Intraoperative corneal thickness change and clinical outcomes after corneal collagen crosslinking: Standard crosslinking versus hypotonic riboflavin

Elan Rosenblat, MD, Peter S. Hersh, MD

PURPOSE: To determine intraoperative changes in corneal thickness and outcomes of corneal collagen crosslinking (CXL) using 2 intraoperative regimens: riboflavin–dextran or hypotonic riboflavin.

SETTING: Cornea and refractive surgery practice, Teaneck, New Jersey, USA.

DESIGN: Prospective randomized case series.

METHODS: Eyes with keratoconus or corneal ectasia were treated. All eyes received preloading with riboflavin 0.1% in 20% dextran. During ultraviolet-A (UVA) exposure, patients were randomly assigned to 1 of 2 study arms; that is, riboflavin–dextran or hypotonic riboflavin. Intraoperative pachymetry was measured before and after the corneal epithelium was removed, after initial riboflavin loading, and after UVA light exposure. Patients were evaluated for maximum keratometry (K), uncorrected distance visual acuity (UDVA), corrected distance visual acuity, corneal thickness, and endothelial cell count (ECC).

RESULTS: Forty-eight eyes were treated. After removal of the epithelium and riboflavin loading, the mean pachymetry was 430 μm and 432 μm in the standard group and hypotonic group, respectively. Immediately after 30-minute UVA administration, the mean pachymetry was 302 μm and 342 μm, respectively. There was no statistically significant difference in the postoperative maximum K change, UDVA, corneal thickness, or ECC between the 2 groups.

CONCLUSIONS: The cornea thinned substantially during the CXL procedure. The use of hypotonic riboflavin rather than riboflavin–dextran during UVA administration decreased the amount of corneal thinning during the procedure by 30%, from 128 μm to 90 μm. However, there were no significant differences in clinical efficacy or changes in ECC or function between groups postoperatively. In general, corneal thinning during CXL did not seem to compromise the safety of the endothelium.

Financial Disclosures: Dr. Hersh is a consultant to Avedro, Inc. Dr. Rosenblat has no financial or proprietary interest in any material or method mentioned.

J Cataract Refract Surg 2016; 42:596–605 © 2016 ASCRS and ESCRS

Corneal collagen crosslinking (CXL)[1] is a treatment to decrease the progression of keratoconus[2] in particular and other corneal thinning processes such as ectasia after laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK).[3] Studies have suggested that CXL also can have beneficial visual and optical effects.[4–6] We have reported a number of studies showing an improvement in corrected (CDVA) and

uncorrected (UDVA) distance visual acuities, maximum and average keratometry (K) values,[4] keratoconus topographic indices,[7] higher-order aberrations (HOAs),[8] and subjective visual function[9] after CXL.

As first described,[1] the CXL protocol uses riboflavin 0.1% in 20% dextran solution, both for initial corneal saturation and during ultraviolet-A (UVA) administration. Hypotonic riboflavin solution (riboflavin in a

distilled water carrier without dextran) has been used to swell corneas before UVA exposure in patients with corneas thinner than 400 μm, with the goal of protecting the corneal endothelium from damage by the UVA–riboflavin interaction.[10]

In previous work,[A] we reported significant thinning of the cornea during the CXL procedure using the riboflavin–dextran formulation. Conversely, it has been suggested that the thicker riboflavin–dextran solution forms a biofilm important for procedure reproducibility and safety by blocking some of the incoming UVA light.[11] This attribute, however, might reduce the crosslinking effect compared with a thinner viscosity solution by attenuating UVA light delivery to the deeper stroma. Given these observations, we attempted to answer 4 questions: (1) How much corneal thinning occurs during the CXL procedure in general? (2) Is this thinning related to the efficacy and safety of the procedure? (3) Is there a difference in the intraoperative thinning of the cornea between treatments using riboflavin in a dextran carrier and hypotonic riboflavin? (4) Is there a difference in efficacy (change in maximum K and visual acuity) or safety (change in endothelial cell count [ECC]) outcomes between the 2 riboflavin solutions regimens?

This randomized controlled clinical trial, therefore, was designed with 2 study arms. The first received standard treatment with riboflavin–dextran solution during both the saturation and UVA phase. The second received hypotonic riboflavin during the 30-minute UVA exposure.

From the Cornea and Laser Eye Institute–Hersh Vision Group, CLEI Center for Keratoconus, Teaneck, and the Department of Ophthalmology, Rutgers-New Jersey Medical School, Newark, New Jersey, USA.

Corresponding author: Peter S. Hersh, MD, Cornea and Laser Eye Institute–Hersh Vision Group, CLEI Center for Keratoconus, 300 Frank W. Burr Boulevard, Teaneck, New Jersey 07666, USA. E-mail: phersh@vision-institute.com.

PATIENTS AND METHODS

Patients with keratoconus and ectasia after LASIK were enrolled as part of a single-center prospective randomized controlled clinical trial.[B] This study was performed under a physician-sponsored Investigational New Drug, was approved and monitored by an investigational review board, and was compliant with the U.S. Health Insurance Portability and Accountability Act. Informed consent was obtained from all patients.

The inclusion criteria included patients 18 years or older with a diagnosis of keratoconus or corneal ectasia after laser refractive surgery and axial corneal topography consistent with keratoconus or ectasia. Exclusion criteria included a history of corneal surgery other than laser refractive surgery, chemical injury, delayed epithelial healing, and a corneal pachymetry (including the epithelium) less than 300 μm. All patients included in the ectasia group were post-LASIK patients; no patient was post-PRK.

Surgical Technique

Crosslinking was performed according to the method described by Wollensak et al.[1] Topical anesthesia was administered, and the corneal epithelium was removed by mechanical debridement over the central 9.0 mm. Riboflavin (0.1% in 20.0% dextran T500 solution, Medio Cross, Peschke Trade GmbH) was then administered topically every 2 minutes for a total of 30 minutes. After riboflavin administration, riboflavin absorption throughout the corneal stroma and anterior chamber was confirmed by slitlamp examination.

Ultrasonic pachymetry was performed. If the cornea was thinner than 400 μm, hypotonic riboflavin (0.1% in sterile water, Medio Cross hypotonic) was administered, 1 drop every 10 seconds for 2-minute sessions, after which ultrasonic pachymetry was performed to confirm that the stroma had swollen to more than 400 μm. This was repeated until a corneal thickness of 400 μm or more was obtained.

The cornea was exposed to UVA 365 nm light (UV-X system, IROC Innocross AG) for 30 minutes at an irradiance of 3.0 mW/cm^2. During UVA exposure, riboflavin drops of 1 of 2 formulations were continued every 2 minutes, as determined by preoperative randomization. The first group continued to receive riboflavin in dextran solution (0.1% in 20.0% dextran T500); the second group received hypotonic riboflavin (0.1% in sterile water) every 2 minutes during UVA exposure.

Postoperatively, antibiotic and corticosteroid drops were administered and a therapeutic soft contact lens (Acuvue Oasys, Vistakon Pharmaceuticals, LLC) was placed. The contact lens was removed after epithelial healing, typically 3 to 5 days postoperatively. Antibiotic drops were continued for 1 week, and corticosteroid drops were continued for 2 weeks.

Pachymetry

Intraoperative corneal thickness measurements were taken by ultrasound (US) pachymetry (Corneo-Gage Plus, Sonogage, Inc.) before the corneal epithelium was removed and after the following steps: epithelium removal, initial 30-minute riboflavin loading just before UVA light exposure, swelling with hypotonic riboflavin if necessary, and 30 minutes of UVA light exposure. At least 5 pachymetry measurements were taken at each timepoint, and the lowest was used for analysis. Pachymetry measurements

preoperatively and 1, 3, 6, and 12 months postoperatively also were taken at the thinnest point using a rotating Scheimpflug camera (Pentacam, Oculus Optikgeräte GmbH).

Visual Acuity

The UDVA and CDVA were measured preoperatively and postoperatively at 1, 3, 6, and 12 months. Visual acuity measurements were obtained under controlled lighting conditions using a modified Early Treatment Diabetic Retinopathy Study visual acuity test (2nd edition, Lighthouse International) with Sloan letters. Patients were tested 4 m from the visual acuity chart. If patients could not read any letters at 4 m, they were tested at 2 m. Visual acuity was recorded and analyzed as the logMAR value.

Topography

Topography measurements were obtained using a rotating Scheimpflug camera. Topographic maximum K was obtained directly from the Scheimpflug data preoperatively and 1, 3, 6, and 12 months postoperatively.

Endothelial Cell Count

The ECC was obtained using specular microscopy (Konan Medical) preoperatively and 12 months postoperatively. Three measurements were taken at both timepoints, and the mean cell count for each eye was used in the analysis. In 2 patients, it was not possible to obtain readable ECCs; these patients were excluded from this part of the analysis.

Statistical Analysis

Statistical analysis using a paired 2-tailed Student t test was used to analyze the postoperative changes compared with baseline and to analyze the changes in postoperative outcomes over time. A P value less than 0.05 was considered statistically significant.

RESULTS

Forty-eight patients with keratoconus (35) or post-LASIK ectasia (13) who had CXL were analyzed; 26 patients received standard riboflavin–dextran solution during UVA light administration, and 22 patients received hypotonic riboflavin solution during UVA light administration. Table 1 the shows baseline characteristics in each treatment group.

Corneal Thickness

Figure 1 shows the mean US pachymetry at different stages. There was no statistically significant difference in the mean US pachymetry measurements preoperatively between the hypotonic group and the standard group (P = 0.7). Immediately after epithelium removal, the mean US pachymetry was 386 μm and 403 μm in the standard and hypotonic riboflavin groups, respectively (P = .15). After 30-minute administration of standard riboflavin (in both groups), if the pachymetry was less the 400 μm in either study group,

Table 1. Baseline patient characteristics.

Characteristic	Standard Riboflavin (n = 26)	Hypotonic Riboflavin (n = 20)
Pathology, n (%)		
Keratoconus	18 (69)	15 (75)
Ectasia	8 (31)	5 (25)
Mean age (y)	29	28
Sex, n (%)		
Male	20 (77)	16 (80)
Female	6 (23)	4 (20)
Mean maximum K (D) $\pm$ SD	58.9 $\pm$ 10.9	61.7 $\pm$ 11.5
Mean UDVA (logMAR) $\pm$ SD	0.77 $\pm$ 0.37	0.87 $\pm$ 0.26
Mean CDVA (logMAR) $\pm$ SD	0.29 $\pm$ 0.26	0.23 $\pm$ 0.13
Mean ECC (cells/mm^2) $\pm$ SD	2475 $\pm$ 269	2512 $\pm$ 309

CDVA = corrected distance visual acuity; ECC = endothelial cell count; K = keratometry; UDVA = uncorrected distance visual acuity

the cornea was swelled with hypotonic riboflavin per the study protocol until the corneal thickness was 400 μm or more. Nine eyes (35%) in the standard riboflavin group and 6 eyes (30%) in the hypotonic study group required hypotonic swelling. After initial corneal riboflavin loading and additional swelling (if required), but before UVA light administration, there was no significant difference in the mean US pachymetry measurements between groups (P = .8). Postoperatively immediately after the 30-minute UVA light administration, the mean pachymetry ranged from 252 to 420 μm and from 271 to 448 μm in the standard group and hypotonic riboflavin group, respectively. During UVA exposure, the standard group thinned by a mean of 128 μm (30%) compared with thinning of 90 μm (21%) in the hypotonic riboflavin group, a finding that was significant (P = .0002).

To determine whether there was any influence of pre-UVA hypotonic swelling to ultimate intraoperative stromal thinning, a subset analysis was performed; for this analysis, all eyes requiring hypotonic riboflavin before UVA light administration were excluded. In this cohort, before removal of the corneal epithelium, the mean US pachymetry measurements were 454 μm and 460 μm in the standard group and hypotonic riboflavin group, respectively (P = .68). Immediately after epithelium removal, the mean pachymetry was 401 μm and 413 μm, respectively (P = .31). After 30-minute administration of standard riboflavin (in both groups), the mean pachymetry was 437 μm and 441 μm in the standard group and hypotonic riboflavin group, respectively (P = .7). Postoperatively, immediately after UVA light administration, the mean pachymetry was 308 μm and 346 μm, respectively. Thus, during UVA exposure, the standard
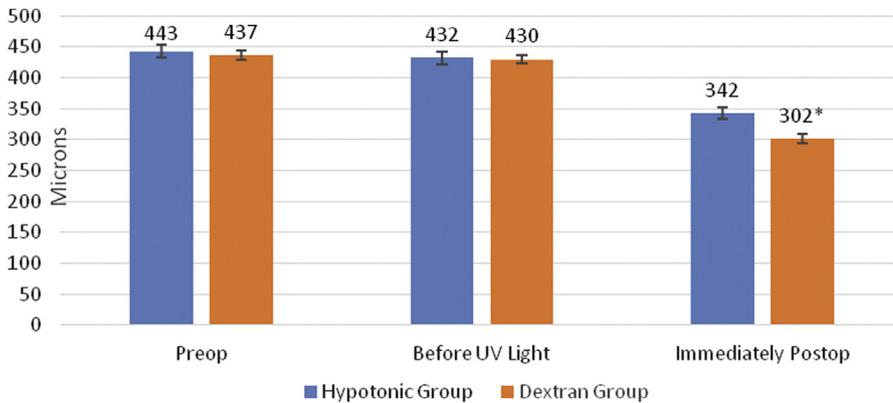
Figure 1. Mean intraoperative pachymetry obtained with US pachymetry measurement (UV = ultraviolet-A light).

group thinned by 129 μm (30%) on average compared with thinning of 95 μm (22%) in the hypotonic riboflavin group ($P = .0026$), corroborating the significant finding in the entire cohort.

Postoperative Corneal Thickness

The postoperative mean thinnest pachymetry by rotating Scheimpflug camera analysis in the standard riboflavin group was 414 μm, 419 μm, 420 μm, and 429 μm at 1, 3, 6, and 12 months, respectively; in the hypotonic group it was 434 μm, 428 μm, 430 μm, and 441 μm, respectively ($P = .189$, $P = .529$, $P = .470$, and $P = .380$, respectively) (Figure 2). The mean thinnest pachymetry in the standard riboflavin group changed from 431 μm preoperatively to 429 μm at 12 months; in the hypotonic riboflavin group, the mean pachymetry changed from 445 μm preoperatively to 441 μm at 12 months. The difference between preoperative and 12-month postoperative pachymetry was −2 μm and −4 μm in the standard group and hypotonic riboflavin group, respectively ($P = .82$). Thus, there was no significant change in Scheimpflug-measured corneal thickness in either group or between treatment groups from preoperatively to 1 year

postoperatively. In both groups, the natural history of postoperative corneal thickness showed thinning at 1 month with recovery to baseline over the year.

Maximum Topographic Keratometry

Figure 3 shows the maximum K values obtained with rotating Scheimpflug corneal topography.

There were no statistically significant differences in the postoperative mean maximum K between the 2 groups at 1, 3, 6, or 12 months ($P = .58$, $P = .42$, $P = .84$, and $P = .54$, respectively). The difference between the preoperative and 12-month postoperative maximum K was −0.80 diopter (D) and −2.07 D in the standard group and hypotonic riboflavin group, respectively ($P = .33$). Thus, although there was an improvement in maximum K in each group over the 1-year after CXL, there was no statistically significant difference in efficacy between the 2 groups.

Figure 4 shows a scatterplot of corneal thickness immediately after UVA administration in individual eyes compared with the change in maximum K in those eyes. There was no association between intraoperative corneal thinning and the 1-year change in maximum K (Figure 5).
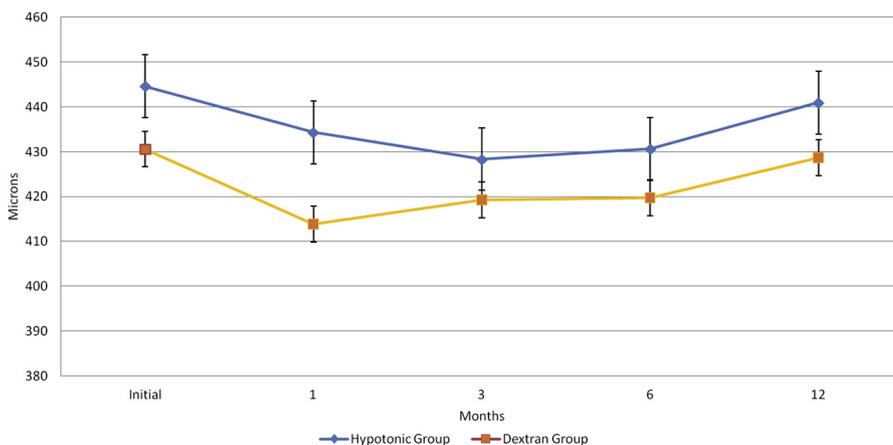

Figure 2. Mean pachymetry over time obtained with rotating Scheimpflug camera measurement.
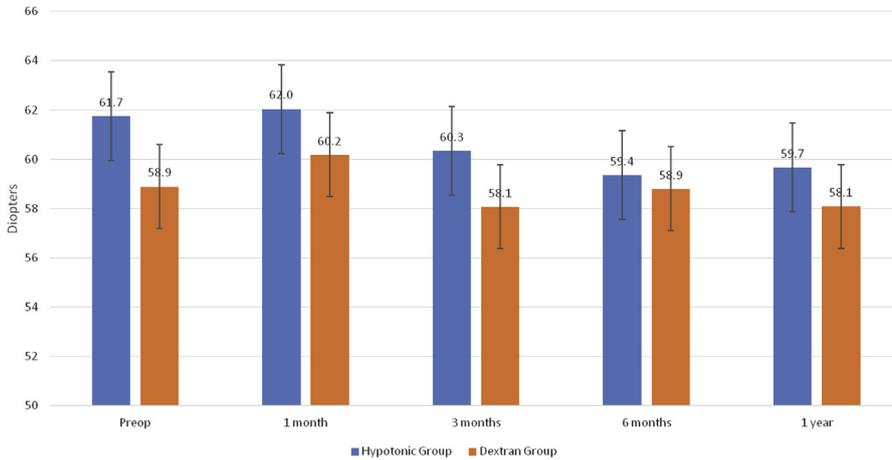
Figure 3. Maximum K obtained with rotating Scheimpflug camera measurement.

Uncorrected Distance Visual Acuity

Figure 5 shows the mean logMAR UDVA preoperatively and after CXL. There were no statistically significant differences in the postoperative mean logMAR UDVA between the 2 groups at 1, 3, 6, or 12 months ($P = .15$, $P = .25$, $P = .31$, and $P = .08$, respectively). The mean UDVA in the standard riboflavin group improved 0.09 logMAR units from preoperatively to 1 year postoperatively. In the hypotonic riboflavin group, the mean UDVA decreased 0.01 logMAR units from preoperatively to 1 year. The change in the mean UDVA from preoperatively to 1 year postoperatively was not statistically significant within groups (standard 12 months versus standard preoperative: $P = .40$; hypotonic 12 months versus preoperative hypotonic: $P = .92$). The mean change between the preoperative and 12-month postoperative UDVA was −0.09 and +0.01 in the standard group and hypotonic riboflavin group, respectively ($P = .17$). Thus, although there was an improvement in UDVA in the standard riboflavin group only, this was not statistically significant, and there was no significant difference in UDVA outcome between the 2 groups.
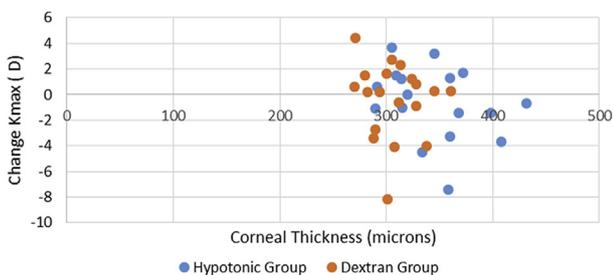

Figure 4. Corneal thickness immediately after UVA administration versus change in maximum K 1 year after CXL (Kmax = maximum keratometry).

Corrected Distance Visual Acuity

Figure 6 shows the mean logMAR CDVA preoperatively and after CXL. There were no statistically significant differences in the postoperative mean logMAR CDVA between the 2 groups at 1, 3, 6, or 12 months ($P = .39$, $P = .78$, $P = .27$, and $P = .28$, respectively). The mean CDVA in the standard riboflavin group improved 0.11 logMAR units from preoperatively to 1 year postoperatively. In the hypotonic riboflavin group, the mean CDVA remained unchanged preoperatively and at 1 year. These findings were not statistically significant within groups (standard 12 months versus standard preoperative: $P = .09$; hypotonic 12 months versus hypotonic preoperative: $P = .80$). The mean change between the preoperative and 12-month postoperative CDVA was −0.11 logMAR and 0.00 logMAR in the standard group and hypotonic group, respectively, a statistically significant finding ($P = .02$).

Endothelial Cell Count

The mean preoperative ECC was 2475 cells/mm^2 ± 309 (SD) and 2512 ± 269 cells/mm^2 in the standard group and hypotonic riboflavin group, respectively ($P = .64$). The mean 1-year postoperative EEC was 2405 ± 363 cells/mm^2 and 2284 ± 438 cells/mm^2, respectively ($P = .49$). The change in EEC was −70 cells/mm^2 (2.8%) and −228 cells/mm^2 (9.0%) in the standard group and hypotonic riboflavin group, respectively ($P = .37$) (Figure 7).

There was no relationship between the immediate postoperative corneal thickness and the 1-year change in EEC (Figure 8). Moreover, there was no relationship between EEC changes and the change in corneal thickness from preoperatively to 1 year postoperatively (Figure 9). Of note, no clinical instance of corneal swelling or endothelial decompensation was seen.
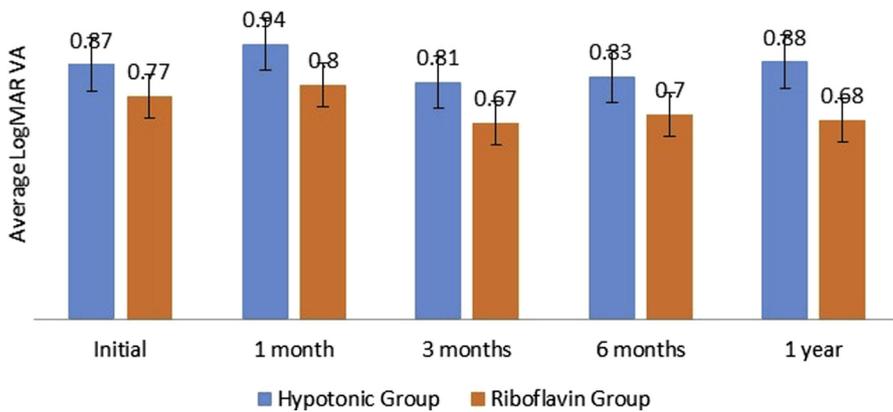
Figure 5. Mean logMAR UDVA preoperatively and after CXL (VA = visual acuity).

DISCUSSION

Collagen crosslinking is a promising new treatment for keratoconus and corneal ectasia. Crosslinking is thought to biomechanically strengthen the corneal stroma and, consequently, slow the progression of keratoconus and ectasia.[12] In many cases, CXL improves the patient's vision[4,9] and topography outcomes[7,8] with few reported complications.[13]

The original parameters for CXL, established in a series of time- and dose-response assays in animal models, suggested a minimum stromal thickness of 400 μm to attenuate the UVA power and thus prevent endothelial damage.[14] Although in general investigators have heeded this admonition before administering UVA light in the CXL procedure, previous studies have shown significant corneal thinning intraoperatively. Kymionis et al.[15] found a mean 75 μm decrease in corneal thickness during the riboflavin loading phase of the CXL procedure. Similarly, using optical coherence tomography, Mazzotta and Caragiuli[16] found a mean 79 μm thinning of the cornea during the first 10 minutes of riboflavin loading.

In a study of 20 eyes treated with hypotonic riboflavin before CXL, Hafezi et al.[10] found that swelling of the corneal stroma could be achieved using a solution with a low colloid osmotic pressure as a result of the hydrophilicity of stromal proteoglycans. Thus, the use of hypotonic riboflavin to swell corneas that are less than 400 μm thick has become the current protocol. Typically, after the corneal epithelium is removed, riboflavin–dextran solution is administered every 2 minutes for 30 minutes. The corneal thickness is then measured and if less than 400 μm, hypotonic riboflavin is administered every 10 seconds for 2-minute sessions until the stroma has reached the 400 μm goal. The patient then has UVA irradiation during which additional riboflavin and dextran solution is administered.

Although corneal swelling with hypotonic riboflavin can effectively swell the cornea, studies suggest that this swelling is short-lived. Kaya et al.[17] showed that the stromal swelling militated by hypotonic riboflavin was transient and decreased significantly after 10 minutes and 30 minutes of UVA light administration, suggesting that the loading process with hypotonic riboflavin was insufficient to protect the corneal endothelium throughout the procedure. Supporting this concern that treating thin corneas might lead to undesired outcomes, Raiskup et al.[18] reviewed cases of permanent corneal haze after CXL, concluding that thin corneas and high preoperative maximum K values predisposed to a worse outcome. Specifically, they found that the mean preoperative corneal thickness in a cohort of patients in whom clinically
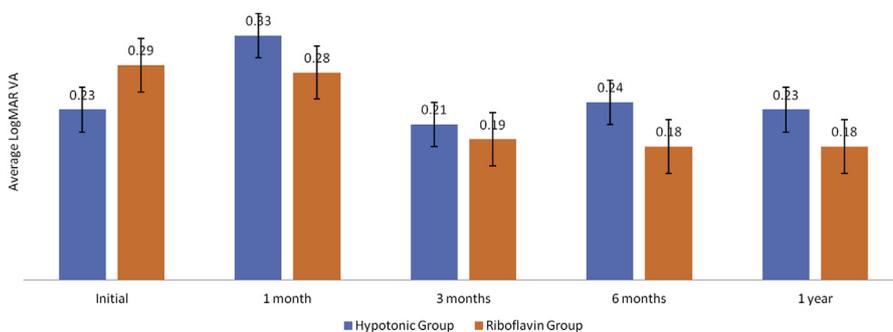

Figure 6. Mean logMAR CDVA preoperatively and after CXL (VA = visual acuity).
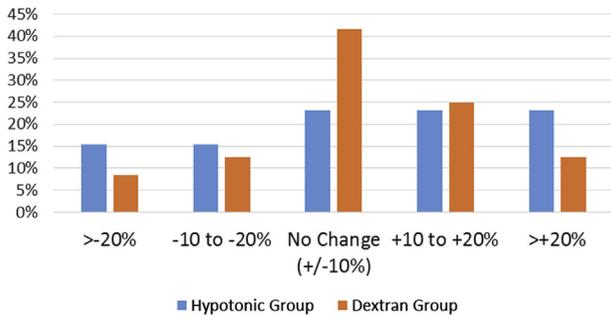
Figure 7. Stratified change in EEC.

Figure 8. Corneal thickness immediately after UVA administration versus change in EEC (EEC = endothelial cell count).

significant stromal haze was found at 1 year was 420 μm compared with 478 μm in patients who had clear corneas without stromal haze at 1 year. Moreover, the mean preoperative K value in the haze group was 71.0 D compared with 62.0 D in the no-haze group. This finding is somewhat belied by our group's previous study,[19] in which eyes with worse a maximum K value were more likely to improve than less advanced eyes.

Raiskup and Spoerl[20] further studied the use of riboflavin solutions in a cohort of eyes with corneas thinner than 400 μm. In these eyes, they loaded the cornea with hypotonic riboflavin and continued to use hypotonic riboflavin during UVA light administration. Their results showed CXL efficacy results similar to those with standard riboflavin preparations.

Given the limited knowledge of corneal thickness changes during the CXL procedure and the clinical implications of using different riboflavin formulations, in this current study, we attempted to answer 4 questions: (1) How much corneal thinning occurs in the CXL procedure in general? (2) Is there a difference in
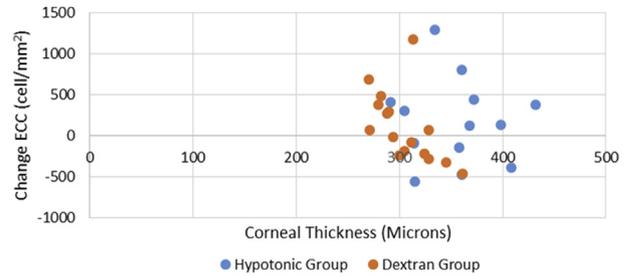
the intraoperative thinning of the cornea between treatments using riboflavin in a dextran carrier and hypotonic riboflavin? (3) Is thinning related to the efficacy (change in maximum K and visual acuity) or safety (change in EEC) of the procedure? (4) Is there a difference in efficacy or safety outcomes between the 2 riboflavin solution regimens? Our study was confined to a standard CXL protocol using 3 mW/cm^2 for 30 minutes. We did not study accelerated CXL using higher irradiance UVA light sources; thus, the results reported here might not be generalizable to accelerated CXL protocols.

With regard to intraoperative changes in corneal thickness, we found that in general, there was substantial stromal thinning over the course of the CXL procedure. In the entire treated cohort, there was thinning of 113 μm (26%), on average. However, there was a significant difference between the standard group and hypotonic riboflavin–treated group—a decrease of 128 μm (30%) versus 90 μm (21%). Thus, the use of hypotonic riboflavin during UVA irradiation does seem to have a mitigating effect on intraoperative
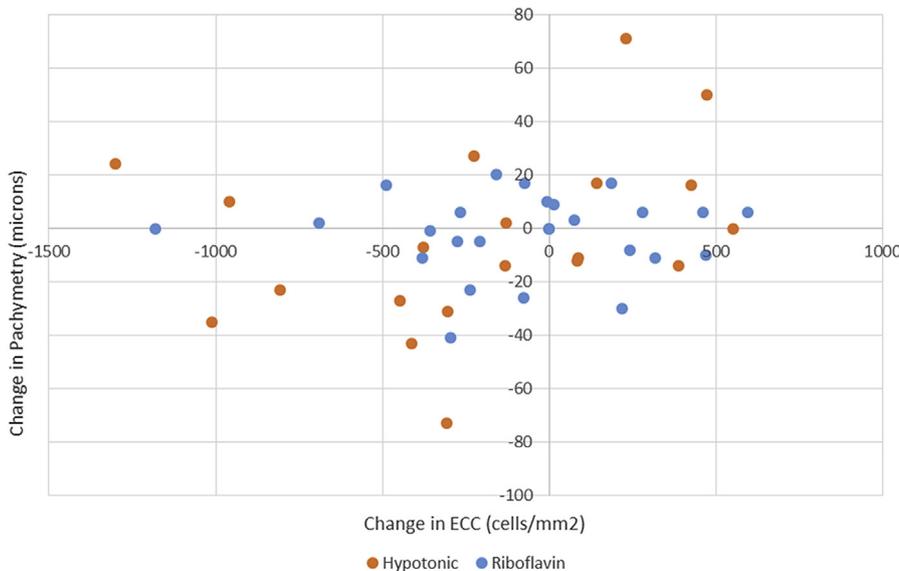

Figure 9. Change in EEC versus change in corneal thickness 1 year after CXL (EEC = endothelial cell count).

corneal thinning. Of note, the need for corneal swelling to the 400 μm starting point for UVA administration did not influence subsequent thinning during the procedure.

The principal outcome that has been used to evaluate the efficacy of CXL is the maximum K value derived from corneal topography analysis. By measuring the maximum steepness of the keratoconic cornea, flattening of the maximum K acts as a proxy for treatment success and steepening of the maximum K is an indication of poorer procedure efficacy. Collagen crosslinking has been shown to flatten the maximum K on average,[4,5] and such flattening has been accompanied by a general improvement in corneal topography and HOAs.[7,8]

Using hypotonic riboflavin instead of the standard riboflavin–dextran solution might be expected to improve effectiveness. Wollensak et al.[11] found that the initial thickness of the riboflavin solution on the surface of the cornea was 70 μm for riboflavin–dextran compared with 40 μm for hypotonic riboflavin. Moreover, the breakup time of the riboflavin film was 22 minutes for riboflavin–dextran but only 90 seconds for hypotonic riboflavin. Thus, there is more absorption of UVA light by the surface riboflavin itself in the setting of riboflavin–dextran dropping than with hypotonic riboflavin dropping. In addition, irradiance to the corneal stroma would be expected to be greater and of deeper penetration in the hypotonic group because the UVA light is not being blocked by the surface riboflavin solution.

Mitigating this potential beneficial effect of hypotonic riboflavin on CXL efficacy, paradoxically, might be the finding that there is less thinning in this group. When the cornea thins, as it does more in the riboflavin–dextran treatments, the compression of the stromal elements might cause more robust CXL; that is, on restoration of normal corneal thickness postoperatively, the depth and efficacy of CXL might be greater in the riboflavin–dextran group. Belying this hypothesis, however, we did not find any relationship between the stromal thickness immediately after CXL and the ultimate change in the maximum K value at 1 year (Figure 4).

The mean 1-year improvement in the maximum K value was 0.8 D in the riboflavin–dextran group and 2.1 D in the hypotonic group. Although this difference appears substantial, it was not statistically significant. The seeming superiority of the hypotonic treatment is skewed by the fact that 1 eye in the hypotonic group had flattening of 23.4 D, from 88.0 D preoperatively to 64.6 D at 1 year. When this patient was excluded from the analysis, the mean maximum K in the hypotonic group changed from 60.36 D preoperatively to 59.41 D at 1 year, a mean improvement of 0.95 D ($P = .87$). Thus, although there was an improvement in maximum K in each group over the 1-year after CXL, there was no significant difference in efficacy between the 2 groups.

With regard to UDVA, there was a trend toward a slight average improvement in the standard riboflavin group; however, it was not statistically significant. Yet, the 1-line improvement in CDVA in the standard riboflavin group was significantly better than the stability in the hypotonic group. There was no obvious cause of these between-group visual acuity differences, and the clinical significance of this finding is unclear.

There was no change in corneal thickness between preoperatively and 1-year postoperatively in both groups. As in our previous work,[21] there was thinning in both groups at 1 month, with recovery to baseline over 1 year.

Of foremost interest in this study is the potential effect of intraoperative thinning on the corneal endothelium. As noted above, a 400 μm minimum stromal thickness has been suggested for the protection of the endothelium from UVA–riboflavin damage.[14] In this study, the mean corneal thickness at the conclusion of UVA administration was 325 μm; 29% were thinner than 300 μm, 46% were between 300 μm and 350 μm, 17% were between 350 μm and 400 μm, and only 8% were above the 400 μm suggested thickness. Despite those many corneas with substantial intraoperative thinning, the endothelium appeared unaffected. Among the entire cohort treated in this study, there was a mean loss of 130 cells/mm^2 (5%), a statistically insignificant change. In addition, there was no significant difference in the change in cell count between the standard group and hypotonic group despite the reduced intraoperative thinning in the group receiving hypotonic riboflavin as well as the putative protective effect of the riboflavin–dextran biofilm in the standard riboflavin group.

To further explore the effect of CXL on the endothelium and endothelial function, additional analyses were performed. There was no relationship between the immediate postoperative corneal thickness and 1-year change in EEC. This suggests that clinically, the endpoint corneal thicknesses that we found are safe to the endothelium. Moreover, there was no relationship between EEC changes and the change in corneal thickness from preoperatively to 1 year postoperatively, suggesting no clinical diminution in endothelial cell function from the procedure. Thus, even though 92% of corneas were thinner than 400 μm at the conclusion of the procedure, there was no evidence of damage to the endothelium. And, importantly, there were no instances of postoperative corneal edema.

The salient conclusions from this study are as follows: (1) The cornea thins substantially over the course of the CXL procedure as currently performed with 30 minutes of UVA exposure. (2) Corneal thickness is better maintained using a hypotonic riboflavin solution during the UVA phase of the procedure. (3) There are no differences in clinical outcomes between the 2 riboflavin regimens used, although there was a suggestion of improved CDVA in the standard riboflavin group. (4) Despite substantial stromal thinning during the procedure, there was no evidence of damage to the endothelium. Further studies in laboratory and clinical settings are necessary to refine the clinical parameters for the safety and efficacy of the CXL procedure.

WHAT WAS KNOWN

- Collagen crosslinking improves corneal topography–measured maximum K values in patients with keratoconus, thus likely decreasing disease progression and long-term prognosis.

- It has been suggested that a 400 μm corneal thickness is the minimum level for safety of the endothelium with CXL.

WHAT THIS PAPER ADDS

- The cornea thinned substantially, below the 400 μm suggested thickness, during the course of the standard CXL technique.

- Stromal thinning during CXL can be mitigated by use of a hypotonic riboflavin solution, decreasing stromal thinning by 30%.

- Intraoperative thinning did not appear to compromise the safety of the endothelium.

- Modulation of intraoperative corneal thinning during CXL did not affect the clinical outcomes.

REFERENCES

1. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A–induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003; 135:620–627. Available at: http://iogen.fi/files/2011/10/Wollensak_et_al_Keratoconus_2003.pdf. Accessed February 1, 2016
2. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998; 42:297–319. Available at: http://www.keratoconus.com/resources/Major+Review-Keratoconus.pdf. Accessed February 1, 2017
3. Seiler T, Koufala K, Richter G. Iatrogenic keratectasia after laser in situ keratomileusis. J Refract Surg 1998; 14:312–317
4. Hersh PS, Greenstein SA, Fry KL. Corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. J Cataract Refract Surg 2011; 37:149–160. Available at: http://www.vision-institute.com/UserFiles/File/CXL%20results%20published2011.pdf. Accessed February 1, 2016
5. Vinciguerra P, Albè E, Trazza S, Rosetta P, Vinciguerra R, Seiler T, Epstein D. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. Ophthalmology 2009; 116:369–378
6. Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet A corneal collagen cross-linking for keratoconus in Italy: the Siena Eye Cross Study. Am J Ophthalmol 2010; 149:585–593
7. Greenstein SA, Fry KL, Hersh PS. Corneal topography indices after corneal collagen crosslinking for keratoconus and corneal ectasia: one-year results. J Cataract Refract Surg 2011; 37:1282–1290
8. Greenstein SA, Fry KL, Hersh MJ, Hersh PS. Higher-order aberrations after corneal collagen crosslinking for keratoconus and corneal ectasia. J Cataract Refract Surg 2012; 38:292–302
9. Brooks NO, Greenstein S, Fry K, Hersh PS. Patient subjective visual function after corneal collagen crosslinking for keratoconus and corneal ectasia. J Cataract Refract Surg 2012; 38:615–619
10. Hafezi F, Mrochen M, Iseli HP, Seiler T. Collagen crosslinking with ultraviolet-A and hypoosmolar riboflavin solution in thin corneas. J Cataract Refract Surg 2009; 35:621–624
11. Wollensak G, Aurich H, Wirbelauer C, Sel S. Significance of the riboflavin film in corneal collagen crosslinking. J Cataract Refract Surg 2010; 36:114–120
12. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin–ultraviolet-A-induced cross-linking. J Cataract Refract Surg 2003; 29:1780–1785
13. Greenstein SA, Fry KL, Bhatt J, Hersh PS. Natural history of corneal haze after collagen crosslinking for keratoconus and corneal ectasia: Scheimpflug and biomicroscopic analysis. J Cataract Refract Surg 2010; 36:2105–2114. Available at: http://www.vision-institute.com/UserFiles/File/CXL%20Haze%20Published.pdf. Accessed February 1, 2016
14. Wollensak G, Spörl E, Reber F, Pillunat L, Funk R. Corneal endothelial cytotoxicity of riboflavin/UVA treatment in vitro. Ophthalmic Res 2003; 35:324–328
15. Kymionis GD, Kounis GA, Portaliou DM, Grentzelos MA, Karavitaki AE, Coskunseven E, Jankov MR, Pallikaris IG. Intraoperative pachymetric measurements during corneal collagen cross-linking with riboflavin and ultraviolet A irradiation. Ophthalmology 2009; 116:2336–2339
16. Mazzotta C, Caragiuli S. Intraoperative corneal thickness measurement by optical coherence tomography in keratoconic patients undergoing corneal collagen cross-linking. Am J Ophthalmol 2014; 157:1156–1162
17. Kaya V, Utine CA, Yılmaz ÖF. Intraoperative corneal thickness measurements during corneal collagen cross-linking with hypoosmolar riboflavin solution in thin corneas. Cornea 2012; 31:486–490
18. Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. J Refract Surg 2009; 25:S824–S828
19. Greenstein SA, Hersh PS. Characteristics influencing outcomes of corneal collagen crosslinking for keratoconus and ectasia: implications for patient selection. J Cataract Refract Surg 2013; 39:1133–1140
20. Raiskup F, Spoerl E. Corneal cross-linking with hypo-osmolar riboflavin solution in thin keratoconic corneas. Am J Ophthalmol 2011; 152:28–32
21. Greenstein SA, Shah VP, Fry KL, Hersh PS. Corneal thickness changes after corneal collagen crosslinking for keratoconus and

corneal ectasia: one year results. J Cataract Refract Surg 2011; 37:691–700

OTHER CITED MATERIAL

A. Hersh PS, Fry KL, "Corneal Thickness Changes During Corneal Collagen Crosslinking," presented at the annual meeting of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, FL, USA, May 2009. Abstract available at: http://iovs.arvojournals.org/article.aspx?articleid=2367856. Accessed February 1, 2016

B. U.S. National Institutes of Health Clinical Trials. Corneal Collagen Crosslinking for Progressive Keratoconus and Ectasia Using Riboflavin/Dextran and Hypotonic Riboflavin. NCT01152541. Available at: https://clinicaltrials.gov/ct2/show/NCT01152541. Accessed February 1, 2016