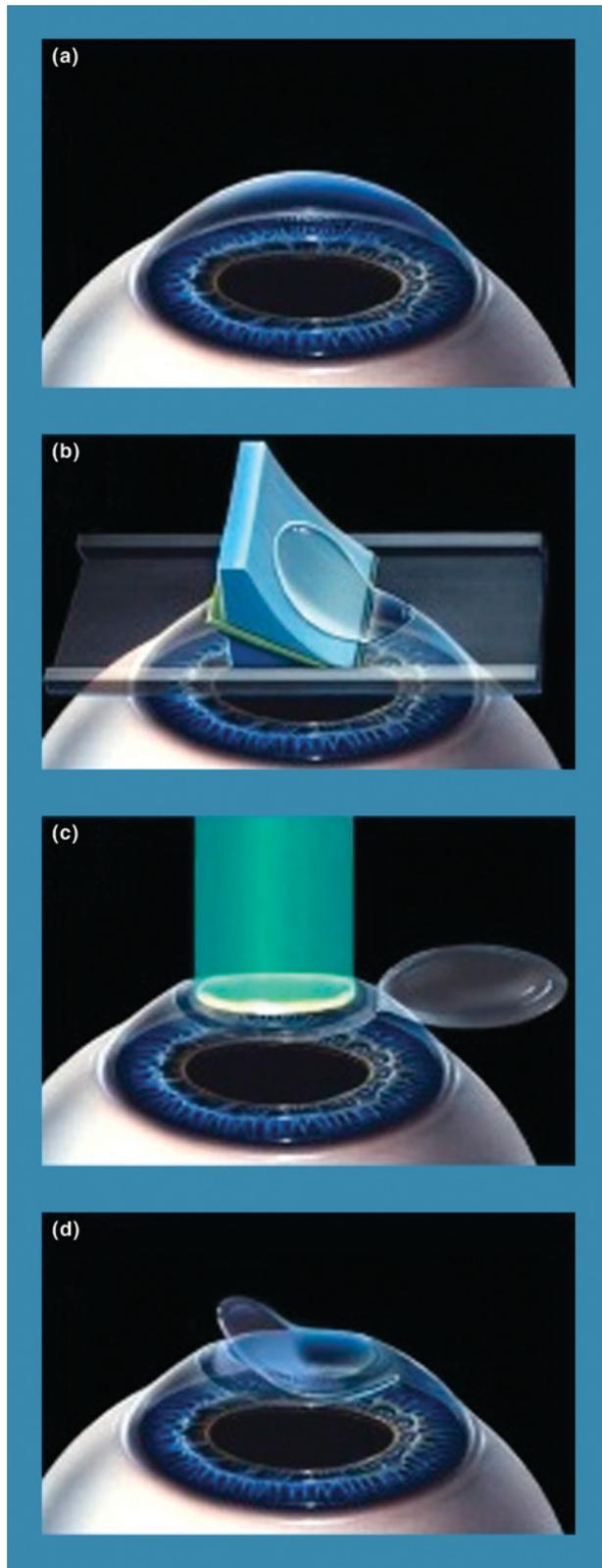
Figure 7. Schematic of LASIK surgery showing the eye (a) receiving a subepithelial cut into the stromal region of the cornea (b). When the flap is lifted and hinged out of the way, laser ablation can occur on the exposed stroma of the cornea (c), following which the flap can be repositioned over the ablated area (d).

occurs in only a fraction of the surgical cases, a therapeutic remedy to prevent or ameliorate its occurrence would be significant.

WOUND HEALING AND uPA

As a consequence of the ablation of corneal tissue, the surface of the cornea is changed; the new surface is composed of injured marginal and proliferating epithelial cells, stromal collagen fibers, extracellular matrix, and keratocytes. After PRK, the wounded cornea stays in contact with the tear film in the first 5 days until the re-epithelialization is completed. After LASIK and LASEK, the corneal flap is repositioned over the wounded cornea and tear fluid diffuses through the flap to the wounded stroma. Through this contact with the wounded surface, the biochemical composition of tears can play a role in the corneal wound healing process.

Wound healing after surgery is regulated by two major systems that are controlled by activators and inhibitors. The first system is the plasminogen activator-plasmin system, which is involved in the degradation and removal of damaged extracellular matrix.[36,37] The second system is the activated keratocyte system, which is involved in the replacement of damaged collagen by synthesizing new collagen and the matrix of glycosaminoglycans that surrounds the collagen fibrils.[9,38] In our work, we have examined the first wound healing system by measuring specific activities of plasminogen activators in tears following refractive surgery.[12,13]

Two types of plasminogen activators are well known: tissue-type plasminogen activator (tPA) and uPA.[39] The tPA does not appear to participate in the corneal wound healing process. The uPA has a pivotal role in extracellular degradation of proteins (proteolysis). In addition, uPA is found as a normal component of tear fluid and corneal cells.[9,11,12,40] If corneal epithelial cell damage occurs, the uPA activity in tears is increased. After corneal wounding, uPA participates in biochemical cascades, leading to both tissue destruction and repair.[12, 41–43] The uPA is a specific serine proteinase that converts inactive plasminogen into active plasmin (Fig. 8) through proteolytic cleavage of the Arg_{560}–Val_{561} bond in plasminogen.[39] Once formed, plasmin, among other substances, degrades fibronectin and laminin in the extracellular matrix, facilitating cell sliding and healing. Plasmin also activates latent procollagenase to collagenase, resulting in collagen molecule degradation. Inactivation of uPA occurs through reaction with plasminogen activator inhibitors.

OBSERVATIONS OF uPA IN POST-PRK TEARS

We conducted three successive studies of uPA in tears related to PRK surgery. In our first study,[12] we observed
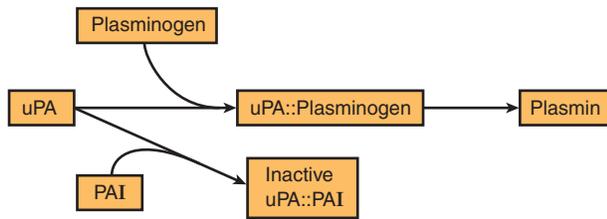
Figure 8. Enzymatic conversion by uPA of plasminogen to plasmin, regulated by plasminogen activator inhibitor (PAI).

patterns of uPA activity during the days following PRK surgery in humans that correlated elevated uPA activity with transparent corneas and a reduced uPA activity with development of haze. As a consequence, we performed our second study[13] using rabbits, which showed that use of a uPA inhibitor to reduce uPA activity after PRK caused haze. Finding that a pregnant rabbit had a low post-operative uPA activity level and developed haze, we chose the pregnant rabbit as an experimental model for our third study,[14] which showed that the pregnant rabbit was susceptible to post-surgical haze formation and that application of uPA eyedrops during the early post-operative period eliminated haze. See the boxed insert for details of the experimental methods and procedures.

EXPERIMENTAL DETAILS

Subjects

In the first study, 77 eyes of 42 human patients undergoing PRK surgery were selected after obtaining informed consent in adherence to the Declaration of Helsinki.[44] Both eyes of 32 healthy New Zealand rabbits, of which 8 were pregnant, were included in the next two studies. Animals were handled and treated in adherence to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.[45]

Surgeries

PRK treatments were essentially similar for the human and animal studies. Using the Schwind Keratom II ArF excimer laser (193 nm), the surgeries were performed by the same surgeon at Vital-Laser LLC, Department of Ophthalmology, University of Debrecen Medical and Health Science Center Faculty of Medicine. De-epithelialization was performed with a blunt keratome blade knife after epithelial marking with a Hoffer trephine (see Fig. 6). Epithelial anesthesia was induced using 0.4% oxybuprocaine hydrochloride eyedrops. General anesthesia was accomplished for the animals by intramuscular injection of ketamine-xylazine. The mean ablation depth of the human PRK surgeries was 48 μm (SD: 22). The animals were treated with an ablation depth of 68 μm.

Post-operative Treatment

The standard post-operative treatment included antibiotic eyedrops, Ciloxan (Alcon), hourly on the first post-operative day and 5 times daily during the next 5 days for each patient and animal. One eye of each of eight surgical rabbits (nonpregnant) received an additional drop of 10,000 KIU/mL aprotinin (Gordox, Richter Gedeon Rt., Budapest) (KIU/mL = kallikrein inactivator unit per milliliter, a measure of the activity of an enzyme inhibitor). The aprotinin was administered during the morning through early evening, 12 times (hourly) on day 1 and 5 times (every 2 h) on post-operative days 2–5 and 7. One eye of each of 24 surgical rabbits (where 8 were pregnant and 16 nonpregnant) was treated with one drop of the antibiotic containing an additional 50 IU/mL uPA (Ukidan, Serono SpA, Unterschleissheim, Germany) (IU/mL = international unit per milliliter, a measure of the concentration of an enzyme) from morning to early evening, 12 times (hourly) on day 1 and 5 times (every 2 h) on post-operative days 2–5 and 7. After the first 7-day period, all rabbits received Flucon (Alcon) and Tears Naturale (Alcon) 5 times daily during the first month, reduced to 4 times daily for the second month and to 3 times daily for the third month. No other treatment was used during this period. All patients and animals received follow-up examinations at 1, 3, and 6 months following the PRK procedure.

Tear Sampling

Tear sampling was performed using glass capillaries,[46] with at least 8 h elapsing after the previous administration of eyedrops to avoid tear sample dilution. Tear samples for uPA analyses were obtained immediately before and immediately after the PRK treatment and on the third and fifth post-operative days. Samples were centrifuged (1800 rpm) for 8–10 min right after sample collection, and supernatants were deep-frozen at −80°C and were thawed only once for measurements.

uPA Determination

The uPA activity in the tear samples was determined, as described by Shimada and coworkers,[47] with modifications according to Tőzsér and coworkers,[9] by a spectrophotometric method using human plasminogen and a plasmin-specific chromogenic peptide substrate, D-valyl-L-leucyl-L-lysine-p-nitroanilide (S-2251). Plasminogen and the S-2251 were purchased from Chromogenix (Môlndal, Sweden). The uPA standard was purchased from Choay (Paris, France). The uPA activity was calculated from absorption measurements at 405 nm using a Labsystem Multiscan MS spectrophotometer.

Clinical Evaluation and Analysis

To eliminate bias, determination of haze, as well as the correlation of uPA activity with haze, was made without knowledge of the uPA activity levels. The haze grading system of Hanna was adopted.[48] Standard statistical procedures[49] were used: t-test for means of correlated pairs and the Yates' correction of the chi-squared test for associations.
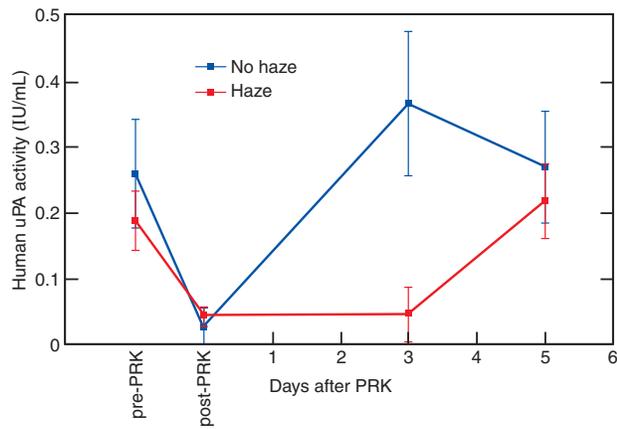
Figure 9. Observed mean (SD) uPA activity in human tears following PRK: 71 eyes with normal uPA activity pattern and no haze (blue curve); 6 with low uPA activity levels through the third day after PRK and subsequent haze (red curve). The difference in uPA activity between the two groups was statistically significant ($p < 0.0001$) on the third postoperative day.
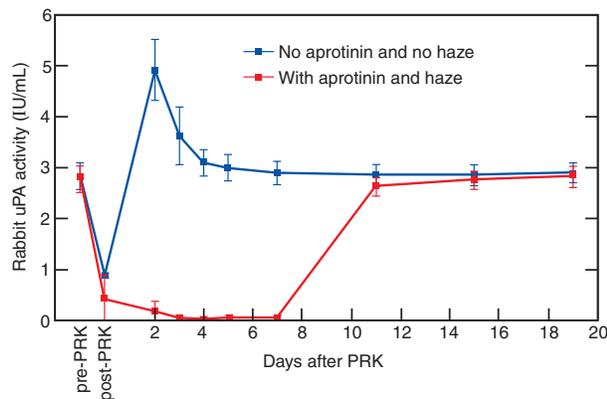
Figure 10. Observed mean (SD) uPA activity in rabbit tears following PRK: eight eyes with normal uPA activity pattern and no haze (blue curve) and eight eyes treated with aprotinin on days 2–7 with subsequent haze (red curve). The difference in uPA activity between the two groups was statistically significant ($p < 0.0001$) on days 2–7.

In our first experiment,[12] the tear uPA activity was lower immediately after PRK compared to pre-operative values (Fig. 9). For 71 (92.2%) human eyes with normal wound healing (clear corneas that remained clear), uPA activity was significantly elevated above the preoperative level on the third post-operative day and returned to the pre-operative level by the fifth day. In contrast, tear uPA activity remained low through the third post-operative day in the six (7.8%) human eyes that eventually developed haze after 3 to 6 months.

In our second study,[13] both eyes of eight New Zealand rabbits underwent PRK treatment, but one eye of each rabbit was treated with aprotinin, a serine protease

inhibitor, to suppress uPA over the first 7 days after PRK. The eight (100%) aprotinin-untreated rabbit eyes did not develop haze and demonstrated a uPA activity pattern over the first week that resembled the healthy human pattern (Fig. 10). For the eight (100%) contralateral rabbit eyes where uPA was suppressed with aprotinin, corneal haze developed after 2 to 3 months.

Serendipitously, we found that a pregnant rabbit exhibited a uPA deprivation in tears following PRK and later developed haze. Our third study[14] involved 8 pregnant and 16 nonpregnant rabbits that underwent PRK treatment on both eyes, where one eye of each rabbit was treated with uPA over the next 7 days. The uPA eyedrops prevented haze in all 24 (100%) uPA-treated rabbit eyes, while in the contralateral uPA-untreated eyes, 7 out of 8 (87.5%) pregnant and 2 out of 16 (12.5%) nonpregnant rabbit eyes developed haze (Fig. 11). Therefore, pregnancy was found to be a risk factor for post-PRK haze with an odds ratio of 49 compared to nonpregnant rabbits. That is, the pregnant rabbit was found to be 49 times more likely to develop haze than the nonpregnant rabbit. Moreover, uPA was found to be an effective prophylactic to prevent haze in our pregnant rabbit model.

DISCUSSION

The exact mechanisms underlying wound healing and complications after laser refractive surgery are not well understood.[50,51] With respect to post-surgical haze, there is no currently accepted treatment to prevent haze, although topical application of mitomycin C following surgery is commonly used to reduce it.[52–54] It is generally suspected that variations among individuals play a
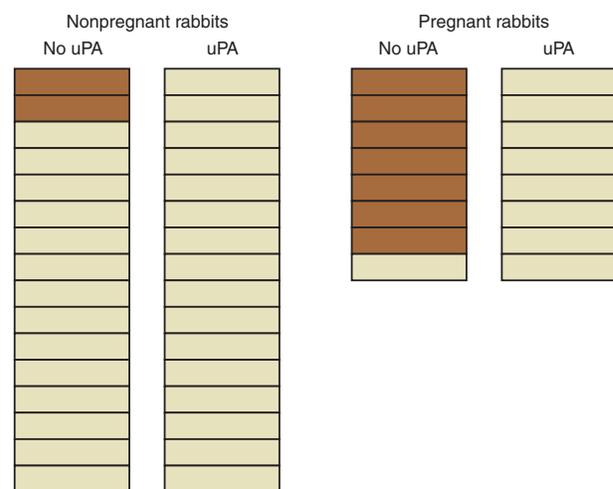
Figure 11. Diagram showing the effect of uPA eyedrops on pregnant rabbits: each rectangle represents an eye, either treated with uPA or not. Darker rectangles indicate an eye that developed haze, and lighter rectangles indicate an eye without haze.

pivotal role in the corneal wound healing response. Some of these variations are found in the biochemical components of tears, which play a role in the subsequent wound healing process following laser refractive surgery.

We have reviewed our observations of the correlation between the measured values of uPA activity in tears during the wound healing process after laser vision correction surgery and the subsequent absence or occurrence of subepithelial haze. In humans, uPA activity follows a pattern similar to that in Fig. 9. Elevated levels of uPA activity following refractive surgery correlate with transparent corneas. Low levels of uPA activity in tears, extending for a few days after the refractive surgery, are correlated with the later development of haze. Our experiments on rabbits show that haze could be induced by administering an inhibitor of the uPA after surgery (Fig. 10) and that haze could be eliminated by administering uPA to post-PRK eyes of pregnant rabbits, which are at high risk for post-PRK haze. Since having an elevated physiological level of uPA during the post-surgical period appears to help the wound healing process maintain a transparent cornea, providing uPA in the form of eyedrops is one way to compensate for the possibility of a depletion or inability to regenerate an adequate quantity of uPA in tears during that period. This suggests that uPA might serve as a therapeutic measure for reducing or preventing the occurrence of haze. Published results[55] indicate that uPA at the dose levels of interest here is benign when applied to the cornea. Since haze formation cannot be predicted, treating patients on a prophylactic basis would be prudent.

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