Corneal Collagen Cross-Linking: a Review

BY MALAIKA DAVID, ASSOCIATE EDITOR

Last year, this column's "Corneal Collagen Cross-Linking for Keratoconus and Ectasia" focused primarily on the science behind cross-linking, because there were few articles available involving long-term human data after treatment. Now, surgeons from around the world are providing promising data to support the use of corneal collagen cross-linking in patients with keratoconus and keractasia after refractive surgery. As many as 2% to 5% of all refractive surgery candidates presenting to our offices with underlying keratoconus and 15% of all corneal transplants performed in the United States are secondary to keratoconus.[1,2] This procedure offers promise to aid these patients surgically by reducing the rate of progressive ectasia and, one hopes, avoiding penetrating keratoplasty. Other applications for corneal cross-linking have also been proposed, including a potential adjunctive therapy for the management of severe infectious corneal ulcers.

Clinical trials have suggested that the minimum corneal thickness to safely treat with collagen cross-linking using riboflavin is 400 µm. The key concern is the negative effects of ultraviolet-A (UVA) light on the deeper corneal keratocytes and endothelium. Research has demonstrated that 94% of UVA light is absorbed by the anterior 400 µm of the cornea when combined with a riboflavin photosensitizer, resulting in minimal-to-no effect on the volume of endothelial cells.[3]

As previously discussed, collagen cross-linking works by increasing the number of covalent bonds present between collagen fibers. Riboflavin serves as a photomediator that, when activated by UVA (3 mW/cm^2), increases the strength of the cornea by more than 300%. There is still much discussion regarding the most beneficial means by which to aid the cornea's absorption of riboflavin. Brian Boxer Wachler, MD, has been performing transepithelial treatments of riboflavin 0.2% after applying topical tetracaine for 5 minutes, which loosens the tight epithelial junctions without the negative side effects of a similar technique using alcohol 20% solution for 25 seconds. Joseph Colin, MD, is using riboflavin 0.1% in dextran 20% solution (Ricrolin; Sooft Italia S.p.A., Montegiorgio, Italy), which is isosmotic to the corneal stroma. At this year's ASCRS meeting in Boston, John Kanellopoulos, MD, discussed altering the application of UVA light from 3 mW/cm^2 to 7 mW/cm^2 in 30-second pulsed fractions for 15 minutes. In addition to optimizing the collagen oxidative deamination reaction, he believes that the shorter procedure may, in fact, result in a lower loss of keratocytes, because fibroblasts are more resistant to higher energy exposure at shorter intervals (15 minutes) compared with lower energy at higher intervals (30 minutes).

I hope you enjoy this installment of "Peer Review," and I encourage you seek out and review the articles in their entirety at your convenience.

—Mitchell C. Shultz, MD, section editor

COMBINED THERAPY

Corneal Collagen Cross-Linking With Riboflavin and UVA Irradiation

A 43-year-old man underwent corneal collagen cross-linking with riboflavin and UVA to treat bilateral pellucid marginal degeneration in his left eye. The eye was examined 1 day; 1 week; 1, 3, and 6 months; and 1 year postoperatively. Epithelial regrowth was complete after his use of a bandage soft contact lens for 4 days. No side effects or damage to the limbal region were observed during re-epithelization or during follow-up visits. Corrected distance visual acuity improved from 20/200 to 20/63 at 3 months and was stable through the 12-month interval. Keratometric astigmatism showed a 1.40 D reduction, and the power of ectasia apex decreased from 82.00 D to 78.00 D.[4]

Two patients who suffered from bullous keratopathy presented to the Eye Institute of Thrace in Alexandroupolis, Greece, with gradually deteriorating, vision-threatening, central corneal ulcers. Neither patient was responding to intense local antibiotic therapy. De-epithelialization of the affected corneas was performed with UVA corneal collagen cross-linking. Thereafter, a mixture of 0.1% riboflavin in 20% dextran solution was administered to the cornea for 30 minutes. A therapeutic contact lens was applied, and local antibiotic therapy was resumed after the procedure. The patients were monitored daily during the first postoperative week and then every 15 days for up to 2 months. Within 24 hours of the treatment, both patients reported a significant subjective improvement in their visual acuity and ocular discomfort. Slit-lamp examinations revealed an improvement in the corneal ulcers and bullous keratopathy, which was associated with a significant decrease in the corneal thickness and haziness.[5]

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In a prospective, nonrandomized, open trial, 363 eyes with progressive keratoconus underwent corneal collagen cross-linking with riboflavin and UVA. Forty-four patients were evaluated preoperatively and at a minimum of 48 months postoperatively in terms of UCVA, BSCVA, spherical spectacle-corrected visual acuity, endothelial cell count, optical and ultrasound pachymetry, corneal topography and surface aberrometry, tomography, posterior segment optical coherence tomography (OCT), and in vivo confocal microscopy. At a minimum of 48 months postoperatively, stable keratoconus was detected in all 44 eyes. More than 65% of fellow eyes showed a mean progression of 1.50 D after 24 months. The K value was reduced by a mean of 2.00 D, and coma decreased in more than 85% of eyes. The mean UCVA and BSCVA improved by a mean of +2.85 ±0.81 and +2.03 ±1.04 Snellen lines, respectively.[6]

In a single-center, prospective, interventional study, 14 patients (14 eyes) with progressive keratoconus underwent corneal collagen cross-linking with riboflavin and UVA. Patients were assessed at 1 week and 1, 3, 6, 9, and 12 months postoperatively. The corneal endothelium was assessed with an endothelial specular microscope. The central retina was assessed with biomicroscopy fundus examination and with OCT using a macular-thickness protocol. After comparing pre- and postoperative results, researchers reported a stable endothelial cell density of 2,730 cells/mm preop-

eratively; 2,793 cells/mm at 6 months postoperatively; and 2,640 cells/mm at 12 months preoperatively. They also reported stable foveal thickness of 203 µm preoperatively, 202 µm at 6 months postoperatively, and 205 µm at 12 months preoperatively. No morphological abnormalities were noted.[7]

In a prospective clinical study, 24 eyes with progressive advanced keratoconus underwent corneal collagen cross-linking with riboflavin and UVA. Ocular Response Analyzer (Reichert, Inc., Depew, NY) parameters were recorded at baseline, intraoperatively, and at 1, 6, and 12 months postoperatively. A statistically significant reduction in the thinnest corneal point from 462 ±23.24 µm was observed at the end of the cross-linking procedure intraoperatively and at 1 and 6 months postoperatively ($P < .05$). A significant increase in the thinnest corneal point to 624 ±31.72 µm was found after re-epithelialization ($P < .05$), and no significant changes were observed 1 year postoperatively. No significant change was noted in mean corneal hysteresis and corneal resistance factor after de-epithelialization, but they significantly increased after cross-linking intraoperatively and at 1 month postoperatively. At 6 and 12 months postoperatively, corneal hysteresis and the corneal resistance factor were not statistically significantly different from the preoperative measurements. Corneal-compensated IOP and Goldmann-correlated IOP had increased at 1 month after corneal collagen cross-linking ($P >. 05$).[8]

Investigators from the Aarhus University Hospital in Aarhus, Denmark, evaluated the distribution of riboflavin in the corneal stroma of 54 porcine eyes. The investigators removed the central corneal epithelium from all of the eyes and applied 0.035%, 0.1%, or 0.2% riboflavin-5-phosphate for 10, 20, or 30 minutes. The procedures were repeated in seven human corneal donor grafts using 0.1% riboflavin-5-phosphate for 20 or 30 minutes. In the porcine corneas, the intensity of fluorescence peaked in the anterior stroma within the first 50 µm, followed by a steep decline to baseline. Raising the riboflavin concentration from 0.1% to 0.2% did not increase stromal depth propagation, although a higher concentration in the anterior 200 µm was reported. Reducing the riboflavin application time from 30 to 20 minutes had no impact on corneal depth propagation or total riboflavin uptake. Reducing the application time by 10 minutes significantly reduced the uptake of riboflavin. In all human corneas, fluorescence peaked within the anterior 50 µm, followed by a steep decline to baseline over the next 200 µm, which was similar to the observation in porcine corneas. Investigators reported that the human corneas

imbibed more riboflavin compared to the porcine corneas.[9]

Corneal Collagen Cross-Linking With Conductive Keratoplasty

Two patients presented to the Vardinoyannion Eye Institute in Crete, Greece, with bilateral keratoconus in both eyes. Investigators assessed patients' UCVA and BSCVA, tonometry, corneal topography, central and peripheral corneal pachymetry, and fundi. Both patients had more advanced keratoconus in one eye. Conductive keratoplasty was performed on the corneas at the periphery of the topographically flatter area. Within 24 hours of the conductive keratoplasty, corneal collagen cross-linking was performed using UVA light. Both patients experienced a significant corneal topographic improvement immediately after conductive keratoplasty. By 3 months postoperatively, the topographic pattern was similar to the preoperative pattern, which indicated that the remodeling after conductive keratoplasty had regressed. By 6 months postoperatively, UCVA, manifest refraction, BSCVA, and corneal topography remained unchanged from baseline.[10]

Corneal Collagen Cross-Linking With PRK

Twelve patients (12 eyes) with keratectasia were treated with topography-guided customized ablation and corneal collagen cross-linking at the University Hospital North Norway, Tromso, Norway. Topography-guided customized ablation was performed using the iVIS Suite (not available in the United States; LIGI Tecnologie Medicali S.p.A., Taranto, Italy), and corneal collagen cross-linking was performed immediately afterward. UCVA, BSCVA, refractive change, corneal topography, and pachymetry were analyzed preoperatively and 3, 6, and 12 months postoperatively. Between the preoperative visit and the 12-month postoperative visit, mean UCVA increased from 20/1000 to 20/125, mean BSCVA increased from 20/57 to 20/35, mean astigmatism decreased from 5.40 ±2.13 D to 2.70 ±1.44 D, and keratometric asymmetry decreased from 6.38 ±1.02 D to 2.76 ±0.73 D. Investigators reported minor changes in the elevation of the posterior corneal surface and stability of refraction.[11]

Twenty-three patients (28 eyes) with keratoconus underwent simultaneous customized PRK followed by corneal collagen cross-linking. Corneas were examined biomicroscopically and with confocal microscopy preoperatively and 1, 3, 6, 9, and 12 months postoperatively. Slit-lamp biomicroscopy revealed posterior linear stromal haze 1 month postoperatively in 13 eyes. No corneal edema or anterior haze was reported. Confocal microscopy revealed a hyperreflective area at the level of the posterior stroma 1 month after combined PRK and corneal collagen cross-linking. A slit-lamp examination and corneal confocal microscopy showed that these hyperreflective structures demonstrated a gradual relocation following a posterior/anterior pattern. By 12 months postoperatively, posterior linear stromal haze, despite its anterior movement and decreased density, had not completely disappeared.[12]

REVIEW OF COLLAGEN CROSS-LINKING AS A TREATMENT PARADIGM

In a review, Snibson reported that corneal collagen cross-linking is one of the more promising developments of this decade for the treatment of keratoconus. Based on the current literature, he said it reduces the morbidity of progressive forms of this disease and may ultimately reduce the need for corneal transplantation. The clinical evidence in support of corneal collagen cross-linking has been limited to small, uncontrolled, retrospective series with relatively short follow-up. He stated that the safety of corneal collagen cross-linking, in relation to the corneal endothelium, will take time to fully ascertain and that long-term observation will be required. Snibson concluded that decisions regarding treatment protocols, riboflavin solution formulation, and UVA delivery should be informed and made with care.[13] ■

1. Eye Banking Statistical Report. In: America EBAo, ed. Washington, DC, 2006.
2. Sray WA, Cohen EJ, Rapuano CJ, Laibson PR. Factors associated with the need for penetrating keratoplasty in keratoconus. *Cornea*. 2002;21(8):784-786.
3. Wollensak G, Spoerl E, Reber F, Seiler T. Keratocyte cytotoxicity of riboflavin/UVA-treatment in vitro. *Eye (Lond)*. 2004;18(7):718-722.
4. Spadea L. Corneal collagen cross-linking with riboflavin and UVA irradiation in pellucid marginal degeneration. *J Refract Surg*. 2010;26(5):375-377.
5. Kozobolis V, Labiris G, Gkika M, et al. UV-A collagen cross-linking treatment of bullous keratopathy combined with corneal ulcer. *Cornea*. 2010;29(2):235-238.
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(4):585-593.
7. Goldich Y, Marcovich AL, Barkana Y, et al. Safety of corneal collagen cross-linking with UV-A and riboflavin in progressive keratoconus. *Cornea*. 2010;29(4):409-411.
8. Vinciguerra P, Albé E, Mahmoud AM, et al. Intra- and postoperative variation in ocular response analyzer parameters in keratoconic eyes after corneal cross-linking. *J Refract Surg*. 2010. http://www.journalofrefractivesurgery.com/showAbst.asp?thing=63072. Accessed June 15, 2010.
9. Aondergaard AP, Hjortdal J, Breitenbach, Ivarsen A. Corneal distribution of riboflavin prior to collagen cross-linking. *Curr Eye Res*. 2010;35(2):116-121.
10. Kymionis GD, Kontadakis GA, Naoumidi TL, et al. Conductive keratoplasty followed by collagen cross-linking with riboflavin-UV-A in patients with keratoconus. 2010;29(2):239-243.
11. Stojanovic A, Zhang J, Chen X, et al. Topography-guided transepithelial surface ablation followed by corneal collagen cross-linking performed in a single combined procedure for the treatment of keratoconus and pellucid marginal degeneration. *J Refract Surg*. 2010;26(2):145-152.
12. Kymionis GD, Portaliou DM, Diakonis VF, et al. Posterior linear stromal haze formation after simultaneous photorefractive keratectomy followed by corneal collagen cross linking. *Invest Ophthalmol Vis Sci*. 2010. http://www.iovs.org/cgi/content/abstract/iovs.09-5105v1. Accessed June 15, 1010.
13. Snibson GR. Collagen cross-linking: a new treatment paradigm in corneal disease – a review. *Clin Experiment Ophthalmol*. 2010;38(2):141-153.