Intraoperative Pachymetric Measurements during Corneal Collagen Cross-Linking with Riboflavin and Ultraviolet A Irradiation

George D. Kymionis, MD, PhD, George A. Kounis, PhD, Dimitra M. Portaliou, MD,
Michael A. Grentzelos, MD, Alexandra E. Karavitaki, MD, Efekan Coskunseven, MD, Mirko R. Jankov, MD,
Ioannis G. Pallikaris, MD, PhD

Objective: To study central corneal pachymetric variations during corneal collagen cross-linking (CXL) treatment with the use of riboflavin and ultraviolet A irradiation (UVA).

Design: Prospective, noncomparative, interventional clinical study.

Participants: Fifteen keratoconic patients (19 eyes) were enrolled.

Methods: All patients underwent riboflavin-UVA–induced corneal CXL. Intraoperative central corneal thickness (CCT) measurements using ultrasound pachymetry were performed during the procedure. Measurements were obtained after epithelial removal, after riboflavin drop instillation, and every 5 minutes (6 interval times) during UVA irradiation (30 minutes).

Main Outcome Measures: Central corneal thickness measurements.

Results: Mean patient age was 26.9 ± 6.5 years (range, 17–40 years). Ten were male and 5 were female. Mean preoperative CCT was 458.5 ± 21.5 μm (range, 427–494 μm; 95% confidence interval [CI], 448–467 μm) and 415.7 ± 20.6 μm (range, 400–468 μm; 95% CI, 406–426 μm) before and after epithelial removal, respectively. There was a statistically significant decrease (mean, 75 μm) of CCT between the epithelial removal interval (415.7 ± 20.6 μm; range, 400–468 μm) and at the end of riboflavin solution instillation (340.7 ± 22.9 μm; range, 292–386 μm; $P<0.001$). There was no statistically significant change in CCT during irradiation ($P>0.05$). There was no statistically significant difference between preoperative and 1-month postoperative endothelial cell count (preoperative, 2780 ± 197 to 1-month postoperative, 2713 ± 116; $P = 0.14$). No intraoperative, early postoperative, or late postoperative complications were observed in this patient series.

Conclusions: During corneal CXL with the use of riboflavin and UVA irradiation, a statistically significant decrease of CCT was demonstrated.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. *Ophthalmology 2009;116:2336–2339 © 2009 by the American Academy of Ophthalmology.*

Keratoconus is a bilateral, progressive, noninflammatory corneal ectatic disorder appearing as a result of corneal biomechanical instability.[1] Progressive corneal thinning, which is the main characteristic of keratoconus, leads to irregular astigmatism and decrement in visual acuity. Until recently, proposed interventions for improving visual function included spectacles, rigid gas contact lenses, and intracorneal ring segments.[2] Penetrating or lamellar keratoplasty[3] may represent a therapeutic treatment when the patient becomes contact lens intolerant and corneal thinning has advanced.

Corneal collagen cross-linking (CXL) with riboflavin and ultraviolet A (UVA) irradiation[4,5] is a new technique with a minimally invasive nature used for the stabilization of different types of corneal ectatic disorders. The aim of this technique is the augmentation of the mechanical rigidity of the cornea by inducing cross-links at the corneal stroma.[6] Wollensak[5] proposed a corneal preoperative thickness of 400 μm as a minimum safety limit to avoid endothelium, lens, and retinal damage. He also reported corneal keratocyte apoptosis after CXL of up to 300 μm depth in rabbit

corneas.[7] This prospective study evaluated the intraoperative pachymetric variations during corneal CXL with the use of riboflavin and UVA irradiation in keratoconic patients.

Patients and Methods

Patient Population

In this prospective clinical study, 15 patients (19 eyes) who underwent riboflavin–UVA irradiation-induced corneal CXL for keratoconus were enrolled. The total number of eyes enrolled in the study was estimated by power analysis. Power analysis was performed in 3 eyes (power, 80%; $\alpha = 0.05$) over an analysis of variance (ANOVA). The total sample size indicated 80 eyes for 8 groups with 0.7 effect size.

Patients with one of the following criteria after the preoperative examination were excluded from the study: age younger than 16 or older than 45 years, corneal scars or opacities, pregnancy or lactation, active anterior segment pathologic features, previous corneal or anterior segment surgery, systemic connective tissue disease, atopic syndrome, ocular or systemic disease that could

Table 1. Intraoperative Central Corneal Pachymetry Variations during Corneal Collagen Cross-Linking

| | | | 95% Confidence Interval for Mean | | | |
	Mean	Standard Deviation	*Lower Bound*	*Upper Bound*	Minimum	Maximum
Post Ep	415.7	20.6	405.7	425.6	400	468
Post Rivo	340.7	22.9	329.7	351.8	292	386
5-minute irrad	325.1	24.8	313.1	337.1	290	377
10-minute irrad	325.3	22.7	314.3	336.2	297	374
15-minute irrad	333.3	23.1	322.2	344.5	287	374
20-minute irrad	324.6	18.7	315.6	333.7	295	360
25-minute irrad	327.3	17.9	318.7	336.0	305	367
30-minute irrad	327.2	19.4	317.9	336.6	281	369

Irrad = irradiation; Post Ep = after epithelial removal; Post Rivo = after riboflavin instillation.

affect the epithelial healing, and dry eye syndrome. All patients were advised to discontinue contact lens wear 1 month before the preoperative evaluation.

Institutional review board approval was obtained and all patients were informed appropriately before their participation in the study and gave written informed consent in accordance with institutional guidelines, according to the Declaration of Helsinki. Preoperative data obtained from the case records included patient age and gender, central corneal thickness (CCT) using ultrasonic corneal pachymetry (Corneo-Gage Plus; Sonogage, Inc., Cleveland, OH), slit-lamp biomicroscopy, and fundus examination.

Surgical Technique

Corneal CXL was conducted under sterile conditions. A speculum was placed at the patient's eye. Before epithelial removal, a CCT measurement was performed. The probe tip of the pachymeter was held perpendicular to the cornea. Three consecutive measurements were obtained at the center of the cornea of each eye; the thinnest measurement was used in the statistical analysis. An 8.5- to 9.0-mm diameter of the corneal epithelium was removed mechanically using a rotating brush. After the removal of the corneal epithelium, 3 CCT measurements were repeated. Riboflavin 0.1% solution was instilled every 3 minutes for approximately 30 minutes, and CCT was measured again. Ultraviolet A irradiation was performed using a commercially available UVA system (UV-X; Peschke Meditrade, GmbH, Huenenberg, Switzerland). Before treatment, the intended 3 mW/cm^2 surface irradiance (5.4 J/cm^2 surface dose after 30 minutes) was calibrated using the UVA meter YK-34UV (Lutron Electronic Enterprise Co, Ltd, Taipei, Taiwan), which is supplied with the UV-X device. Irradiance was performed for 30 minutes. During treatment, riboflavin solution was applied every 5 minutes and CCT measurements were obtained every 5 minutes as well. The CCT measurements were obtained during irradiation in between riboflavin drop instillation. At the end of the procedure, a silicon–hydrogel bandage contact lens (Lotrafilcon B; Air Optix, Ciba Vision, Duluth, GA); 14.0 mm diameter, 8.6 base curvature, Dk = 140 barrers) was applied until full reepithelialization (which typically occurred after 4 days).

Postoperative medication included diclofenac sodium 0.1% (Denaclof, Novartis, Hellas, Greece) for 2 days as well as antibiotic and corticosteroid (tobramycin and dexamethasone) drops (Tobradex; Alcon Laboratories, Inc., Fort Worth, TX) 4 times daily until the removal of the bandage contact lens. After the removal of the contact lens, patients received corticosteroid drops (fluorometholone 0.1%; Falcon Pharmaceuticals, Fort Worth, TX) tapering for the next 3 weeks. Patients were encouraged to use artificial tears at least 6 times daily for 3 months after surgery.

Statistical Analysis

Eight groups were created for pachymetric measurements in different steps during the CXL procedure. Those groups were: after epithelial removal, after riboflavin drops application (30-minute of isotonic riboflavin instillation), and 5, 10, 15, 20, 25, and 30 minutes during irradiation. Measurements were obtained at the center of the cornea by the same surgeon (GDK).

An ANOVA was used for the statistical analysis. All assumptions for the use of ANOVA were met, because the 8 groups were following normal distributions ($P > 0.05$, Kolmogorov test) and the variances between groups were even ($P > 0.05$, Levene test). Images of the endothelium were acquired (before and 1 month after the treatment) with a modified confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph II/Rostock Corneal Module; Heidelberg Engineering, GmbH, Dossenheim, Germany).

Results

Mean patient age was 26.9 ± 6.5 years (range, 17–40 years). Ten were male and 5 were female. Mean preoperative CCT was 458.5 ± 21.5 μm (range, 427–494 μm; 95% confidence interval, 448–467 μm) and 415.7 ± 20.6 μm (range, 400–468 μm; 95% confidence interval, 406–426 μm) before and after epithelial removal, respectively (Table 1). An ANOVA revealed that there is a statistically significant difference between the 8 groups ($F = 40$, degrees of freedom between groups, 7; $P < 0.001$). There was a statistically significant decrease (mean, 75 μm) of CCT after riboflavin solution instillation (CCT after epithelial removal, 415.7 ± 20.6 μm; range, 400–468 μm) to the end of riboflavin
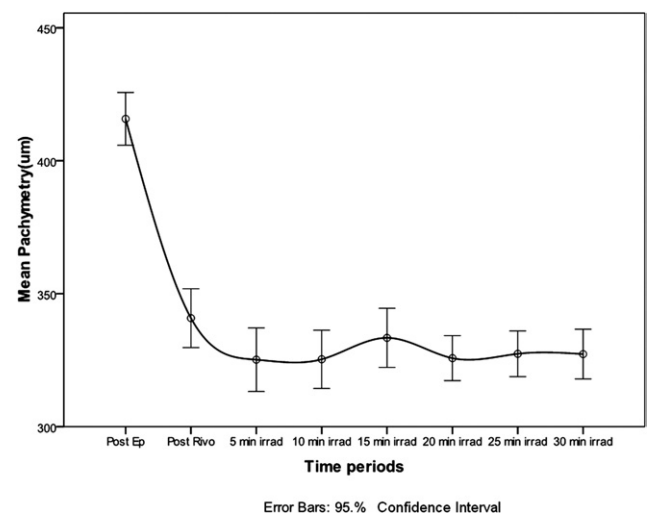

Figure 1. Graph showing variations in central corneal thickness with time during corneal collagen cross-linking. Irrad = irradiation; Post Ep = after epithelial removal; Post Rivo = after riboflavin instillation.

instillation (340.7±22.9 μm; range, 292–386 μm; $P<0.001$). There was no statistically significant difference between riboflavin instillation and any interval during the 30-minute UVA irradiation ($P>0.05$; Fig 1).

The mean values of pachymetry for all eyes in which all 3 measurements made in each interval were considered was 359±36.5 μm. An ANOVA revealed a variance component for repeatability at 1.68% for Gauge R&R analysis.[8]

No intraoperative, early postoperative, or late postoperative complications were observed in this patient series. There was no statistically significant difference between preoperative and 1-month postoperative endothelial cell count (preoperative, 2780±197 cells/mm^2; to 1-month postoperative, 2713±116 cells/mm^2; $P = 0.14$].

Discussion

Corneal CXL treatment is a relatively new, minimally invasive procedure for the stabilization of corneal ectatic disorders such as keratoconus or post-LASIK ectasia. The combined use of riboflavin and UVA irradiation creates new chemical bonds (cross-links) between collagen fibrils at the corneal stroma, resulting in an increase of the biomechanical stability of the corneal tissue.

The role of riboflavin in corneal CXL is dual. It works as a photosensitizer for the induction of cross-links and at the same time protects the underlying tissues from the harmful influence of UVA irradiation. Riboflavin (vitamin B$_2$) has 3 peaks in its absorption spectrum at 270, 366, and 445 nm. During CXL, the corneal tissue is illuminated with ultraviolet light at a frequency of 370 nm, a wavelength that is strongly absorbed by riboflavin. During treatment, riboflavin has a dual action of producing free radicals that cause cross-linking of the stromal collagen (strengthening the cornea), as well as acting as a shield to prevent significant levels of ultraviolet light from penetrating the eye. More than 90% of the ultraviolet light is absorbed in the cornea, in addition the anterior chamber, and riboflavin reduces the ultraviolet intensity to a level that is a factor of 1000 smaller than the official safety level. It has been proven by Wollensak et al[9] that the cell damage threshold of UVA irradiation combined with riboflavin is 10 times lower than that of UVA irradiation alone.

It is well established that keratocytes are exposed to UVA irradiation during treatment and that cell death occurs. Animal experiments[7] and clinical studies[10,11] conclude that keratocyte apoptosis 24 hours after treatment is found at the anterior 300 μm of the cornea stroma. This cell apoptosis follows the Lambert–Beer law, according to which smaller irradiances can lead to shallower cell death.

The structural alterations of the cornea after corneal CXL have been described by means of confocal microscopy analysis.[12,13] Confocal images of the cornea reveal keratocyte absence from the anterior 300 μm of the stroma 3 months after CXL treatment and gradual keratocyte repopulation in the anterior and midcorneal stroma 6 to 12 months after the procedure. Furthermore, endothelium was found intact and no endothelial cell damage was evident.

Wollensak,[5] in a previous study, proposed a preoperative corneal thickness of 400 μm as a minimum safety limit to avoid posterior corneal tissue damage during CXL. Spoerl et al[14] state clearly that a safety threshold of 400 μm corneal thickness including riboflavin distribution is necessary to minimize UVA irradiance (less than 1 J/cm^2) at the level of the corneal endothelium, anterior chamber, lens, and retina.

In this prospective clinical study, CCT variations were evaluated during corneal CXL with the use of riboflavin and UVA irradiation. The patients underwent subsequent ultrasound pachymetry measurements before and after epithelial removal, after 30 minutes of isotonic solution of 0.1% riboflavin and 20% dextran instillation, and every 5 minutes during UVA irradiation. A decrease of approximately 20% in CCT was evident after riboflavin drops were instilled that remained stable until the completion of the procedure. In certain cases, measurements were less than 300 μm, with a minimum of 270 μm in one eye. Although pachymetric values decreased by approximately 20%, reaching values of approximately 300 μm during treatment, patients' postoperative examinations showed no early (1-month) side effects such as endothelial cell toxicity. Nevertheless, these findings do not mean that the CXL indications could be expanded in patients with preoperative corneal thickness of less than 400 μm. The original protocol for CXL may have undertaken these CCT variations while no side effects were demonstrated. Treating thinner corneas (less than 400 μm) with the current protocol may cause serious complications because it has been shown in the current study that there is a decrease in CCT during the procedure. Other treatment protocols (such as using hypo-osmolar riboflavin solution[15]) may represent another treatment option for thinner corneas. In this study, a hypotonic solution was not used to explore intraoperative pachymetric differences according to the original and standard protocol used.

A few potential limitations were apparent in this study. First, whether all measurements were obtained at the same location—at the center of the cornea and not at the mid periphery— is not certain. Second, ultrasound pachymetry measurements are not always accurate and may depend, in some grade, on the corneal stroma hydration.

In conclusion, during the intraoperative course of CXL, a significant pachymetric decrease was demonstrated in this study. Nevertheless, this finding was not related to complications attributed to the procedure in the patients.

References

1. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998;42:297–319.
2. Rabinowitz YS. Intacs for keratoconus. Curr Opin Ophthalmol 2007;18:279–83.
3. Pramanik S, Musch DC, Sutphin JE, Farjo AA. Extended long-term outcomes of penetrating keratoplasty for keratoconus. Ophthalmology 2006;113:1633–8.
4. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620–7.
5. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol 2006;17:356–60.

6. Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye Res 1998;66:97–03.
7. Wollensak G, Spoerl E, Wilsch M, Seiler T. Keratocyte apoptosis after corneal collagen cross-linking using riboflavin/UVA treatment. Cornea 2004;23:43–9.
8. Barrentine LB. Concepts for R&R studies. 2nd ed. Milwaukee, WI: Quality Press; 2003;25–32.
9. Wollensak G, Spörl E, Reber F, et al. Corneal endothelial cytotoxicity of riboflavin/UVA treatment in vitro. Ophthalmic Res 2003;35:324–8.
10. Caporossi A, Baiocchi S, Mazzotta C, et al. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A rays induced cross-linking of corneal collagen: preliminary refractive results in an Italian study. J Cataract Refract Surg 2006; 32:837–45.
11. Mazzotta C, Traversi C, Baiocchi S, et al. Conservative treatment of keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: qualitative investigation. Eur J Ophthalmol 2006;16:530–5.
12. Mazzotta C, Balestrazzi A, Traversi C, et al. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructural analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. Cornea 2007;26:390–7.
13. Kymionis GD, Diakonis VF, Kalyvianaki M, et al. One-year follow-up of corneal confocal microscopy after corneal cross-linking in patients with post laser in situ keratomileusis ectasia and keratoconus. Am J Ophthalmol 2009;147:774–8.
14. Spoerl E, Mrochen M, Sliney D, et al. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007;26:385–9.
15. 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;35:621–4.

Footnotes and Financial Disclosures

Correspondence:
Dimitra M. Portaliou, MD, Institute of Vision and Optics (IVO), University of Crete, Medical School, 71003 Heraklion, Crete, Greece. E-mail: mimi24279@gmail.com.