Effect of Topical Immunomodulatory Interleukin 1 Receptor Antagonist Therapy on Corneal Healing in New Zealand White Rabbits (Oryctolagus cuniculus) After Photorefractive Keratectomy

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Objectives: To compare topical interleukin 1 receptor antagonist (IL-1ra) to steroid treatment following photorefractive keratectomy (PRK) in rabbit eyes.

Methods: Our study is a randomized, investigator-masked study that was approved by the Institutional Animal Care and Use Committee. Following standard PRK, 48 eyes of 24 rabbits were divided into 5 arms: 4 treatment arms and 1 control arm. The right eye of each rabbit served as the treatment eye, and the left eye served as a control. Eyes in treatment arms were randomized to receive either fluorometholone, 0.1%, 4 times a day (Falcon, Fort Worth, Texas), or 2.5, 1.25, or 0.25 mg of IL-1ra 4 times a day. Control eyes received only moxifloxacin hydrochloride, 0.5% (Vigamox; Alcon, Fort Worth, Texas), and a solution of polyethylene glycol 400, 0.4%, and propylene glycol, 0.3% (Systane; Alcon), 4 times a day. Primary outcome measures included weekly evaluation of subjective haze formation and time to corneal reepithelialization with clinic examinations, objective haze formation using Pentacam technology (Oculus, Lynnwood, Washington), as well as histological examination for haze thickness 7 weeks after PRK.

Results: There was no difference among treatment groups in time to reepithelization. The IL-1ra treatment groups showed a statistically significant reduction in haze formation ($P < .001$, determined by repeated-measures analysis of variance) on corneal evaluation using the Pentacam 3 weeks after PRK compared with the control group. This effect was comparable to that in the steroid treatment group. There was also a statistically significant effect of the treatment on subjective haze evaluation at weeks 4 and 5 ($P < .05$, determined by repeated-measures analysis of variance), but this effect lost statistical significance when the steroid group was excluded from the evaluation. In addition, there was no statistically significant difference in histologic evaluation of haze thickness among treatment groups ($P = .997$).

Conclusion: Further studies are needed to determine the efficacy and adverse effect profile of topical IL-1ra in human eyes.

Clinical Relevance: IL-1ra therapy may be an alternative to steroid treatment following PRK.

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tectomy (PRK) are the most common refractive procedures. However, alternative techniques, including laser epithelial keratomileusis, PRK with mitomycin C, and epithelial laser in situ keratomileusis, have been developed in an attempt to overcome common complications. Clinical outcomes are directly related to the corneal healing process, and a number of common complications are based on the unpredictable nature of the associated corneal cellular response to excimer laser injury. Overall complications include overcorrection, undercorrection, regression, corneal stromal opacification, and other adverse effects that are based on the biologic response to surgery. Postoperative histological studies have demonstrated that the corneal healing process consists of corneal epithelial and endothelial cell loss and a large amount of inflammatory cell infiltration into the corneal stroma.

Recent advances in understanding the biologic and molecular processes that contribute to the healing response demonstrate that inflammation and corneal wound healing is highly associated with increased cytokine levels, especially interleukin-1 (IL-1). IL-1 is a potent pro-inflammatory cytokine that has a wide range of activities, including the critical mediation of the acute phase response, chemotaxis and activation of inflammatory and antigen-presenting cells, upregulation of adhesion molecules, costimulatory factors on cells, and stimulation of neovascularization. In the eye, IL-1 activity has been correlated with corneal neovascularization, endotoxin-mediated uveitis, corneal collagenase and metalloproteinase expression, corneal injury in vitamin A deficiency, and herpetic stromal keratitis.

IL-1 is an attractive target for therapeutic intervention in immunogenic inflammatory diseases. Blocking IL-1 receptors in the cornea has been shown to inhibit proliferation of fibroblast-like corneal and conjunctival cells as well as suppress intercellular adhesion molecule 1 expression on vascular endothelial cells. Furthermore, clinical evidence has shown that IL-1 receptor antagonist (IL-1ra) therapy can significantly decrease corneal inflammation and lead to enhanced corneal transparency following traumatic damage. Excessive scarring and fibrotic repair in the cornea are currently addressed by the topical or systemic use of steroids, which carry the usual risks of unpredictable and delayed wound healing and increased risks of infections, cataract formation, and increased intraocular pressure.

At our center, which is the US Department of Defense’s largest refractive surgery center, more than 26,000 refractive surgical procedures have been performed since the inception of the program in 2001. In our hands, the rate of haze (greater than trace) is 0.5%. This is not an insignificant number, and currently these patients are treated with steroids. Therefore, the purpose of our study is to investigate a novel approach toward improving corneal healing following PRK. An alternative therapy with a better adverse effect profile would be an attractive option for patients.

Table. Medication Dosing Regimen for Treatment and Control Eyes of 24 White Rabbits

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treated Right Eyes (n=24)</th>
<th>Control Left Eyes (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Mox, 2.5 mg of IL-1ra</td>
<td>Mox, BSS</td>
</tr>
<tr>
<td>Group 2</td>
<td>Mox, 1.25 mg of IL-1ra</td>
<td>Mox, BSS</td>
</tr>
<tr>
<td>Group 3</td>
<td>Mox, 0.25 mg of IL-1ra</td>
<td>Mox, BSS</td>
</tr>
<tr>
<td>Group 4</td>
<td>Mox, Fml</td>
<td>Mox, BSS</td>
</tr>
</tbody>
</table>

Abbreviations: BSS, balanced salt solution; Fml, fluorometholone, 0.1%; IL-1ra, interleukin 1 receptor antagonist; Mox, moxifloxacin hydrochloride.

STUDY DESIGN

Our study was approved by the Wilford Hall Medical Center Institutional Animal Care and Use Committee. Before surgery, each rabbit underwent corneal evaluation using the Pentacam (Oculus, Lynnwood, Washington) rotating Scheimpflug camera to measure the shape, thickness, clarity, and contour of the cornea. Prior to ablation, a standard 8.5-mm epithelial defect was created in all eyes using 20% denatured ethanol. Forty-eight eyes of 24 rabbits underwent standard PRK with an ablation depth of 100 µm using the VISX STAR S4 Eximer Laser System (Abbott, Abbott Park, Illinois). Following PRK, the rabbits were fitted with Elizabethan collars for 36 hours to prevent them from rubbing their eyes. The 48 eyes were divided into 5 arms: 4 treatment arms and 1 control arm. The right eye of each rabbit served as the treatment eye, and the left eye served as a control. Eyes in treatment arms were randomized to receive standard steroid treatment with fluorometholone, 0.1% (Falcon, Fort Worth, Texas), 4 times a day or experimental therapy with 2.5, 1.25, or 0.25 mg of IL-1ra (TheraKine Inc, Atlanta, Georgia) 4 times a day. Thus, each treatment group was made up of 6 right eyes, and the control group was made up of 24 left eyes.

The IL-1ra treatment solutions were prepared via serial dilution in polyethylene glycol 400, 0.4%, and propylene glycol, 0.3%, solution (Systane; Alcon, Fort Worth, Texas). The solutions were prepared by an independent contractor not directly associated with our study to ensure that the identity of each treatment solution was blinded to study personnel. Each solution underwent repeated biologic testing to confirm the dose accuracy. The order of application of the solutions was identical in each group. Topical ophthalmic moxifloxacin hydrochloride, 0.5% (Vigamox; Alcon), was applied first to each eye, followed by balanced salt solution in the control group, fluorometholone in the steroid group, or IL-1ra in the experimental group. All drops were separated in time by at least 5 minutes, and all medications were dosed 4 times per day for 1 week. See the Table for the dosing schedule.

OUTCOME MEASURES

Four primary outcome measures were used to determine the effect of the different treatments on corneal healing. First, the rabbit eyes were examined daily postoperatively to determine the number of days required for full reepithelization. Second, the eyes were evaluated for haze formation and scattering via a slitlamp examination. Each cornea was evaluated independent by 3 separate staff ophthalmologists at weekly intervals after surgery for 6 weeks. A standard clinical haze grading scale from 0 to 4 was used, with grade 0 representing a clear cornea, grade 1 representing trace haze, grade 2 representing mild haze, grade 3 representing moderate haze, and grade 4 representing severe haze. Third, objective haze was evaluated in each cornea using the densitometer function of the Pentacam. This evaluation was repeated at weeks 1, 2, 3, and 7.

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RESULTS

There was no difference among treatment groups in time to reepithelization. The mean healing times in the control group, the steroid group, and the IL-1ra treatment groups were 5.3, 5.4, and 5.3 days, respectively. These results were consistent with previous reports of epithelial healing time in rabbits after PRK.27,28

Figure 1 depicts the results of the subjective haze analysis from weeks 3 through 6. There was no significant difference between treatment and control groups at week 1 (P = .17, determined by repeated-measures analysis of variance), week 2 (P = .32), or week 3 (P = .39). However, at week 3, there was a trend for decreased haze in the steroid group, followed by the IL-1ra groups, and then finally the control group. At weeks 4 and 5, the steroid group had a significant decrease in haze formation compared with the IL-1ra and control groups (P < .01). However, this result was short-lived, and at week 6, the differences in haze formation were no longer significantly different (P = .54).

On Pentacam density evaluation, shown in Figure 2, there was no difference in haze density among treatment groups at weeks 1 and 2 (P = .63 and P = .38, respectively). However, at week 3, there was a statistically significant reduction in haze formation (P < .001) among the treatment groups. The lower doses of IL-1ra reduced haze density comparable to the steroid group, whereas the higher doses of IL-1ra (2.5 mg) reduced haze less effectively. All treatment groups reduced haze compared with the control group at week 3. By week 7, there was no statistically significant difference in treatment groups on Pentacam evaluation, but a trend similar to the results at week 3 was observed (P = .44).

Finally, at week 7, the corneas were harvested for histologic evaluation. Figure 3 shows an example of the subepithelial repair layer that was measured in every cornea by an ocular pathologist at both the central cornea and the thickest area of the foam layer. Thickness among treatment groups was not statistically significant.
tion of neovascularization. IL-1 and tumor necrosis factor (TNF) are the primary regulators of the limbal Langerhans cell, the chief antigen-presenting cell on the ocular surface. These cells are capable of infiltrating the cornea in response to various inflammatory insults to initiate the T-cell–mediated immune response. Trauma, infections, and surgical manipulation are all insults that can trigger IL-1 and the subsequent inflammatory cascade.

IL-1ra is a naturally occurring isoform of IL-1 that has a high affinity for the IL-1 receptor but produces no agonist activity. Studies show that IL-1ra is expressed natively in the corneal epithelium and stroma. Epithelial IL-1ra may have a role to play in the suppression of the inflammatory response to stimuli and thereby may also play a role in the maintenance of corneal transparency. A polymorphism in intron 2 of IL-1ra has been shown to be associated with several diseases of epithelial origin, including ulcerative colitis, alopecia areata, and ichthyosis. This association between IL-1ra polymorphisms and inflammatory diseases of epithelial origin suggests that IL-1ra plays a role in maintaining tonic suppression of inflammation in the cornea.

Just as the native form of IL-1ra can suppress corneal inflammation, topical administration of IL-1ra has been shown to suppress antigen-presenting cell migration in the cornea, slowing the inflammatory cascade. It has also proven to decrease corneal inflammation and lead to enhanced corneal transparency following traumatic damage. To our knowledge, there have been no published studies to date looking at the effects of IL-1ra on corneal inflammation following refractive surgery. Steroids are the current “gold standard” for treating postoperative inflammation. However, unwanted adverse effects such as glaucoma, delayed wound healing, cataracts, and risk of secondary infection can occur. For this reason, an effective anti-inflammatory topical medication with an improved adverse effect profile could serve as an alternative treatment.

Dana recently compared topical IL-1ra and TNF receptor with prednisolone sodium phosphate, 1%, following penetrating keratoplasty in a mouse model. Results showed that topical IL-1ra and prednisolone were comparable in their capacity to promote graft survival. Furthermore, combination IL-1ra and steroid therapy added only minimal efficacy compared with either agent alone. Both topical medications promoted graft survival better than the TNF. Although we used a different steroid control, our clinical outcomes comparing topical IL-1ra with steroids were in agreement with the results of Dana. Our analysis of haze included densitometry as measured by the Pentacam rotating Scheimpflug camera. Following acquisition of the 3-dimensional images by the Scheimpflug camera, the corneal density was measured at both the central cornea and the peak density recording. Although the Pentacam densitometer provides a quantitative measurement, its exact correlation to clinical corneal haze remains unknown. Takacs et al recently used the Pentacam densitometer to compare corneal haze in human eyes undergoing PRK. They found a positive correlation between subjective haze and Pentacam density. Greenstein et al used Scheimpflug densitometry to assess corneal haze following collagen cross-linking in patients with keratoconus. Although the haze associated with corneal cross-linking may be of a different character than the haze from refractive surgery, they also found a close approximation between densitometry findings and slit-lamp haze. Pentacam densitometry appears to be a promising tool in the quantification of corneal haze, but further studies are needed to confirm its accuracy and reproducibility.

Although our subjective haze evaluation favored steroid treatment at weeks 4 and 5, there was no difference between steroid treatment and IL-1ra therapy by week 6. We did not have Pentacam data at weeks 4 and 5, and in retrospect, it would have been beneficial to have had a weekly Pentacam evaluation. The significant difference between treatment and control groups in Pentacam density at week 3, as well as the trend on slitlamp examination, prompted us to look for a correlation between the subjective haze score and the Pentacam density for each group. At week 3, we did find a correlation between these values. The mean grades on slitlamp examination for the steroid, IL-1ra, and control groups at week 3 were 1.0, 1.27, and 1.5, respectively. The mean Pentacam density for the groups at week 3 were 46.25, 58.54, and 94.97, respectively. Although there does not appear to be a 1:1 relationship, it is promising to note the agreement between the mean grades from slitlamp examination and the mean Pentacam densities. It would be beneficial in the future to compare these methods in human subjects who can provide visual acuity and subjective complaints (ie, glare and halos).

It was interesting to note that, on Pentacam evaluation, the lower doses of IL-1ra were more effective than the higher doses. We hypothesize that the IL-1 receptors on the ocular surface were saturated at the higher dose, leading to upregulation of more receptors. With fresh receptors available, the IL-1 would again be able to initiate its inflammatory cascade. The increased inflammation in turn resulted in more haze.

Postoperative histological studies have demonstrated that the corneal healing process consists of a large amount of inflammatory cell infiltration into the corneal stroma. Following refractive surgery, a subepithelial healing layer can be observed on microscopic sections (Figure 1). This layer is thought to be made up of myofibroblasts, interstitial fluid, and collagen debris. Our purpose in taking histologic sections following the treatment course was to determine if this repair layer was influenced by topical anti-inflammatory therapy. However, results showed no difference between the control and treatment groups. Given the statistically significant difference between treatment and control groups in both clinical and Pentacam data, there does not appear to be a correlation between the thickness of the repair layer and clinical haze. It may be that some other aspect of the repair layer correlates with the clinical examination, such as total area of corneal involved or exact composition of the repair layer.

The importance of corneal clarity cannot be overstated when discussing refractive surgery outcomes. Haze formation, undercorrection or overcorrection, corneal edema, neovascularization, and opacity can lead to decreased visual performance and unsatisfied patients. Clinical outcomes are directly related to the corneal healing process and the corneal cellular response to excimer laser
injury. Our results indicate that topical IL-1ra may be an alternative to steroids for treating corneal inflammation and haze following refractive surgery. Further studies are needed to determine the effect in the human cornea, including the effect of haze reduction on visual acuity and glare in patients treated with IL-1ra compared with patients treated with steroids. Because IL-1ra therapy did not increase the epithelial healing time in our study, future studies are also needed to determine the adverse effect profile of topical IL-1ra in human eyes. Our study also shows that Pentacam density may be a useful tool in measuring corneal haze after refractive surgery. Further studies may help delineate a numerical relation between Pentacam density and haze as measured at the slitlamp.

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