Effect of Hyaluronic Acid on Corneal Haze in a Photorefractive Keratectomy Experimental Model

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ABSTRACT

PURPOSE: To evaluate the ability of topical hyaluronic acid to decrease corneal opacity after excimer laser photorefractive keratectomy (PRK) in hens.

METHODS: Twenty-four white hens underwent bilateral 193-nm excimer laser PRK to correct -9.00 D of myopia. One eye received postoperative treatment with topical 1% hyaluronic acid six times daily for 3 days; the other eye received phosphate buffered saline. Slit-lamp evaluation by a masked observer was performed for 6 months after PRK, and electron microscopy was carried out at the end of the study.

RESULTS: There were no significant differences in postoperative haze between the eyes treated with hyaluronic acid and those treated with phosphate buffered saline.

CONCLUSION: Topical administration of hyaluronic acid had no effect on the development of corneal haze following PRK in hens. [J Refract Surg 2001;17:549-554]

Excimer laser photorefractive keratectomy (PRK) achieves good visual results in the treatment of myopia, hyperopia, and astigmatism, especially in patients with mild to moderate myopia. However, development of corneal opacity and refractive regression are still the main obstacles, especially when higher corrections are attempted.1,2

Although most series report only mild to no haze at the final postoperative examination, it has been estimated that corneal scarring after PRK reduces visual acuity by two or more lines in about 1% of cases.3 This phenomenon may be related to cellular activity, collagen synthesis, and epithelial hyperplasia. Therefore, there is a considerable interest in pharmacologic modification of these healing responses.4,5

Research has been directed toward controlling postoperative development of haze with drugs such as corticosteroids, nonsteroidal anti-inflammatory agents, mitomycin, idoxuridine, topical interleukin alpha 2b, and others.6-17

Hyaluronic acid, a glycosaminoglycan capable of binding considerable amounts of water, is present in trace amounts in the corneal endothelium, but it is not normally found in the corneal epithelium or stroma.18-21 Endogenous hyaluronan production is seen in the entire spectrum of corneal disorders.18,19,22 and it is thought to play an important role in development, wound healing, and inflammation.20,23-25 It has been reported that hyaluronic acid is reactively formed in the wound area following excimer laser photoablation,6,18,21,26,27 radial keratotomy,28 during healing after extracapsular lens extraction,18,28,29, and after corneal alkali burn.18,29 Moreover, topical administration of hyaluronic acid has been shown to facilitate the healing process.22,23,30,31 Reports have also suggested that hyaluronic acid decreases the inflammatory response following skin incisions in rabbits.32

This study explored whether the topical application of hyaluronic acid has any effect on the development of corneal haze after PRK in an experimental hen model.

MATERIALS AND METHODS

Twenty-four white hens (weight, approximately 1.3 kg) were used in the study. All animals were treated in accordance with the Association for Research in Vision and Ophthalmology (ARVO) regulations for the use of animals in ophthalmic and vision research.

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All surgery was performed using a commercially available medical excimer laser system (Summit Technologies, Waltham, MA). This argon fluoride laser (wavelength, 193 nm) emits light with a fluence of 180 mJ/cm².

The animals were anesthetized with an intramuscular mixture of 1:7.5 xylazine (20 mg/ml; Rompun, Bayer, Barcelona, Spain) and ketamine (50 mg/ml; Ketolar, Parke Davis S.A., Barcelona, Spain) at a dose of 1.5 ml/kg body weight. The animals also received one drop of topical oxybuprocaine and tetracaine (Colirisci Anestésico Doble, Alcon-Cusi, Barcelona). The third lid was cauterized and excised. A circular central de-epithelialization (6.5-mm diameter) was performed mechanically. The denuded surface was carefully cleaned under the operating microscope with surgical cellulose sponges. Ablations were done according to our experimental model of haze (unpublished data). All ablations (5 mm in diameter and 87 μm in central depth) were bilateral and programmed to treat -9.00 diopters (D) of myopia using a pulse rate of 10 Hz.

Both eyes of each animal were randomly assigned to one group: control group, 30-μl applications of phosphate buffered saline (n = 24 eyes) or treatment group, 30-μl applications of 1% hyaluronic acid in phosphate buffered saline (n = 24 eyes).

All treatments were administered once immediately after ablation and subsequently six times daily during the first 3 postoperative days.

Six animals died during follow-up, and were excluded from analysis.

Evaluation of Corneal Opacity

Slit-lamp (Haag Streit 900, Berne, Switzerland) microscopic observations to grade corneal haze began 2 weeks after surgery and continued at 1, 1.5, 2, 3, 4, 5, and 6 months thereafter. One observer performed all examinations in a masked fashion. The density of the corneal haze was graded as follows on a subjective scale of 0 to 5, regardless of visualization of iris details: 0, completely clear cornea; 1, faint haze; 2, mild opacity; 3, moderate opacity; 4, dense opacity; and 5, very dense opacity.

Transmission Electron Microscopy

The animals were killed at the end of 6 months, and six corneas, three from treated and three from control eyes were obtained for transmission electron microscopy. A masked expert observer performed the examinations.

Statistical Analysis

The differences between the two groups were analyzed using a two-tailed Student’s t-test for paired samples. The results were considered significant when the P-value was less than .05.

RESULTS

All corneas had grade 4 to 5 haze 2 weeks after surgery, which progressively decreased during follow-up and did not resolve by the end of the study. The curves of the mean values of anterior stromal haze obtained for phosphate buffered saline controls and hyaluronic acid-treated eyes showed an almost identical course over time (Fig 1). In both the treated and control groups, haze appeared to decrease with time. There was no statistically significant difference in haze between the hyaluronic acid and placebo groups at any timepoint (P > .05 for all) (Table).

Electron microscopy photographs further illustrated the similar intensity of the corneal opacity in the control and the treated eyes (Figs 2 and 3). Six months after ablation, the epithelium overlying the
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C, control; T, treated. - excluded from analysis

Ablated cornea was of normal thickness and firmly adhered to a basal lamina, which was not continuous at small focal sites. Bowman's layer was absent and collagen fibrils were less organized than in a normal corneal stroma. Prominent extracellular electronlucent areas were apparent between the collagen bundles. During the entire observation period, the haze was restricted to the subepithelial area of the anterior stroma. Changes regarding the deeper parts of the stroma or the endothelium were not detected.

**DISCUSSION**

Because the excimer laser removes corneal tissue in the optical zone, the wound-healing process after PRK has been extensively studied clinically and histopathologically. These investigations support the hypothesis of a close relationship between corneal wound healing and the resultant optical clarity and stability of the intended refractive change. Many advances in laser technology have contributed to a continuous improvement of these problems. Pharmacological modulation of the healing response has been studied in animal models, mostly in rabbits. However, rabbits are a poor model of the human cornea since they do not have a Bowman's layer.

Partial loss of corneal clarity is common after PRK. The rate and severity of corneal haze are influenced by the amount of attempted correction, with corrections of high myopia (over -6.00 D) associated with greater corneal opacity. The severity of haze after excimer laser is also time-dependent, with maximal haze usually observed between 3 and 6 months postoperatively, with progressive clearing of haze at about 1 year after treatment. In this study, however, the course of the haze reached its peak shortly postoperatively and slowly regressed during follow-up. This behavior has been reported by other authors, and it is
Figure 2. Electron microscopy of hyaluronic acid-treated cornea: epithelium and superficial stroma (scale = 1 μm/cm; magnification X3,150). Image shows the epithelium (star), discontinuous basal lamina with hemidesmosomes (asterisk), and cicatrical collagen (cross).

Figure 3. Electron microscopy of control cornea: epithelium and superficial stroma (scale = 0.4 μm/cm; magnification X10,000). Image shows epithelium (star), discontinuous basal lamina with hemidesmosomes (asterisk), and cicatrical collagen (cross).
perhaps the result of aggressive ablation (-9.00 D) compared to the thickness of the cornea in the hen.

Hyaluronic acid is a disaccharide polymer composed of glucuronic acid and N-acetylglucosamine. It is a ubiquitous molecule, present in the connective tissue of all higher animals and even produced by some bacteria. It is present in trace amounts on the corneal endothelium, and it is not normally found in the epithelium or stroma. Endogenous hyaluronan production is seen in the entire spectrum of corneal disorders and is thought to play an important role in embryology, wound healing, and corneal inflammation. The waves of nuclear crest cells in the stroma are associated with hyaluronic acid production. The action of hyaluronidase later leads to stromal dehydration and corneal clarity. Hyaluronic acid may create hydrated spaces for cell movement and also may form a coating around migrating cells that blocks or weakens adhesion to other cells, preventing immobilization or precocious development. Corneal wound healing is characterized by cell movement, stromal remodeling, and proteoglycan changes. The discovery of the presence of hyaluronic acid after various types of trauma supports the notion that corneal wound healing recapitulates ontogenic events.

Hyaluronic acid may contribute to haze formation by changing the water balance in the cornea, thereby disturbing the surrounding lamellar architecture and creating focal changes in the index of refraction. However, the fact that hyaluronic acid appears during the first days after PRK cannot explain why maximum haze occurs so late, which suggests that this is not its role.

Rapid epithelial healing seems to be an important condition required for anterior stromal wound healing of good quality. The positive effect of hyaluronic acid on anterior stromal healing may be explained in part by rapid epithelial closure that allows improved reorganization of the basement membrane and the anterior stromal lamellae. It also could substitute for Bowman’s layer in the ablated area by acting as a barrier that blocks cellular interaction between the epithelium and the kerocytes.

Hyaluronic acid has been topically applied in other clinical situations. Reim and Lenz reported satisfactory results after using sodium hyaluronate as part of combined medical and surgical management of caustic and thermal ocular injuries. Nakamura and colleagues and Inoue and Katakami found that topical hyaluronic acid, combined with fibronectin, promotes corneal epithelial wound healing in rabbits. Chung and colleagues proposed hyaluronic acid use after alkali burns. Algawi and colleagues investigated whether topical sodium hyaluronate application, following PRK for myopia in humans, had an effect on postoperative pain and epithelial healing. They reported negative results, but the hyaluronic acid was smeared on the surface thickly, which could have obstructed penetration.

The efficacy of a pharmacologic substance to modulate stromal healing appears to depend on early postoperative onset of treatment. This hypothesis can be supported because the beginning of the cellular response leading to the development of subepithelial haze seems to occur in the first hours or days after surgery. We administered hyaluronic acid only the first 3 days postoperatively because its high molecular weight limited penetration through the intact corneal epithelium.

In the present study, we found no effect of 1% hyaluronic acid on corneal haze after PRK, which could be the result of the lack of binding of the topical agent to the cornea. However, according to the report of Sugiyama and colleagues, this substance is retained in the cornea when the basement membrane is absent, although its concentrations were 0, 1, and 0.25% in that study. Therefore, if the cornea binds topical hyaluronic acid during healing, it is reasonable that it does not play a role in these opacities.

Since the data presented are negative, there is also the possibility that the model chosen is inappropriate. Perhaps, given these results, we should have studied the effect of topical corticosteroids using this model as a positive control, since it is known that corticosteroids reduce haze. If corticosteroids were ineffective as well, then the results using hyaluronic acid would also seem invalid.

It is evident that the future success of photorefractive procedures depends on our ability to modulate the corneal wound healing response. Improved control of postoperative wound healing will improve corneal clarity and stability of visual outcomes and will enable more precise titration of refractive results.

REFERENCES


