Collagen Cross-linking in Early Keratoconus With Riboflavin in a Femtosecond Laser-created Pocket: Initial Clinical Results

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ABSTRACT

PURPOSE: To evaluate the safety and efficacy of a novel femtosecond laser-assisted technique for intracorneal administration of riboflavin and higher fluence ultraviolet A (UVA) light in collagen cross-linking (CXL) for keratoconus.

METHODS: Ten cases of early keratoconus were treated with CXL and followed for a mean of 26 months (range: 18 to 36 months). Using a femtosecond laser, a 100-μm deep, 7-mm diameter intracorneal pocket was created. Two 0.1-mL doses of 0.1% riboflavin sodium phosphate solution were infused into the pocket and, with a 2 minute soak period, the cornea was irradiated with 7 mW/cm^2 UVA light of mean 370 nm wavelength for 15 minutes. Changes to uncorrected (UCVA) and best spectacle-corrected visual acuity (BSCVA), refraction, keratometry (K), corneal thickness, Pentacam topography, endothelial cell count, and intraocular pressure were evaluated.

RESULTS: Mean UCVA improved from 20/50 to 20/40, BSCVA was unchanged at 20/20, mean sphere was reduced by 0.50 diopters (D), mean cylinder was reduced by 0.90 D, maximum mean K reduced from 49.50 to 48.10 D, no ectasia progression (defined as increase in K over 3-month follow-up) was noted during the follow-up time studied, and no statistically significant change in endothelial cell count was seen. The mean thinnest corneal thickness appeared to initially reduce but the mean returned to at least the preoperative level by 18 months. All patients returned to full activities within 1 day postoperative. No adverse effects were noted in any of the cases studied.

CONCLUSIONS: This novel epithelium-sparing, rapid soak-and-treat method of intrastromal riboflavin instillation and higher fluence UVA light for CXL appears to be safe and effective. No negative biomechanical effect (ectasia/epithelial ingrowth) was noted due to the femtosecond laser-created pocket. Because minimal epithelial injury occurs using this technique, postoperative pain appears to be significantly reduced. [*J Refract Surg.* 2009;xx:xxx-xxx.] doi:10.3928/1081597X-2009

The management of keratoconus with collagen cross-linking (CXL) utilizing ultraviolet A (UVA) light irradiation and simultaneous topical riboflavin administration has been studied at length both in the laboratory as well as clinically[1-14] and received CE marking in December 2006 for clinical use in the European Union countries. The standard technique involves partial or complete central epithelial removal followed by topical administration of riboflavin 0.1% solution to achieve intrastromal penetration.[1]

It was theorized that by instilling the riboflavin solution paracentrally within the cornea, with minimal epithelial surface disturbance, and by using a higher fluence UVA light source the following could be targeted: 1) faster re-epithelialization and visual rehabilitation for the patient with significant reduction in pain from the procedure; 2) faster diffusion/soak period as the (large molecule, in regard to the Bowman's layer barrier) riboflavin is directly injected at depth via the intrastromal pocket, providing greater shielding near the endothelium; and 3) selective CXL of the anterior two-thirds of the cornea. We expected minimal toxicity to the endothelium, as no significant concentration of riboflavin with this technique was expected to be activated by stray UVA light at that level.

PATIENTS AND METHODS

Ten consecutive "early" keratoconic corneas were selected with the following criteria: topographic evidence of keratoconus (keratometry [K] >48.00 diopters [D] and/or inferior steepening >1.00 D) in the superior half of the cornea, minimum corneal thickness $\geq$500 µm, and patient age $\geq$18 years.

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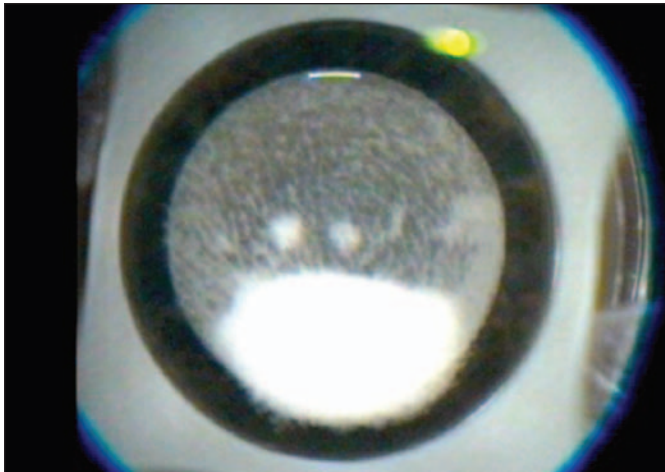
Figure 1. Clinical photograph demonstrating the intracorneal "pocket" created with the IntraLase FS60 femtosecond laser. The cornea has been laminated at 100 μm-depth, in a 7-mm diameter disk, and a side-cut of 10°, which can be seen at the top of the picture, depicting the actual vertical separation that will make it possible to enter the pocket from the corneal surface.

SURGICAL TECHNIQUE

Intrastromal Pocket Creation. Under topical 1% proparacaine anesthesia (Alcaine; Alcon Laboratories Inc, Ft Worth, Tex), the IntraLase FS60 (Abbott Medical Optics, Irvine, Calif) femtosecond laser was used to create an intrastromal pocket with a nasal hinge at 100-µm corneal depth, 7-mm diameter centered at the pupil, and a 10° temporal side-cut (Fig 1).

Following the pocket creation, a small Jameson muscle hook (Rumex International Co, St Petersburg, Fla) was used to enter and bluntly dissect the pocket.

Collagen Cross-linking. A 0.1-mL dose of 0.1% riboflavin solution was administered twice with a 25-gauge air cannula into the intrastromal pocket until it was clearly visualized that the entire pocket was colored bright yellow from the presence and stromal infiltration of the riboflavin solution (Fig 2). After 2 minutes, the UVA light exposure was started. The slit lamp confirmed rapid homogeneous riboflavin diffusion within the corneal stroma. Ultraviolet A irradiation source of ~370 nm wavelength (365 to 375 nm) was used to irradiate the corneal surface. The effective fluence at the corneal surface was calibrated at 7 mW/cm² for a duration of 15 minutes. The total amount of UVA irradiation delivered at the corneal plane of a 9-mm diameter was calculated to be 6.3 J.

Postoperative medications included topical ofloxacin and 1% prednisolone acetate four times a day for 1 week. Patients were evaluated at postoperative day 1, week 1 , months 1 and 3, and every 6 months thereafter to last follow-up.
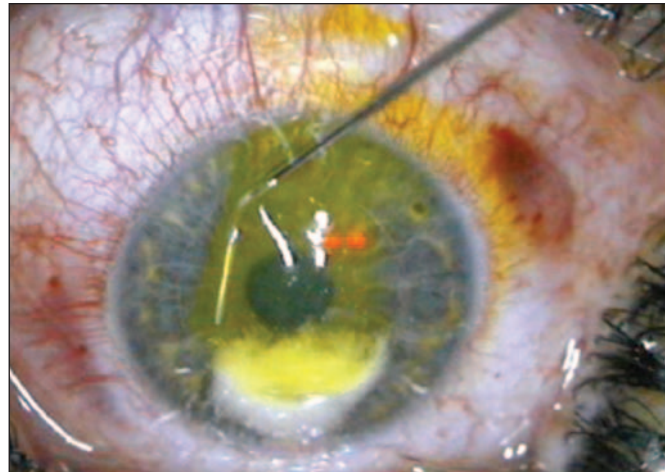
Figure 2. Clinical photograph demonstrating the infusion of 0.1 mL of 0.1% riboflavin solution within the femtosecond laser-created intrastromal pocket to facilitate intrastromal cross-linking.

RESULTS

The Table shows pre- and postoperative uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), steepest K, minimum K, endothelial cell count, and corneal thickness.

In mean values, UCVA improved from 20/50 to 20/40, BSCVA was unchanged at 20/20, mean sphere was reduced by 0.50 D, mean cylinder was reduced by 0.90 D on average, steepest K was reduced from 49.50 to 48.10 D, and no ectasia progression or change in endothelial cell count was noted in any patient during follow-up. An initial reduction of corneal thickness at 1 month at approximately 5% of preoperative measurement was noted. The mean thinnest corneal thickness appeared to increase at 18 months by 5% on average. All patients returned to full activities within 1 day postoperative. No adverse effects were noted in any of the patients studied. Mean follow-up was 26 months (range: 18 to 36 months).

Slit-lamp microscopy revealed no epithelial defects or diffuse light scattering in the anterior two-thirds of the corneal stroma, which is consistent with our clinical findings using the standard CXL technique (Fig 3).

DISCUSSION

Collagen CXL utilizing UVA irradiation and riboflavin solution has been applied clinically for the treatment of corneal ectasia, keratoconus, and bullous keratopathy over the past 6 years.[12-16] We have presented the implementation of this intervention modality as a means to stabilize corneal ectasia associated with LASIK and keratoconus.[14] The documented benefit in corneal ectatic disorders is nevertheless associated with the shortcomings of epithelial removal and topi-

TABLE

Pre- and Postoperative Results of Eyes That Underwent Femtosecond Laser-assisted Cross-linking With Intrastromal Riboflavin and UVA Light for Keratoconus

Case (F/U [mo])	UCVA		Keratometry (D)		ECC Change (cells/mm^2)	Corneal Thickness (μm)	
	Preop	Postop	Preop	Postop		Preop	Postop
1	20/40	20/30	49.00	48.00	50	505	514
2	20/50	20/40	50.00	48.50	50	523	529
3	20/30	20/30	49.00	48.50	0	504	506
4	20/40	20/30	49.50	49.00	100	510	515
5	20/30	20/30	47.50	46.00	50	545	548
6	20/50	20/30	49.00	48.50	0	511	509
7	20/40	20/25	48.00	47.00	50	527	533
8	20/25	20/30	46.00	46.00	0	532	523
9	20/60	20/40	50.00	49.00	0	509	510
10	20/40	20/40	48.50	48.00	0	524	525
Mean	20/40.5	20/32.5	48.70	47.90	+30	519	521

F/U = follow-up, UCVA = uncorrected visual acuity, ECC = endothelial cell count

cal riboflavin administration, lengthy soak/exposure durations under speculum, etc, such as postoperative pain, delayed epithelial healing, and delayed visual rehabilitation for 1 to 2 weeks postoperative.

Is it really necessary to remove the epithelium to achieve high intrastromal levels of riboflavin and facilitate CXL? Riboflavin is the activating element, but is also use as a "shield" for the underlying ocular tissues such as the endothelium, iris, and the crystalline lens. However, it may act as a potential hazard if it couples in the level of those structures with enough UVA irradiation as it will generate free radicals. Lastly, the contributing elements in the collagen CXL photo-oxidative and biochemical reactions are riboflavin, UVA light, and oxygen and stromal collagen. Does topical administration of riboflavin on the cornea during the standard CXL procedure coupled with UVA light absorb critical amounts of oxygen? If riboflavin was already present in the corneal stroma, the UVA light would pass through the corneal endothelium and produce the photochemical reaction in the corneal stroma and not on the eye surface, thereby facilitating more efficient intrastromal cross-linking.

There have been reports of using riboflavin solution administered topically through intact epithelium. Is there a way to establish riboflavin placement intrastromally within a more selective fashion so it will absorb and activate UVA light and achieve CXL without posing a threat to the endothelium and crystalline lens?

In the standard CXL technique, the riboflavin-soaked corneal stroma absorbs the UVA light and with oxygen produces a photo-oxidative reaction (type 2). The product is a free oxygen radical creation, which then facilitates a biochemical reaction within stromal collagen; it generates fiber-to-fiber bonds of stromal collagen and leads to higher stromal rigidity.

Disadvantages of the standard CXL technique include the necessity of epithelial removal as well as the localization of the cross-linking in the anterior corneal surface where most of the UVA light is absorbed by the "denser" concentration of the photosensitizer, riboflavin. The 3- to 4-day re-epithelialization interval poses the significant risk of infectious keratitis. We have reported, along with several investigators, on our initial clinical experience utilizing this technique in ectasia after LASIK and primary keratoconus.

The novel method described here—selective riboflavin instillation for CXL and higher fluence (but of shorter exposure duration) UVA light used—appears to be safe and effective in stabilizing keratoconus.

The femtosecond laser cornea-dissection step, performed on the ectatic cornea, may raise concern for further biomechanical destabilization. There is growing evidence that in a femtosecond laser-created flap, it is the final side-cut that changes the corneal biomechanics and not the lamellar corneal separation (pocket). In our opinion, the creation of a pocket with just a 10° side-cut at the 7-mm diameter may minimize this concern.

Marshal et al[17] reported that a femtosecond laser-generated pocket without a side-cut does not alter cor-

neal biomechanics. These findings suggest that there may not be a biomechanical effect from a femtosecond laser-created pocket (very small side-cut).

We did not anticipate, or find, a negative biomechanical effect from the femtosecond laser-created pocket as it is associated with a minimal side-cut of 10° at the 7-mm diameter. The CXL was effective in reversal of the ectatic process, although longer follow-up is necessary to validate this finding.

Our novel technique appears to be significantly less painful for patients as it does not require de-epithelialization of the cornea and appears to offer "LASIK-like" rapid rehabilitation. On postoperative day 1, no discomfort was reported and all patients returned to preoperative visual function with no epithelial defect.

We have given consideration to the fact that the traditional technique of "soaking" the de-epithelialized cornea with topical riboflavin solution provides a formidable concentration of riboflavin in the stroma so the photochemical reaction between riboflavin and UVA light takes place. Additionally, it provides high concentrations of riboflavin in the endothelium and anterior chamber to protect these structures from "stray" UVA light that passes through the overlaying cornea. It is our opinion that UVA light of this fluence without the presence of riboflavin is of no harm to the intraocular structures. The presence of adequate concentration of riboflavin in the 50- to 300-μm corneal thickness range (calculated from the epithelial surface) will absorb most, if not all, UVA light irradiated during the CXL process. Would there be a reduced stromal keratocyte loss to that reported with the standard technique[18] as there is no epithelial removal and a shorter duration of UVA exposure with higher fluence? Most human cells are believed to be more resistant to short or pulsed exposure to UVA light rather than longer and continuous exposure. In our early clinical experience, this novel technique has been rewarding in regard to patient comfort and evaluated cross-linking effect. It may diminish epithelial healing complications associated with "standard" CXL such as scars and infectious keratitis.

The potential risks of the femtosecond laser application (suction, corneal dissection, and potential microbial implantation) should be weighed by the clinician. This novel technique may become an alternative for the prevention of cornea transplantation in corneal ectasias. It could possibly be applied as an adjunct prophylactic treatment in routine LASIK cases when cornea ectasia may be a possible concern. Further studies and longer follow-up are needed to validate these data.

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.

2. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. *Curr Opin Ophthalmol.* 2006;17:356-360.

3. Mazzotta C, Balestrazzi A, Traversi C, Baiocchi S, Caporossi T, Tommasi C, Caporossi A. Treatment of progressive keratoconus by riboflavin-UVA-induced cross-linking of corneal collagen: ultrastructual analysis by Heidelberg Retinal Tomograph II in vivo confocal microscopy in humans. *Cornea.* 2007;26:390-397.

4. Kanellopoulos AJ. Post-LASIK ectasia. *Ophthalmology.* 2007;114:1230.

5. Kanellopoulos A, Binder PS. Collagen cross-linking (CCL) with sequential topography-guided PRK: a temporizing alternative for keratoconus to penetrating keratoplasty. *Cornea.* 2007;26:891-895.

6. Spoerl E, Mrochen M, Sliney D, Trokel S, Seiler T. Safety of UVA riboflavin cross-linking of the cornea. *Cornea.* 2007;26:385-389.

7. Seiler T, Hafezi F. Corneal cross-linking-induced stromal demarcation line. *Cornea.* 2006;25:1057-1059.

8. 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.

9. Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. *Exp Eye Res.* 1998;66:97-103.

10. Hafezi F, Kanellopolous J, Wiltfang R, Seiler T. Corneal collagen crosslinking with riboflavin and ultraviolet A to treat induced kerectasia after laser in situ keratomleusis. *J Cataract Refract Surg.* 2007;33:2035-2040.

11. Kanellopoulos AJ. Cross-linking plus topography-guided PRK for post-LASIK ectasia management. In: Garg A, ed. *Mastering Advanced Surface Ablation Techniques.* New Dehli, India: Jaypee Brothers Medical Publishers Ltd; 2007:

12. Kanellopoulos AJ. Video technique on sequential topography-guided partial PRK followed by collagen cross-linking in keratoconus. In: Garg A, ed. *Video Atlas of Ophthalmic Surgery.* New Dehli, India: Jaypee Brothers Medical Publishers Ltd; 2007.

13. Kanellopoulos AJ. PRK and C3-R In: Boxer Wachler B, ed. *Modern Management of Keratoconus.* New Dehli, India: Jaypee Brothers Medical Publishers Ltd; 2008:219-228.

14. Kanellopoulos AJ. Managing highly distorted corneas. Presented at International Society of Refractive Surgery of the American Academy of Ophthalmology Refractive Surgery Subspecialty Day; November 12-13, 2007; New Orleans, La.

15. Krueger R, Esteban RJ, Kanellopoulos AJ. Staged, intrastromal delivery of riboflavin with UVA cross-linking in advanced bullous keratopathy: laboratory investigation and first clinical case. *J Cataract Refract Surg.* 2008;24:S730-S736.

16. Kanellopoulos AJ. Cross-linking plus topography-guided PRK for post-LASIK Ectasia Management. In: Garg A, Rosen E, eds. *Instant Clinical Diagnosis in Ophthalmology Refractive Surgery.* New Dehli, India: Jaypee Brothers Medical Publisher Ltd; 2008:

17. Marshal et al. Presented at: International Society of Refractive Surgery of the American Academy of Ophthalmology Refractive Surgery Subspecialty Day; November 10-11, 2007; New Orleans, La.

18. Wollensak G, Spoerl E, Reber F, Seiler T. Keratocyte cytotoxicity of riboflavin/ultraviolet-A treatment in vitro. *Eye.* 2004;18:718-722.