

Staged Intrastromal Delivery of Riboflavin With UVA Cross-linking in Advanced Bullous Keratopathy: Laboratory Investigation and First Clinical Case

Ronald R. Krueger, MD, MSE; Jerome C. Ramos-Esteban, MD; A. John Kanellopoulos, MD

ABSTRACT

PURPOSE: To evaluate the safety and efficacy of staged ultraviolet A (UVA) cross-linking following intrastromal 0.1% riboflavin administration in eyes with advanced corneal edema.

METHODS: Ten eye bank corneas divided in two groups ($n=5$) were placed on a pressurized artificial anterior chamber following Descemet's membrane stripping. Two consecutive corneal pockets (350- and 150- μm depth) were sequentially created using a femtosecond laser. Sequential intrastromal injections of 0.1% riboflavin (0.2 mL) followed by either UVA irradiation (15 mW/cm²) for 7 minutes or exposure to air were performed for each pocket. Corneal clarity and central thickness were measured before and after the two UVA cross-linking steps. The same steps were clinically applied in an 84-year-old woman with bullous keratopathy prior to corneal transplantation and followed for 6 months.

RESULTS: The corneal clarity improved in the treated but not the control eyes. The mean central corneal thickness was significantly reduced by 256 μm (ultrasound, $P=.0002$) and 273 μm (Scheimpflug, $P=.0004$) in treated eyes, but only 100 μm (ultrasound, $P=.048$) and 107 μm (Scheimpflug, $P=.075$) in the control eyes. The clinical treatment of corneal edema showed improved clarity and reduced central corneal thickness from 675 to 550 μm (ultrasound) and 696 to 571 μm (Scheimpflug) at 1 month. Best spectacle-corrected visual acuity improved from finger counting to 20/80 at 1 week and beyond, postponing corneal transplantation for >6 months.

CONCLUSIONS: Staged UVA cross-linking (15 mW/cm²) with femtosecond laser facilitated intrastromal 0.1% riboflavin administration may be a safe (no corneal scarring) and effective (marked reduction of edema) temporizing alternative method for managing bullous keratopathy. [*J Refract Surg.* 2008;24:S730-S736.]

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phakic/pseudophakic bullous keratopathy is a visually disabling corneal disorder due to endothelial cell dysfunction and decompensation.¹ Without sufficient endothelial pump function, the cornea swells and fluid accumulates in the extracellular spaces between collagen fibers and lamellae. The altered corneal fiber spacing affects corneal transparency and leads to light scatter with a reduction of visual function. The condition worsens as the epithelium becomes edematous with the formation of bullae, leading to profound visual loss and disabling pain. Although corneal deturgescence can be partially achieved with exposure to hypertonic saline and chemical agents, such as dextran, permanent and complete compaction of fibers with restoration of corneal transparency cannot be achieved in these edematous corneas, except by the surgical replacement of endothelial cells after penetrating or posterior lamellar keratoplasty.^{2,3}

Riboflavin 0.1% ultraviolet A (UVA) collagen cross-linking is a photochemical reactive process that has been clinically used for stabilizing progressive keratoconus⁴⁻⁷ and postoperative LASIK ectasia^{8,9} over the past 5 years. The technique typically requires the removal of the corneal epithelium and saturation of the corneal stroma with riboflavin, a low molecular weight compound, which easily diffuses through the cornea into the anterior chamber. The riboflavin molecule collects alongside the collagen to be cross-

From Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, Ohio (Krueger, Ramos-Esteban); NYU Medical School, New York, NY (Kanellopoulos); and Laservision.gr Institute, Athens, Greece (Kanellopoulos).

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Correspondence: A. John Kanellopoulos, MD, Laservision.gr Institute, 15-17 Tsocha St, Athens, Greece 11521. Tel: 30 210 7472777; Fax: 30 210 7472789; E-mail: laservision@internet.gr

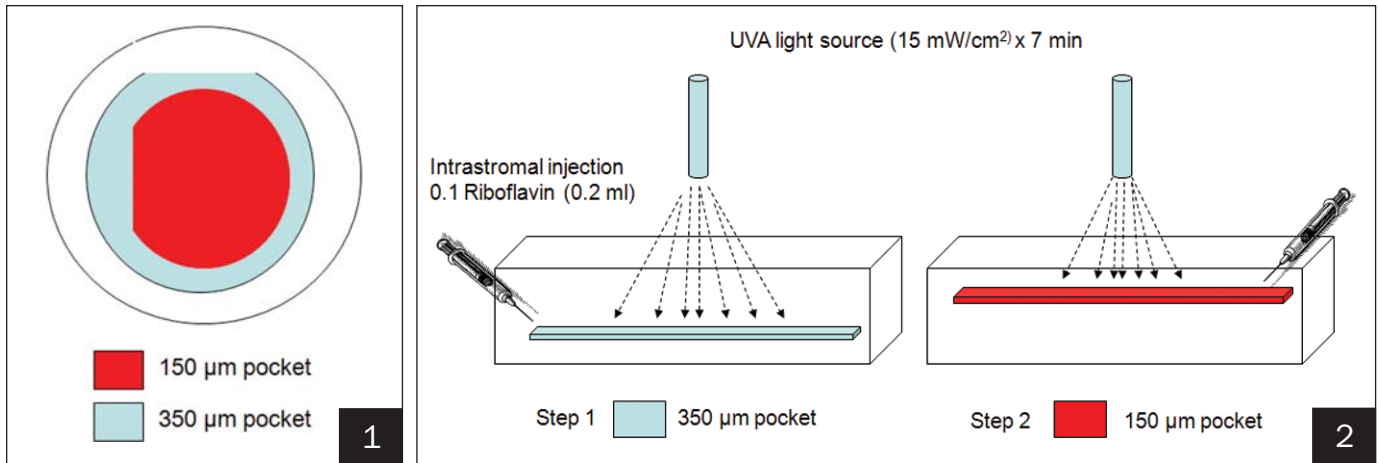


Figure 1. Schematic representation of the pockets created during staged intrastromal injection of 0.1% riboflavin and UVA cross-linking. **Figure 2.** Staged intrastromal injection of 0.1% riboflavin and UVA cross-linking.

linked and is photosensitized by the exposed UVA light in the presence of oxygen to create a reactive singlet oxygen species that induces collagen cross-linking. Although stromal absorption of riboflavin is best achieved with repeated topical application after epithelial cell removal due to evaporative losses and bleaching effects, some investigators choose to keep the epithelium in place and attempt to loosen the epithelial tight junctions to facilitate riboflavin diffusion with prolonged use of topical anesthetics.

One author in our group (A.J.K.) recently conceptualized a new method of riboflavin application in keratoconic eyes through a femtosecond laser–created central corneal pocket. In this technique, a superficial (100 µm) lamellar pocket was generated; together with a 5° to 10° externalizing side cut with the assistance of a femtosecond laser (IntraLase FS60; Advanced Medical Optics [AMO], Irvine, Calif). The pocket allowed for superficial intrastromal administration of riboflavin (0.1%) solution without removal of the epithelium, and after cross-linking with 365 nm UVA light (6 mW/cm² for 15 minutes), produced similar results compared to published studies⁴⁻⁸ as well as our own traditional epithelial removal technique.

Because of our success with this method and greater thickness present in an edematous cornea, we decided to apply this concept in two stages and at two deeper stromal locations to achieve deeper penetration of collagen cross-linking in corneas with experimental bullous keratopathy.

In this study, we propose that riboflavin UVA collagen cross-linking of edematous corneas in the posterior and mid corneal stroma can strengthen inter-fiber attachments and reduce the potential space for fluid accumulation, which is characteristic in bullous keratopathy. We believe this may lead to improved

stromal compaction and optical function in edematous corneas.

MATERIALS AND METHODS

EXPERIMENTAL MODEL

Ten human cadaver corneas were prepared by stripping Descemet’s membrane from their posterior surface and placing them in an artificial anterior chamber constantly pressurized (20 mmHg [27.2 cm H₂O]) (Katena Products Inc, Denville, NJ). Each cornea was then positioned and applanated beneath an IntraLase FS60 femtosecond laser, where two consecutive corneal pockets were created using a raster pattern with a pulse energy of 1.15 µJ and a spot and line separation of 6 µm. The first pocket was set at 350-µm depth and 7.5-mm diameter with a superior side cut of 10° (350° hinge angle) and a side cut angle of 70°. The next pocket was set at 150-µm depth and 7-mm diameter, also with a 10° side cut (350° hinge angle), but 90° away from the initial side cut (Fig 1). Following blunt dissection of the first pocket with a Jameson muscle hook (Rumex International, St Petersburg, Fla) at 350 µm, a single dose (0.2 mL) of 0.1% riboflavin phosphate sodium solution was instilled. Within 5 minutes, yellow “soaking” of the middle and posterior stroma was visualized, and the first stage of UVA exposure was begun (Fig 2). Injection of riboflavin 0.1% solution was determined to be the best method to ensure the delivery of an adequate concentration of the medication to the target tissue. High irradiance UVA exposure (15 mW/cm²) was selected to quickly achieve intrastromal cross-linking prior to clearance of the single dose of riboflavin from the stroma. Intrastromal injection minimizes evaporative losses that can occur on the surface of the cornea. In addition, this delivery method also reduces bleaching artifacts as the

TABLE 1

Mean Corneal Clarity Score and Central Corneal Thickness Measurements With Ultrasound and Scheimpflug Pachymetry Before and After Staged UVA Cross-linking and Sham Treatment

Group	Mean ± Standard Deviation (P Value)					
	Clarity Score		Ultrasound CCT (μm)		Scheimpflug CCT (μm)	
	Before	After	Before	After	Before	After
UVA	2±0	3.2±0.4 (<i><.01</i>)	844±56.8	588±19.4 (.0002)	852±57.8	579±29.2 (.0004)
Air exposure	2±0	2.2±0.4 (.37)	814±92.4	714±28.8 (.048)	822±92.4	715±23.5 (.075)

CCT = central corneal thickness

Note. Statistical differences (P value) in relation with measurements before and after staged intrastromally delivered riboflavin-UVA collagen cross-linking treatment are represented in parentheses.

deeper pocket (350 μm) is irradiated first and the most superficial pocket (150 μm) is irradiated last.

The 10 corneas were then divided into two groups. In the first group (UVA group), 5 corneas were exposed to 365 nm UVA light at an irradiance of 15 mW/cm² for 7 minutes (PriaVision Inc, Menlo Park, Calif) to achieve deep collagen cross-linking. In the second group (air exposure group), 5 corneas were exposed to air as a sham control. Following the first session of cross-linking, the second pocket at 150 μm was dissected with a blunt Jameson muscle hook and an additional single dose (0.2 mL) of 0.1% riboflavin solution instilled and allowed to “soak” into the middle and superficial stroma. Corneas in the UVA group then received the second session of UVA exposure at 15 mW/cm² for 7 minutes, whereas the corneas in the air exposure group were again exposed to air as a sham control.

Slit-lamp examination, ultrasound pachymetry (Corneoscanner; Sonogauge, Cleveland, Ohio), and Scheimpflug corneal tomography (Pentacam; Oculus Optikgeräte GmbH, Wetzlar, Germany) were performed to assess corneal clarity and central corneal thickness both before IntraLase pocket creation and after the two sessions of UVA versus air exposure. Corneal clarity was graded by visual slit-lamp inspection according to an arbitrary scale, referred to as corneal clarity score, ranging from 1 (very opaque cornea difficult to visualize underlying structures), 2 (relatively opaque cornea with some visualization of underlying structures), 3 (diffuse corneal edema with relative clarity to visualize underlying structures), and 4 (clear cornea). Corneal clarity was graded by one of the investigators (A.J.K.), who was blinded to clinical data. This corneal clarity scale is used by the investigator in his daily clinical practice.

CLINICAL CASE

After obtaining appropriate informed consent, an 84-year-old female patient with pseudophakic bullous keratopathy underwent the staged cross-linking procedure in her left eye (surgeon, A.J.K.). The risks, benefits, and alternatives to this procedure were explained to the patient. The patient was scheduled for an elective penetrating keratoplasty in her left eye to relieve symptoms of painful epithelial bullae and decreased visual acuity. The patient decided to undergo the cross-linking procedure to relieve pain symptoms and prevent intraoperative complications associated with a penetrating keratoplasty such as a suprachoroidal hemorrhage or endophthalmitis. The patient understood that this procedure is still in its experimental stages and that if it failed she would have to undergo the scheduled penetrating keratoplasty.

Preoperative ultrasound central corneal thickness was 675 μm and Scheimpflug central corneal thickness was 696 μm. After signing an informed consent and receiving topical anesthetic in the left eye, the patient had two lamellar pockets created in a similar manner to the experimental model, and a single dose (0.2 mL) of 0.1% riboflavin was instilled into the deepest (350 μm) pocket first followed by cross-linking using a KeraCure UVA light delivery system (PriaVision Inc) at 15 mW/cm² for 7 minutes. Afterwards, the second cross-linking procedure was performed in the same manner, following instillation of 0.1% riboflavin into the 150-μm pocket. The eye was then prospectively examined at 1 day, 1 week, 1 month, and 6 months. Corneal clarity scores were determined by slit-lamp examination, and central corneal thickness was prospectively followed using ultrasound and Scheimpflug pachymetry.

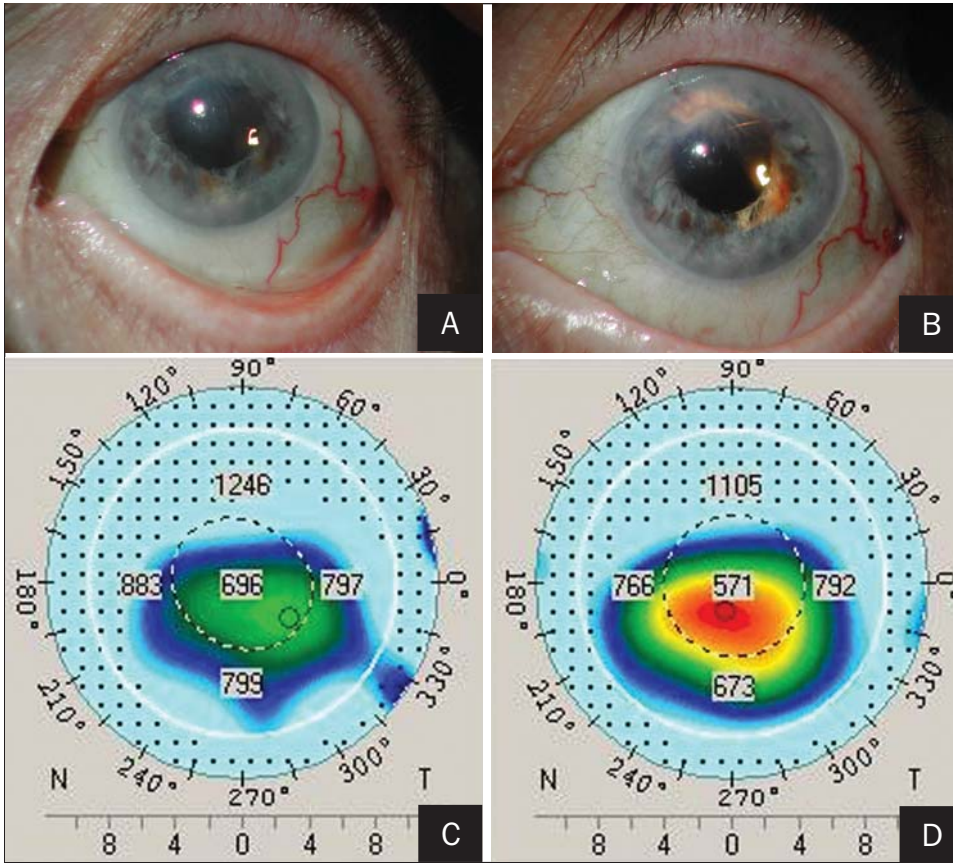


Figure 3. Clinical photographs and Scheimpflug corneal thickness maps of an 84-year-old woman with pseudophakic bullous keratopathy **A, C**) before and **B, D**) 1 month after staged intrastromally delivered riboflavin-UVA collagen cross-linking.

RESULTS

EXPERIMENTAL MODEL

The mean central clarity score and mean central corneal thickness for the UVA and air exposure groups are recorded before and after staged intrastromal cross-linking in Table 1. Before pocket creation, mean clarity score for both experimental groups was 2 ± 0 , and mean ultrasound and Scheimpflug central corneal thicknesses were $844 \pm 56.8 \mu\text{m}$ and $852 \pm 57.8 \mu\text{m}$ for the UVA group and $814 \pm 92.4 \mu\text{m}$ and $822 \pm 84.9 \mu\text{m}$ for the air exposure group, respectively. After UVA versus sham (air) exposure, mean clarity score was 3.2 ± 0.4 for the UVA group and 2.2 ± 0.4 for the air exposure group, whereas mean ultrasound and Scheimpflug central corneal thicknesses were $588 \pm 19.4 \mu\text{m}$ and $579 \pm 29.2 \mu\text{m}$ for the UVA group and $714 \pm 28.8 \mu\text{m}$ and $715 \pm 23.5 \mu\text{m}$ for the air exposure group, respectively.

MEAN CORNEAL CLARITY SCORE

A statistically significant increase in mean clarity score of 1.2 ($P < .001$) occurred after UVA riboflavin cross-linking in the UVA group, whereas a modest but not statistically significant increase of 0.2 in mean clarity score was also observed in the air exposure

group after sham treatment, which was mainly due to a change in clarity in one of the corneas.

MEAN ULTRASOUND CENTRAL CORNEAL THICKNESS

A statistically significant decrease in mean ultrasound central corneal thickness of $256 \mu\text{m}$ ($P = .0002$) occurred after UVA riboflavin cross-linking in the UVA group, and in the air exposure group, ultrasound central corneal thickness was also statistically reduced by $100 \mu\text{m}$ ($P = .048$) after sham treatment.

MEAN SCHEIMPFLUG CENTRAL CORNEAL THICKNESS

A statistically significant decrease in the mean Scheimpflug central corneal thickness of $273 \mu\text{m}$ ($P = .0004$) occurred after UVA riboflavin cross-linking in the UVA group, whereas in the air exposure group, Scheimpflug central corneal thickness was reduced by $107 \mu\text{m}$ after sham treatment, which was not statistically significant ($P = .075$).

CLINICAL CASE

Prior to staged intrastromal cross-linking, the patient's left cornea was thickened and swollen with mild epithelial edema, resulting in persistent pain and diminished visual acuity of finger counting. Baseline corneal clarity score was recorded as 2, and ultrasound and

TABLE 2

Clinical Outcome Before and After Staged Intrastromally Delivered Riboflavin-UVA Collagen Cross-linking for Pseudophakic Bullous Keratopathy in an 84-year-old Woman

	Preop	Day 1	Week 1	Month 1
BSCVA	CF	20/100	20/80	20/80
Corneal clarity score	2	3	3	3
Ultrasound/Scheimpflug CCT (μm)	675/696	516/520	545/549	550/571

BSCVA = best spectacle-corrected visual acuity, CF = counting fingers, CCT = central corneal thickness

Scheimpflug central corneal thicknesses were 675 μm and 696 μm , respectively.

After staged intrastromal cross-linking, the patient's symptoms improved remarkably, as well as best spectacle-corrected visual acuity (BSCVA), achieving a level of 20/100 after the first day and 20/80 by the first week and beyond. After 1 month, corneal clarity score increased to 3 and ultrasound and Scheimpflug central corneal thicknesses uniformly improved to 550 μm and 571 μm , respectively (Table 2, Fig 3). As BSCVA remained stable at the 20/80 level for at least 6 months, the scheduled keratoplasty was postponed due to the significant visual and symptomatic improvement. Additionally, a funduscopy examination at 6-month postoperative follow-up showed no retinal pathology or change from the preoperative examination.

DISCUSSION

Since its first introduction into ophthalmology 5 years ago,⁴ the use of riboflavin UVA collagen cross-linking has been gaining popularity in the treatment of progressive keratoconus, and current estimates suggest that >1000 procedures are performed each month worldwide (personal communication, Michael Mrochen, PhD, February 16, 2008). With this growing popularity, other novel applications are being proposed for the use of riboflavin UVA cross-linking in ophthalmology, such as the treatment of postoperative LASIK ectasia,^{8,9} strengthening of recalcitrant corneal ulcerations,¹⁰ halting of progressive axial myopia,¹¹ and even stiffening the peripapillary sclera for neuroprotection as a possible future therapy for low tension glaucoma.¹² In each of these applications, the formation of interfiber collagen cross-links, through singlet oxygen reactivity, leads to a stiffer, tougher collagen matrix that maintains its rigidity over a period of several years. In corneal edema, where the interfiber collagen spacing has been increased due to fluid accumulation, the concept of stromal compaction, with an enhanced resistance to osmotic and hydrostatic fluid accumula-

tion,¹³ introduces an attractive, new application for the cross-linking technique.

The challenge faced in this application of cross-linking, however, is the delivery of UVA light through the thickened cornea, where effective absorption by riboflavin through the superficial layers impedes delivery of UVA light to the deeper layers. To overcome this limitation, we introduced the concept of intrastromal riboflavin administration through a deep stromal pocket created using an IntraLase laser, which would facilitate UVA light transmission through the superficial cornea layers, allowing for riboflavin absorption and photoactivation where it is needed, posteriorly. We also did not seek to use surface application of riboflavin at standard dosing and UVA exposure as a control for our study, but rather, we used the same steps of riboflavin delivery together with exposure to ambient air as a sham procedure. Only in this way could we compensate for all of the variables introduced with this new technique.

We also introduced the concept of staging the intrastromal riboflavin administration through a second, more anterior stromal pocket after the deeper cross-linking is achieved, so that the superficial layers may also undergo stromal compaction and reduce the fluid accumulation anteriorly. The added benefit of this staging process is that delivery of riboflavin does not require the removal of the corneal epithelium.

The benefit of this staged cross-linking technique in reducing the corneal thickness of our experimental bullous keratopathy model was shown to be effective in the 5 treated corneas (UVA group), which underwent statistically significant reductions of 256 μm ($P=.0002$) in ultrasound central corneal thickness and 273 μm ($P=.0004$) in Scheimpflug central corneal thickness compared to baseline values after UVA cross-linking. Although these data seem compelling, we have also shown a mean statistically significant reduction in ultrasound corneal thickness of 100 μm ($P=.048$), but to a lesser extent, in the 5 corneas (air exposure group)

undergoing sham treatments with staged exposure to air, rather than UVA light. We can explain this latter reduction by the process of intrastromal pocket creation using the IntraLase laser. The suction ring and appplanation plate of the laser's delivery system applies pressure to the edematous corneas, which squeezes out the fluid, relative to the pre-treatment state. Eventually, we anticipate that the pre-treatment thickness would be regained in these latter experimental corneas, but our experimental studies do not allow for longer follow-up to verify this point.

The limitation of studying the immediate effect of staged intrastromal UVA riboflavin cross-linking in this experimental model still leaves questions regarding the long-term duration of such an effect. Although the long-term healing and stability of cross-linked eyes with corneal edema is unknown, we have 6-month longitudinal clinical follow-up in our first patient with advanced bullous keratopathy, showing that stromal compaction with cross-linking reduces central corneal thickness and improves the visual potential, corneal clarity, and painful symptomatology of bullous keratopathy starting on the first postoperative day. Although the functional benefit relative to keratoplasty techniques is yet undetermined, this novel technique may at least prove to be an effective palliative therapy prior to penetrating or posterior lamellar keratoplasty in the management of pain and corneal opacification secondary to bullous keratopathy.

The improvement of corneal clarity achieved with stromal compaction due to cross-linking demonstrates its relative safety in comparison to the cross-linking of keratoconus, where early haze and loss of clarity are often noted and occasionally persistent.¹⁴ As the cornea with advanced bullous keratopathy is usually opaque and has already lost its clarity, the haze associated with cross-linking is of a minor concern, and both our experimental and clinical evaluation demonstrated improvement in clarity rather than loss.

The adequate saturation of riboflavin when injected as a single, intrastromal (0.2 mL) dose, can be explained in accordance with the established safety criteria of Spoerl et al¹⁵ in reference to the safety of its shielding of the endothelium, lens, and retina. Although the saturation level for maximum UVA absorption along 400 μm of tissue requires a diffusion time of 30 minutes when applying riboflavin drops to the de-epithelialized corneal surface, intrastromal delivery in a thickened cornea allows for both anterior and posterior diffusion, achieving adequate saturation within a much shorter time period (~5 to 7 minutes). Injecting additional doses of riboflavin and waiting a longer period (~30 minutes) prior to UVA exposure would

only block the posterior stroma from the UVA-induced cross-linking effect.

In both the experimental and clinical application of staged intrastromal cross-linking, we used a high UVA irradiance of 15 mW/cm^2 for 7 minutes, rather than the standard dose of 3 mW/cm^2 for 30 minutes, because of the diminished practicality of intrastromally reapplying the riboflavin every 5 minutes. In a collaborative study with Krueger, Spoerl, and Herekar, we have shown that riboflavin/UVA induced cross-linking is equally effective and safe when exposed to a total UVA radiant exposure of 5.4 J/cm^2 , regardless of whether it is a short dose of high irradiance or the standard UVA exposure (unpublished data, October 2007). Not only does the high irradiance exposure reduce the amount of time required for effective cross-linking, but it also proportionately increases the rate of oxygen consumption (and cross-links produced) while photoactivating the existing riboflavin in the cornea. Although another dose of riboflavin just prior to the UVA exposure might be optimal, the additional intrastromal dosing of riboflavin (in response to its clearance from the cornea) is not necessarily required, due to the short time of UVA exposure and the nonevaporative dose of riboflavin within the intrastromal pocket.

Staged intrastromally delivered collagen cross-linking is a new and plausible method for strengthening the interfiber collagen attachments in edematous corneas and facilitating stromal compaction with a reduction of fluid accumulation. The intrastromal administration of riboflavin into two IntraLase laser-created stromal pockets followed by exposure to high energy UVA irradiation, during two sequentially staged sessions, allows us to overcome the limited depth of penetration of riboflavin UVA cross-linking, making it possible to achieve stromal compaction throughout the edematous cornea. The optical and functional benefits experienced experimentally and in this pilot case awaiting transplantation make this technique a possible alternative to consider in corneas with advanced bullous keratopathy. Further experimental and clinical studies with longer follow-up are needed to clinically validate these early findings.

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