Urokinase-Type Plasminogen Activator to Prevent Haze after Photorefractive Keratectomy, and Pregnancy as a Risk Factor for Haze in Rabbits

Adrienne Csutak,[1] *David M. Silver,*[1,2] *József Tözsér,*[3] *Ziad Hassan,*[1] *and András Berta*[1]

Purpose. To observe the effect of urokinase-type plasminogen activator (uPA) on the development of subepithelial haze after photorefractive keratectomy (PRK) and to assess pregnancy as a risk factor for haze.

Methods. In 30 rabbits, both eyes of 27 were subjected to PRK and both eyes of 3 served as the nonsurgical control. In the first part of the experiment, for 7 days after PRK, three rabbits (one was pregnant) received aprotinin in one eye and no aprotinin in the contralateral eye. uPA activity was measured by a spectrophotometric method from tear samples in these eyes. In the second part, for 5 days after PRK, 24 rabbits (8 were pregnant) were treated with uPA in one eye and no uPA in the contralateral eye. Haze grading was performed according to the system of Hanna.

Results. In the first experiment, the aprotinin-treated eyes and the aprotinin-untreated eye of the pregnant rabbit showed development of haze. In the second, there were clear corneas in 24 of 24 uPA-treated eyes and in 15 of 24 uPA-untreated eyes. Post-PRK haze formed in 9 of 24 uPA-untreated eyes (7 of the 9 haze observations in pregnant rabbits). Within the uPA-untreated group, haze formed in corneas of 7 of 8 pregnant versus 2 of 16 nonpregnant rabbits. There was a strong association between the uPA treatment and clear corneas ($P = 0.003$) and between pregnancy and haze ($P = 0.002$).

Conclusions. The present results suggest that pregnancy is a risk factor for post-PRK haze in untreated rabbit eyes and that uPA is effective in preventing the occurrence of haze. (*Invest Ophthalmol Vis Sci.* 2004;45:1329–1333) DOI:10.1167/iovs.03-0881

Photorefractive keratectomy (PRK) involves removal of the corneal epithelium and application of an excimer laser to the Bowman layer and the anterior stroma. The resultant reshaping of the cornea alters its optical power (approximately 10 μm ablation depth corrects 1 D of myopia). Re-epithelialization of the cornea occurs usually within 3 to 5 days after surgery. Postsurgical complications of PRK include excessive myopic regression and disturbances in corneal transparency.

From the Departments of [1]Ophthalmology and [3]Biochemistry and Molecular Biology, University of Debrecen Medical and Health Science Center Faculty of Medicine, Debrecen, Hungary; and [2]Applied Physics Laboratory, The Johns Hopkins University, Laurel, Maryland.

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Corresponding author: David M. Silver, Johns Hopkins University, Applied Physics Laboratory, 11100 Johns Hopkins Road, Laurel, MD 20723-6099; david.m.silver@jhuapl.edu.

When post-PRK subepithelial corneal haze or cloudiness occurs, it is observed after a few weeks to a few months after surgery. Its duration can be from weeks to months, with some occurrences lasting more than a year.[1] It can be mild to severe.[2] Even in clear corneas, belated changes in the corneal stroma have been observed 8 to 43 months after PRK.[2] There is a wide variability in the reported prevalence of haze.[3,4] A recent study[5] found an 8% occurrence of mild haze after PRK. Another study found a 29% incidence among brown-eyed individuals and a 5% incidence among blue-eyed subjects.[6] Post-PRK haze is not correlated with the patient's age[7] but haze appears to be highest in the patient with high myopia who is undergoing PRK with a greater ablation depth.[8] The degree of patient satisfaction with PRK is negatively correlated with the degree of corneal haze.[9]

Urokinase-type plasminogen activator (uPA) is a serine protease originating in conjunctival and corneal epithelial cells[10–14] and is a normal component of tear fluid.[15] In previous work on humans,[5] levels of uPA activity in tear fluid were measured during corneal re-epithelialization after excimer laser PRK. Tear samples were collected with glass capillaries from 77 eyes of 42 human patients before and during the 5-day period after PRK. In 20 patients, the contralateral eye was similarly sampled as a control. The uPA activity in the tear samples was measured by a spectrophotometric method using human plasminogen and chromogenic peptide substrate S-2251. The tear uPA activity was lower immediately after PRK, compared with preoperative values. In 71 human eyes with normal wound healing (clear corneas), uPA activity was significantly elevated above the preoperative level on the third postoperative day and then returned to the preoperative level by the fifth postoperative day. In contrast, tear uPA activity remained low through the third postoperative day in the six human eyes in which haze eventually developed after 3 to 6 months. In previous human[16] and rabbit[17] studies, post-PRK corneal haze was found to be greater in eyes treated with aprotinin, a serine protease inhibitor.

Profound changes in the fibrinolytic system have been observed to occur during pregnancy, resulting from increased plasma concentrations of plasminogen activators and their inhibitors compared with nonpregnant levels.[18,19] The uPA inhibitory activity of pregnancy plasma continues from pregnancy through 1 to 2 days postpartum.[19,20] Natural alteration of the plasminogen activator system attending pregnancy suggests the possibility that pregnancy may be a risk factor for altering the plasminogen cascade in corneal wound healing. One purpose of the present work was to explore this possibility.

In the previous human[5] studies, low levels of uPA activity, extending a few days beyond the surgery, correlate with the later development of corneal healing abnormality (haze). This suggests the possibility that replacing (eye drops) uPA may serve as a therapeutic measure for reducing or preventing the occurrence of haze. A second purpose of the present work was to examine the effect on haze formation of providing uPA in

Table 1. Haze Grading and Time Progression at 1, 3, and 6 Month Follow-up Examinations after PRK

Name	Pregnant	Eyes (*n*)	Tear Sampling	1 mo	3 mo	6 mo
Aprotinin-treated	No	2	Yes	−	2	2
Aprotinin-treated	Yes	1	Yes	−	2	3
Aprotinin-untreated control (contralateral eyes)	No	2	Yes	−	−	−
Aprotinin-untreated control (contralateral eyes)	Yes	1	Yes	2	2	2
Non-surgical control	No	6	Yes	NA	NA	NA
uPA-treated	No	16	No	−	−	−
uPA-treated	Yes	8	No	−	−	−
uPA-untreated control (contralateral eyes)	No	14	No	−	−	−
uPA-untreated control (contralateral eyes)	No	2	No	+	1–2	1–0
uPA-untreated control (contralateral eyes)	Yes	1	No	−	−	−
uPA-untreated control (contralateral eyes)	Yes	7	No	+	1–2	0.5–1

−, Clear corneas; +, trace of haze; NA, not applicable.

the form of eye drops after PRK surgery in pregnant and nonpregnant rabbits.

Methods

Thirty healthy New Zealand rabbits (3.0–3.5kg) were included in the study. PRK surgery was performed on both eyes of 27 rabbits, of which 9 were pregnant. Three rabbits underwent no surgery and served as the control. The distribution of rabbit eyes into various groups is shown in Table 1. Animals were handled and treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

All PRK surgeries were identical: 6-D spherical correction with a 6.0-mm ablation zone and an ablation depth of 68 μm with an excimer laser (193 nm Keratom II ArF; Schwind, Kleinostheim, Germany), 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 6.0- to 6.5-mm Hoffer trephine. The epithelium was scraped gently from periphery to center. Residual epithelial debris was removed with a sterile microsponge. Topical anesthetic (0.4% oxybuprocaine hydrochloride) eye drops were administered twice before the surgery. General anesthesia was accomplished by intravenous injection of ketamine-xylazine at a ratio of 60 to 5 mg/kg. The PRK surgeries were performed on the morning of day 1.

The postoperative treatment of both eyes included antibiotic eye drops (Ciloxan; Alcon, Ft. Worth, TX), administered from morning to early evening, 12 times (hourly) on day 1 and 5 times (every 2 hours) on five additional days (postoperative days 2 to 5 and 7). After the seventh day, a corticosteroid (fluorometholone; Flucon; Alcon) and artificial tears (Tears Naturale; Alcon) were also administered from morning to early evening, five times daily (every 2 hours) during the first postoperative month, reduced to four times daily (every 3 hours) for the second month and to three times daily (every 4 hours) for the third month.

Six eyes of three rabbits underwent no surgery and were labeled nonsurgical control eyes. The six nonsurgical control eyes received antibiotic, corticosteroid, and artificial tears on the same schedule and with the same procedure as described for the surgical eyes. Because the antibiotic, corticosteroid, and artificial tears regimen is considered to be standard postoperative care for corneal surgery, these elements were not separately controlled for in this study.

One eye of each of three surgical rabbits received 1 drop of 10,000 kIU/mL aprotinin (Gordox, Richter Gedeon Rt., Budapest, Hungary). The aprotinin was administered during the morning through early evening, 12 times (hourly) on day 1 and 5 times (every 2 hours) on postoperative days 2 to 5 and 7. This was designated the aprotinin-treated group.

One eye of each of 24 surgical rabbits was treated with 1 drop of the antibiotic containing 50 IU/mL uPA (Ukidan, Serono SpA; Unterschleissheim, Germany) from morning to early evening, 12 times (hourly) on day 1 and 5 times (every 2 hours) on postoperative days 2 to 5 and 7. This was designated the uPA-treated group.

The three contralateral surgical eyes in the aprotinin-treated group (labeled aprotinin-untreated control) and the 24 contralateral surgical eyes in the uPA-treated group (labeled uPA-untreated control) were treated with the same antibiotic, corticosteroid, and artificial tear eye drop regimen after PRK, but without aprotinin or uPA, respectively.

For the three aprotinin-treated and three aprotinin-untreated control eyes, tear samples for uPA activity analyses were obtained on the day before PRK surgery (day 0). Tear samples were collected within minutes after PRK (day 1), before treatment with any eye drops. Daily, on postoperative days 2 to 5, on postoperative day 7, and every fourth day thereafter for 3 months, tears were sampled in the morning before the administration of eye drops on the given day. Tear samples were also collected from the nonsurgical control eyes on the same schedule and with the same procedure as described for the surgical eyes. Samples consisted of tears collected with glass capillaries[21,22] using intramuscular injection of pilocarpine hydrochloride (5 mg/kg) for stimulation. Tears were taken from the lower tear meniscus, and care was taken not to touch the conjunctiva. The same collection method was used throughout the study. The duration of the sampling time was recorded, and the secretion rate was calculated in microliters per minute, dividing the obtained tear volume by the time of sample collection. Samples used in this investigation had secretion rates of 10 to 50 μL/min. Samples were centrifuged (1800 rpm) for 8 to 10 minutes right after sample collection, and supernatants were deep frozen at −80°C and were thawed only once for measurements.

The uPA activity was measured in the tear samples by a spectrophotometric method using human plasminogen and a plasmin-specific chromogenic peptide substrate, D-valyl-L-leucyl-L-lysyl-*p*-nitroanilide (S-2251).[23] This assay is sensitive predominantly to uPA.[21] The plasminogen and the S-2251 were purchased from Chromogenix (Milan, Italy). The uPA standard was purchased from Choay (Paris, France). This assay is suitable for measuring plasmin activity but can also be used for determining uPA activity by adding plasminogen to the reagents. The uPA activity was measured as described by Shimada et al.[23] with the modifications reported by Csutak et al.[5] Tõzsér et al.,[21] and Tõzsér and Berta.[24]

All rabbits received follow-up examinations at 1, 3, and 6 months into the investigation, plus weekly assessment of corneal clarity and haze. Determination of haze was made using the grading system of Hanna.[25] The Yates correction for the χ^2 test for associations was used to analyze prognostic outcomes.[26] The animals were killed at 6 months, and histopathologic sections were prepared, to assess the epithelial and stromal thickness, cell density, and cell characterization.

Results

For comparison purposes, the range of haze grading assessments for the rabbits in each of the groups is summarized in

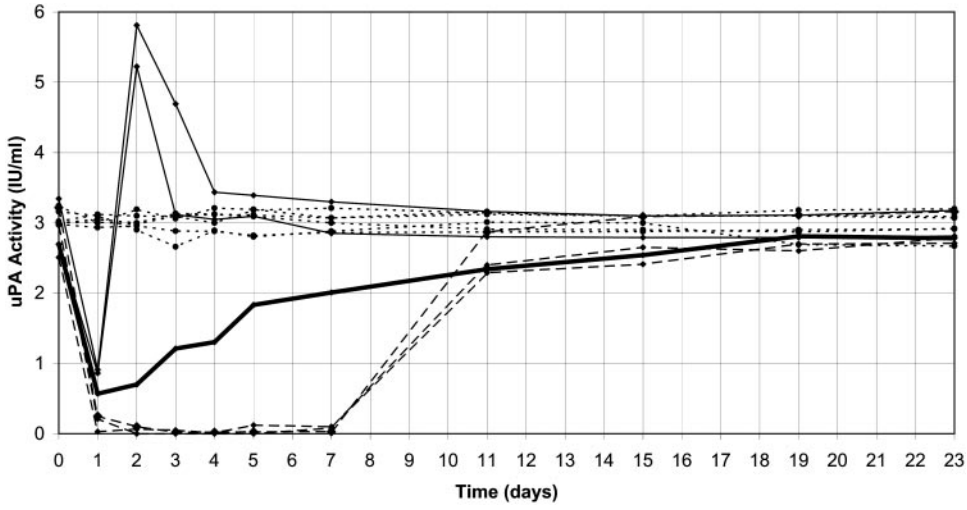

FIGURE 1. Time course of uPA activity measured in rabbit tears relative to PRK: three aprotinin-treated eyes (*dashed curves*), two nonpregnant aprotinin-untreated control eyes (*light solid curves*), one pregnant aprotinin-untreated eye (*heavy solid curve*), and six nonsurgical control eyes (*dotted curves*).

Table 1 corresponding to 1-, 3-, and 6-month follow-up examinations.

The time course of measured uPA activities is shown in Figure 1 for the aprotinin-treated, aprotinin-untreated control, and nonsurgical control eyes. Aprotinin inhibited uPA activity in the aprotinin-treated eyes over the 7 days of aprotinin administration. Two aprotinin-treated eyes (one from the pregnant rabbit) showed development of grade-2 haze by the third month and remained at this haze grade through the 6-month follow-up period. The third aprotinin-treated eye showed grade-2 haze by the third month, progressed to grade 3 by the fourth month, and remained at that level through month 6. Two of the aprotinin-untreated control eyes exhibited the expected normal uPA activity, shown in Figure 1, decreasing immediately after surgery, rising to an elevated level on the day after surgery, and then returning to presurgical values. The corneas of these two eyes remained clear (haze free) during the follow-up. The remaining aprotinin-untreated control eye followed a different pattern, shown in Figure 1, where the uPA activity declined immediately after surgery and remained low for several days after surgery until finally returning to presurgical values after almost 2 weeks. In this rabbit grade-2 haze developed by the first month and remained at grade 2 over the 6-month follow-up (the contralateral aprotinin-treated eye had grade-2 haze by month 3, remaining through month 6). This rabbit was pregnant. The nonsurgical control rabbit eyes underwent no surgery or aprotinin treatment and displayed essentially level uPA activities over the observation period, as shown in Figure 1.

As indicated in Table 1, there were clear corneas in 24 of 24 uPA-treated eyes (8 of the 24 were in pregnant rabbits) and in 15 of 24 uPA-untreated eyes (1 of the 15 was in a pregnant rabbit). Post-PRK haze (maximum of grade 1 to 2 at 3 months) formed in 9 of 24 uPA-untreated eyes (seven of the nine haze observations were in pregnant rabbits). Within the pregnant rabbit group, six eyes showed the appearance of haze by the first month, progressing to grade-2 haze by the third month, and receding to haze grade 0.5 to 1.0 by month 6. An additional pregnant rabbit began showing haze by the first month, progressed to grade 1.0 by month 3, and remained at that level through month 6. Regarding the two nonpregnant rabbits, haze also began to appear by the first month. One animal progressed to haze grade 2 by month 3 and receded to 0 by month 6, and the other progressed to haze grade 1 by month 3 and remained at that level through month 6. Epithelial interface debris but no haze was observed in 1 uPA-treated eye and in 1 uPA-untreated eye (neither rabbit was pregnant).

A summary of the post-PRK corneal conditions during the follow-up period is presented in Table 2 for both uPA-treated and -untreated groups, separately accounting for pregnant and nonpregnant rabbits. None of the uPA-treated eyes had haze, whereas haze developed in 38% of the contralateral uPA-untreated eyes. The results in Table 2 show a strong association between the use of uPA treatment and clear corneas ($P = 0.003$). In the uPA experiments, 7 of 8 pregnant rabbits versus 2 of 16 nonpregnant rabbits had haze. This represents a strong association between pregnancy and haze ($P = 0.002$). Moreover, these outcomes show an odds ratio[26] of 49 to 1 that pregnant rabbits had a greater tendency toward development of haze than nonpregnant rabbits.

The histopathologic sections revealed no difference between the uPA-treated and non–uPA-treated groups with respect to epithelial thickness, stromal thickness, and cell density. The character of basal epithelial cells and activated fibrocytes in the anterior stroma were indistinguishable between the uPA- and non–uPA-treated clear corneas (no haze). Among the non–uPA-treated eyes that had haze, more dark basal epithelial cells were identified, with a larger number of activated fibrocytes in the anterior stroma.

DISCUSSION

Previous analyses of rabbit tears have revealed uPA activity levels of 2.0 ± 0.6 IU/mL[27] and 4.0 ± 2.5 IU/mL[24] in the preoperative, normal, healthy animal. The equilibrium uPA activity levels in the present experiments were close to that range (approximately 3 IU/mL) as seen in Figure 1.

TABLE 2. Number of Pregnant and Nonpregnant Rabbits Having Haze or Clear Post-PRK Corneal Conditions and Percentage of All Eyes in the uPA-Treated and uPA-Untreated Groups

	8 Pregnant		16 Nonpregnant		24 Total	
	Haze	Clear	Haze	Clear	Haze	Clear
uPA-Treated	0	8	0	16	0%	100%
uPA-Untreated control (contralateral Eyes)	7	1	2	14	38%	62%

Interface debris has been found as an early postoperative complication after laser refractive surgery.[28] In the present study, epithelial interface debris was seen in both a uPA-untreated eye and a uPA-treated eye (in animals neither pregnant nor showing haze). The occurrence of interface debris in 2 of 54 of the surgical eyes in this study is interpreted to be independent of the uPA treatment.

Various substances have been considered as agents for the treatment of corneal haze, including steroids, nonsteroidal anti-inflammatory drugs, growth factors, basement membrane components, regulators of collagen structure, aldose reductase inhibitors, antioxidants, immunomodulators, antiallergics, and antimicrobials.[29] For instance, the synthetic inhibitor of metalloproteinase was reported to reduce corneal haze in rabbits by controlling the synthesis of type III collagen[30] and to reduce scar deposition in a rabbit model during glaucoma filtration surgery.[31] The topical application of β-methasone (a corticosteroid), acting as an antiinflammatory agent, decreased haze formation in rabbits but did not eliminate it.[32] Mitomycin C, an alkylating agent with antineoplastic and antibiotic activities, has been reported to reduce haze after PRK by suppressing keratocyte proliferation.[33–35] Elevated levels of transforming growth factor (TGF)-β have been found to correlate with stromal haze formation in humans,[36] rabbits,[37,38] and rats.[39] Topical interferon-α 2b in conjunction with dexamethasone, an adrenocortical steroid, reduces haze in rabbits,[40] but used alone in humans, interferon-α 2b appears to have a short-term benefit in patients who undergo a correction of 5.0 D or greater.[41] Hydrocortisone acetate with vitamin E was shown to reduce the wound-healing response in rabbits after PRK.[42]

The natural depression of uPA in the pregnant rabbit (Fig. 1) and the artificial inhibition of uPA activity in the aprotinin-treated eyes (Fig. 1 and Csutak et al.[17]) correlate with the occurrence of corneal haze after PRK. The pattern of depressed uPA activity in these eyes for several days after PRK mimics that in human eyes in which haze develops after PRK.[5] In contrast, post-PRK clear corneas follow a pattern of elevated uPA activity over that same period (Fig. 1 and Csutak et al.[5,17]). These experiments suggest a significant role for uPA in the post-PRK wound-healing process relative to haze formation.

The biochemical cascades leading to both tissue destruction and repair are triggered by uPA, which is responsible for the activation through proteolytic cleavage of plasminogen to plasmin. Plasmin 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, and participates in feedback to fibroblast cells inducing them to secrete more uPA.[43–45] However, uPA has been shown to have no direct effect on glycoprotein and collagen in basement membrane.[46] The influence of the plasminogen-plasmin-fibronectin-laminin-collagenase cascade on the wound-healing process is governed by the uPA available to initiate and continue these processes. The immediate conclusion is to provide uPA in the form of eye drops in situations in which a deficiency of uPA might otherwise lead to haze.

In the present uPA experiments, the uPA was administered at a concentration of 50 IU/mL, with a dosage of 1 drop per hour on day 1 and 1 drop every 2 hours on days 2 to 5. The intention was to use a higher than normal dosage, but well within a safe level. Doses as high as 2500 IU/mL have been shown to be nontoxic to the cornea in vitro.[14] Other experiments showed no meaningful swelling of rabbit corneas after in vitro perfusion of endothelium with uPA at concentrations of 1000, 2500, and 5000 IU/mL for 3 hours.[47] After these perfusions, scanning electron microscopy demonstrated normal endothelial mosaic.[47] Extrapolation of the rabbit data to humans led to the conclusion[47] that uPA could be used safely at concentrations of 2500 IU/mL to irrigate hyphemas from the anterior chamber of the eye without causing direct corneal damage. With this background, the concentration of uPA chosen in this study for therapeutic or prophylactic use appears to be reasonable.

The present results suggest that pregnancy is a risk factor for post-PRK haze in uPA-untreated rabbit eyes. For this reason, the pregnant rabbit is a useful animal model in which to assess the efficacy of haze-reduction therapies and procedures after PRK, without having to resort to extremely deep ablation depths to produce haze.

The absence of haze in the corneas of the uPA-treated group, whereas 38% of the uPA-untreated contralateral eyes had haze, suggests that uPA is effective in reducing the incidence of haze after PRK. In our previous work, we observed that uPA deficiency in the first few days after PRK surgery correlates with the development of haze in humans[5] and rabbits.[17] In the present work, we observed that administering uPA over the first few days after PRK to rabbits (pregnant) that are prone to haze correlated with absence of haze.

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References

1. Katlun T, Wiegand W. Haze and regression after photorefractive keratectomy (PRK). *Ophthalmologe*. 2000;97:487–490.
2. Bohnke M, Thaer A, Schipper I. Confocal microscopy reveals persisting stromal changes after myopic photorefractive keratectomy in zero haze corneas. *Br J Ophthalmol*. 1998;82:1393–1400.
3. Fisher EM, Ginsberg NE, Scher KS, Hersh PS. Photorefractive keratectomy for myopia with a 15 Hz repetition rate. *J Cataract Refract Surg*. 2000;26:303–304.
4. Siganos DS, Katsanevaki VJ, Pallikaris IG. Correlation of subepithelial haze and refractive regression 1 month after photorefractive keratectomy for myopia. *J Refract Surg*. 1999;15:338–342.
5. Csutak A, Tözsér J, Békési L, Hassan Z, Berta A, Silver DM. Plasminogen activator activity in tears after excimer laser photorefractive keratectomy. *Invest Ophthalmol Vis Sci*. 2000;41:3743–3747.
6. Tabbara KF, El-Sheikh HF, Sharara NA, Aabed B. Corneal haze among blue eyes and brown eyes after photorefractive keratectomy. *Ophthalmology*. 1999;106:2210–2215.
7. Hefetz LH, Domnitz Y, Haviv D, et al. Influence of patient age on refraction and corneal haze after photorefractive keratectomy. *Br J Ophthalmol*. 1997;81:637–638.
8. Møller-Pedersen T, Cavanagh HD, Petroll WM, Jester JV. Corneal haze development after PRK is regulated by volume of stromal tissue removal. *Cornea*. 1998;17:627–639.
9. Brunette I, Gresset J, Boivin JF, et al. Functional outcome and satisfaction after photorefractive keratectomy. Part 2: survey of 690 patients. *Ophthalmology*. 2000;107:1790–1796.
10. Thorig L, Wijngaards G, Van Haeringen NJ. Immunological characterization and possible origin of plasminogen activator in human tear fluid. *Ophthalmic Res*. 1983;15:268–276.
11. Hayashi K, Sueishi K. Fibrinolytic activity and species of plasminogen activator in human tears. *Exp Eye Res*. 1988;46:131–137.
12. Berta A, Tözsér J, Holly FJ. Determination of plasminogen activator activities in normal and pathological human tears: the significance of tear plasminogen activators in the inflammatory and traumatic lesions of the cornea and conjunctiva. *Acta Ophthalmol* (*Copenh*). 1990;68:508–514.
13. Tripathi RC, Tripathi BJ, Park JK. Localization of urokinase-type plasminogen activator in human eyes: an immunocytochemical study. *Exp Eye Res*. 1990;51:545–552.
14. Lohmann CP, Marshall J. Plasmin- and plasminogen-activator inhibitors after excimer laser photorefractive keratectomy: new con-

cept in prevention of postoperative myopic regression and haze. *Refract Corneal Surg*. 1993;9:300–302.
15. Barlati S, Marchina E, Quaranta CA, et al. Analysis of fibronectin, plasminogen activators and plasminogen in tear fluid as markers of corneal damage and repair. *Exp Eye Res*. 1990;51:1–9.
16. O'Brart DPS, Lohmann CP, Klonos G, et al. The effects of topical corticosteroids and plasmin inhibitors on refractive outcome, haze, and visual performance after photorefractive keratectomy. *Ophthalmology*. 1994;101:1565–1574.
17. Csutak A, Silver DM, Tözsér J, Facskó A, Berta A. Plasminogen activator activity and inhibition in rabbit tears after photorefractive keratectomy. *Exp Eye Res*. 2003;77:675–680.
18. Kruithof EK, Tran-Thang C, Gudinchet A, et al. Fibrinolysis in pregnancy: a study of plasminogen activator inhibitors. *Blood*. 1987;69:460–466.
19. Wright JG, Cooper P, Astedt B, et al. Fibrinolysis during normal human pregnancy: complex inter-relationships between plasma levels of tissue plasminogen activator and inhibitors and the euglobulin clot lysis time. *Br J Haematol*. 1988;69:253–258.
20. Walker JE, Gow L, Campbell DM, Ogston D. The inhibition by plasma of urokinase and tissue activator-induced fibrinolysis in pregnancy and the puerperium. *Thromb Haemost*. 1983;49:21–23.
21. Tözsér J, Berta A, Punyiczki M. Plasminogen activator activity and plasminogen independent amidolytic activity in tear fluid from healthy persons and patients with anterior segment inflammation. *Clin Chim Acta*. 1989;183:323–331.
22. van Haeringen NJ, Glasius E. The origin of some enzymes in tear fluid, determined by comparative investigations with two collection methods. *Exp Eye Res*. 1976;22:267–272.
23. Shimada H, Mori T, Takada A, et al. Use of chromogenic substrate S-2251 for determination of plasminogen activator in rat ovaries. *Thromb Haemost* (*Stuttgart*). 1981;46:507–510.
24. Tözsér J, Berta A. Urokinase-type plasminogen activator in rabbit tears: comparison with human tears. *Exp Eye Res*. 1990;51:33–37.
25. Hanna KD, Pouliquen YM, Savoldelli M, et al. Corneal wound healing in monkeys 18 months after excimer laser photorefractive keratectomy. *Refract Corneal Surg*. 1990;6:340–345.
26. Bland M. *An Introduction to Medical Statistics.* (2nd ed). Oxford, UK: Oxford University Press; 1995.
27. Van Setten GB, Salonen EM, Vaheri A, et al. Plasmin and plasminogen activator activities in tear fluid during corneal wound healing after anterior keratectomy. *Curr Eye Res*. 1989;8:1293–1298.
28. Vinciguerra P, Azzolini M, Radice P, Sborgia M, De Molfetta V. A method for examining surface and interface irregularities after photorefractive keratectomy and laser in situ keratomileusis: predictor of optical and functional outcomes. *J Refract Surg*. 1998; 14:S204–S206.
29. McDonald MB, Steinert RF, Bafna S. Laboratory and clinical studies of excimer laser refractive surgery. In: Wu HK, Thompson VM, Steinert RF, Hersh PS, Slade SG, eds. *Refractive Surgery.* New York: Thieme; 1999:225–246.
30. Chang JH, Kook MC, Lee JH, Chung H, Wee WR. Effects of synthetic inhibitor of metalloproteinase and cyclosporin A on corneal haze after excimer laser photorefractive keratectomy in rabbits. *Exp Eye Res*. 1998;66:389–396.
31. Wong TT, Mead AL, Khaw PT. Matrix metalloproteinase inhibition modulates postoperative scarring after experimental glaucoma filtering surgery. *Invest Ophthalmol Vis Sci*. 2003;44:1097–1103.
32. Kaji Y, Amano S, Oshika T, et al. Effect of anti-inflammatory agents on corneal wound-healing process after surface excimer laser keratectomy. *J Cataract Refract Surg*. 2000;26:426–431.
33. Talamo JH, Gollamundi S, Green WR, DeLaCruz Z, Filatov V, Stark WJ. Modulation of corneal wound healing after excimer laser keratomileusis using topical mitomycin C and steroids. *Arch Ophthalmol*. 1991;109:1141–1146.
34. Xu H, Liu S, Xia X, Huang P, Wang P, Wu X. Mitomycin C reduces haze formation in rabbits after excimer laser photorefractive keratectomy. *J Refract Surg*. 2001;17:342–349.
35. Carones F, Vigo L, Scandola E, Vacchini L. Evaluation of the prophylactic use of mitomycin-C to inhibit haze formation after photorefractive keratectomy. *J Cataract Refract Surg*. 2002;28: 2088–2095.
36. Lee JB, Choe CM, Kim HS, Seo KY, Seong GJ, Kim EK. Comparison of TGF-β_1 in tears following laser subepithelial keratomileusis and photorefractive keratectomy. *J Refract Surg*. 2002;18:130–134.
37. Kaji Y, Soya K, Amano S, Oshika T, Yamashita H. Relation between corneal haze and transforming growth factor-β_1 after photorefractive keratectomy and laser in situ keratomileusis. *J Cataract Refract Surg*. 2001;27:1840–1846.
38. Faktorovich EG, Badawi DY, Maloney RK, Ariyasu RG. Growth factor expression in corneal wound healing after excimer laser keratectomy. *Cornea*. 1999;18:580–588.
39. Chen C, Michelini-Norris B, Stevens S, et al. Measurement of mRNAs for TGFβ and extracellular matrix proteins in corneas of rats after PRK. *Invest Ophthalmol Vis Sci*. 2000;41:4108–4116.
40. Morlet N, Gillies MC, Crouch R, Maloof A. Effect of topical interferon-α 2b on corneal haze after excimer laser photorefractive keratectomy in rabbits. *Refract Corneal Surg*. 1993;9:443–451.
41. Gillies MC, Garrett SK, Shina SM, Morlet N, Taylor HR. Topical interferon α 2b for corneal haze after excimer laser photorefractive keratectomy. *J Cataract Refract Surg*. 1996;22:891–900.
42. Bilgihan K, Ozdek S, Ozogul C, Gurelik G, Bilgihan A, Hasanreisoglu B. Topical vitamin E and hydrocortisone acetate treatment after photorefractive keratectomy. *Eye*. 2000;14:231–237.
43. Berman M, Leary R, Gage J. Evidence for a role of the plasminogen activator–plasmin system in corneal ulceration. *Invest Ophthalmol Vis Sci*. 1980;19:1204–1221.
44. Berta A, Holly FJ, Tözsér J, Holly TF. Tear plasminogen activators: indicators of epithelial cell destruction—the effect of scraping, n-heptanol debridement, and alkali burn of the cornea on the plasminogen activator activity of rabbit tears. *Int Ophthalmol*. 1991;15:363–369.
45. Berman MB. Regulation of corneal fibroblast MMP-1 collagenase secretion by plasmin. *Cornea*. 1993;12:420–432.
46. Liotta LA, Goldfarb RH, Brundage R, Siegal GP, Terranova V, Garbisa S. Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. *Cancer Res*. 1981;41:4629–4636.
47. Hull DS, Green K. Effect of urokinase on corneal endothelium. *Arch Ophthalmol*. 1980;98:1285–1286.