Treatment Protocols and Safety Issues

Until safety is clearly established, only surgeons with knowledge of corneal wound healing should perform CXL—and only when the indication is clearly documented.

BY FARHAD HAFEZI, MD, PHD

Inter- and intrafibrillar collagen crosslinks are a major factor ensuring the mechanical stiffness of connective tissues. A number of techniques can be used to induce additional crosslinks: exposure to aldehydes (glutaraldehyde or aldehyde sugars), enzymatic treatment (lysyloxydase), and photopolymerization using UV-A light.[1] However, some approaches are either too toxic, such as exposure to glutaraldehyde or UV-A alone, or too time-consuming, such as the application of ribose 0.5 molar for 14 days, for use in the cornea.[2,3]

In 1995, a promising technique to enhance mechanical stiffness of connective tissue was proposed: photopolymerization by generation of free radicals. In this technique, a nontoxic and soluble photomediator needed to (1) absorb UV-A light enough to generate free radicals that induce crosslinks and (2) protect deeper ocular structures from the potential hazards of free radical formation. These parameters were achieved by using a 0.1% aqueous solution of riboflavin phosphate (vitamin B2). This solution provides an adequate shielding effect at a wavelength of 370 nm and generates sufficient free radicals to induce formation of additional collagen crosslinks. These crosslinks are intramolecular rather than intermolecular, as suggested by an increase in the collagen molecule diameter of up to 10% following corneal collagen crosslinking (CXL).[4]

To reduce stromal swelling, riboflavin solution was diluted in dextran 20%, a carrier iso-osmolar to the corneal stroma . Since riboflavin is a macromolecule, the corneal epithelium represents a barrier that decreases the absorption rate.[5] Thus, the corneal epithelium should be removed prior to instillation of riboflavin. (For an alternate method that does not remove the epithelium, see *Transpithelial Tensioactive Mediated CXL*, by Roberto Pinelli, MD; and Hytham Ib El-Shawaf, MD, on page 37.) The intensity of UV-A irradiation was set to 3 mW/cm^2, corresponding to a surface dose of 5.4 J/cm^2 to induce corneal crosslinks to a depth of 310 µm. A stromal thickness of at least 400 µm was called for in the original protocol, ensuring the irradiation intensity is two times lower than the damage threshold.[6,7]

TAKE-HOME MESSAGE

- Modified treatment parameters for CXL may be used in patients with advanced keratectasia who can still achieve satisfying visual acuity with contact lenses.
- Hypo-osmolar riboflavin solution can be used to increase corneal depth in corneas too thin for standard protocol.
- The treatment must prevent damage to the corneal endothelium, iris, lens, and retina.

THE STANDARD PROTOCOL

CXL using iso-osmolar 0.1% riboflavin solution. Generated by diluting vitamin B2-riboflavin-5-phosphate 0.5% (G. Streuli & Co. AG, Uznach, Switzerland) with dextran T500 20% (402.7 mOsmol/L), this solution must be protected from light and used within 2 hours. After a 9-mm diameter abrasion of the corneal epithelium, the iso-osmolar 0.1% riboflavin solution with dextran is applied on the cornea every 3 minutes for 30 minutes. Then, ultrasound pachymetry (five repetitive measurements) is performed on the deepithelialized cornea at the thinnest point, ensuring a minimal stromal thickness of 400 µm.[6,7] Successful penetration of riboflavin through the cornea is assured by visualization of riboflavin in the anterior chamber by slit-lamp biomicroscopy (Figure 1). Riboflavin saturation ensures the formation of free radicals, whereas riboflavin shielding ensures the protection of deeper ocular structures, such as the corneal endothelium.[6] The eye is then irradiated for 30 minutes with UV-A at a working distance of 5 cm (UV-X; IROC Medical, Zurich, Switzerland; distributed by Peschke Meditrade GmbH, Nuremberg, Germany). An isotonic 0.1% riboflavin solution and topical anesthetic (oxybuprocaine 0.4%) are administered every 5 minutes to saturate the cornea with riboflavin.

THE MODIFIED PROTOCOL

CXL in thin corneas using hypo-osmolar riboflavin solution. As mentioned in the last section, the current CXL

inclusion criteria require a minimal stromal thickness (without the corneal epithelium) of 400 µm, as a safety margin. However, in many cases of advanced progressive keratectasia, patients still achieve a satisfying visual acuity with contact lenses, and a low minimal stromal thickness is the only parameter prohibiting safe CXL. To treat these patients, we have modified the treatment parameters, using a hypo-osmolar riboflavin solution to induce stromal swelling and increase the stromal thickness prior to CXL in cases with preoperatively thin corneas.[8]

Hypo-osmolar 0.1% riboflavin solution is generated by diluting vitamin B2-riboflavin-5-phosphate 0.5% with physiologic salt solution (sodium chloride 0.9% solution; 310 mOsmol/L; B. Braun Medical AG, Sempach, Switzerland). Hypo-osmolar riboflavin solution does not contain dextrane. Again, the solution must be protected from light and used within 2 hours.

After removal of the corneal epithelium and 30 minutes of instillation of iso-osmolar riboflavin solution, the corneal stromal thickness was measured using ultrasound pachymetry. In cases where the remaining stromal bed was thinner than 400 µm, hypo-osmolar riboflavin was applied every 20 seconds for 5 additional minutes, and the corneal thickness was again checked by ultrasound pachymetry. Hypo-osmolar riboflavin solution was again administered until the minimal corneal thickness reached 400 µm, which usually occurred within 5 to 15 minutes.

The absolute increase in corneal thickness observed in this case series ranged from 36 to 110 µm, and the thinnest cornea was 323 µm after removal of the epithelium and prior to instillation of hypo-osmolar riboflavin solution

TRANSEPITHELIAL TENSIOACTIVE MEDIATED CXL

BY ROBERTO PINELLI, MD; AND HYTHAM IB EL-SHAWAF, MD

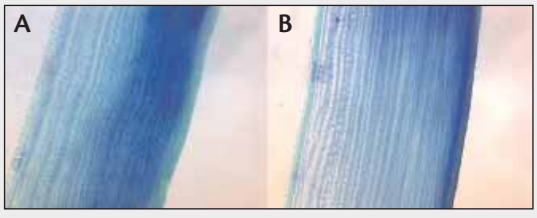

Figure 1. Light microscopy of (A) control and (B) treated corneas.

Not long ago, riboflavin/UV-A corneal crosslinking (CXL) was introduced to ophthalmic practice. Initially aimed for the treatment of ectatic corneal conditions, including keratoconus, pellucid marginal degeneration, and postlaser keratectasia, it is now also used to treat for bullous keratopathy, infectious keratitis, and corneal melting, and for the stabilization of post-radial keratotomy hyperopic shifts. Crosslinking forms new covalent bonds between collagen fibrils; hence, corneal rigidity improves.

The standard protocol for CXL requires mechanical removal of the epithelium. The drawbacks of this step include prolonged surgical time, increased incidence of herpetic activation and haze development, corneal edema, postoperative pain and discomfort, and reduced visual acuity until epithelialization is complete and corneal edema is resolved. We have been motivated to develop a variant technique in which the epithelium is not removed.

In our technique, riboflavin containing benzalkonium chloride (BAK) is applied directly onto the intact epithelium. As a tensioactive substance, BAK changes the surface tension value, facilitating penetration of other substances through biological membranes. In CXL, BAK allows riboflavin to penetrate into the corneal stroma without removal of the epithelium.

We have demonstrated the results of tensioactive CXL in rabbit corneas (Figure 1) and compared it with the standard CXL protocol (control group).[1,2] With our treatment, the epithelial surface retained its curvature due to increased stiffness and was more compact. In the stroma, the fibers not only showed more compact arrangement but were also straighter than the wavy fibers of the control corneas. This effect was noticed in the upper 50% of the stroma. Additionally, the deep stroma adjacent to the endothelium looked the same as the control.

BAK, which is contained in many eye drops prescribed to patients, can cause lifting and peeling of some superficial epithelial cells, exposing the second layer of cells; however, it does not cause change in the basal epithelial or endothelial cells. We conclude that the benefits of using BAK to avoid epithelial removal in CXL outweigh the drawbacks of epithelial removal. Tensioactive CXL is a less invasive procedure with fewer complications and increased patient comfort.

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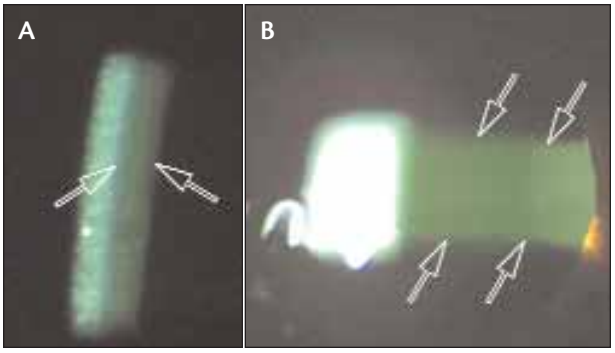

Figure 1. Riboflavin application to the cornea. (A) After 30 minutes of instillation of riboflavin 0.1% drops in 20% dextrane, slit-lamp inspection of the cornea reveals complete penetration of the corneal stroma by riboflavin. (B) Slit-lamp inspection of the anterior chamber reveals a green Tyndall phenomenon. The riboflavin has penetrated the anterior chamber, which is an indicator of riboflavin shielding.

(Figure 2). In the past 2 years, we have used this technique in 20 patients with progressive keratoconus and iatrogenic keratectasia after refractive laser surgery; results are similar to those in patients in whom the standard protocol (ie, an isoosmolar solution) was used.[8]

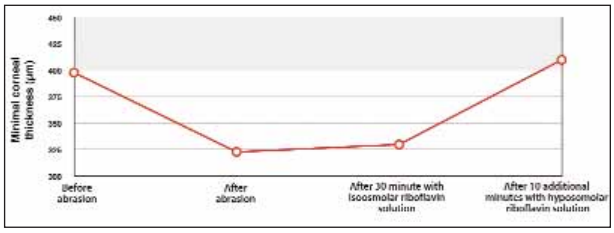

Figure 2. Stromal swelling prior to CXL, as measured by ultrasound pachymetry.

After treatment, an antibiotic ointment (ofloxacin) and a bandage contact lens soaked with preservative-free antibiotic (ofloxacin 0.3%) are applied until healing of the corneal epithelium is complete. Afterward, the patient is instructed to apply fluorometholone eye drops twice daily for 6 weeks.[9] A slight haze, comparable with the healing reaction following corneal abrasion in PRK, can be seen in the first 6 to 8 weeks following surgery. Postoperative follow-up is performed daily until the epithelium is completely healed; at 1, 3, 6, and 12 months postoperatively; and yearly thereafter.

Techniques used to monitor CXL are corneal topography, corneal pachymetry (ultrasound or optical), and corneal confocal microscopy. In the latter, the depth of effective treatment can be monitored by the difference in the

reflectivity/refractive index of treated versus untreated corneas. Furthermore, we have recently shown that the depth of effective treatment may be detected biomicroscopically in slit-lamp examination (Figure 3).[10] At 3 and 6 months, corneal topography may reveal an arrest in progression of the ectatic process. Wollensak and coworkers showed a regression of maximal keratometry values by an average of 2.00 D in 70% of patients. Regression took up to 30 months to stabilize.[11]

Although corneal topography measures only indirectly the biomechanical changes related to CXL, it currently is the technique of choice to monitor the effect of CXL on the cornea. Future detection methods may include devices that directly measure corneal biomechanics, such as the Ocular Response Analyzer (ORA; Reichert Ophthalmics, Depew, New York). Noguera and colleagues recently showed a significant increase in corneal hysteresis in human cadaver eyes after CXL when compared with untreated controls.[12]

SAFETY ISSUES

Crosslinking of the cornea consists of irradiation of the tissue with UV-A light and generation of free radicals that induce the additional crosslinks. Prevention of damage to the corneal endothelium and deeper ocular structures, such as the iris, the lens, and the retina is mandatory.

Wollensak et al studied the cytotoxicity of the riboflavin/UV-A standard treatment on keratocytes and endothelial cells.[7,11,13] In rabbit corneas, keratocyte apoptosis was detected up to 300 µm depth at 24 hours following standard CXL treatment. Smaller irradiances led to shallower cell depth following Lambert-Beer's law.[13] In cell cultures established from porcine keratocytes, the damage threshold of the irradiance of UV-A in combination with 0.025% riboflavin solution was found to be 0.45 mW/cm^2, which is 10 times lower than for UV-A irradiation alone.[7] A similar experimental set-up was used to measure the damage threshold for porcine endothelial cells.[14] At an irradiance of 0.3 mW/cm^2, no signs of cell damage were detected; however, at 0.35 mW/cm^2, 98% of the cells stained positively for both trypan blue and yo-pro fluorescent dye in their nuclei. The authors concluded that when using the standard riboflavin/UV-A technique, a preoperative minimal corneal thickness of 400 µm after removal of the epithelium is mandatory to avoid damage to the corneal endothelium.

Spoerl et al have unambiguously demonstrated that the UV-A intensity used during CXL is far below the damage threshold for the corneal endothelium, iris, lens, and retina.[6] The structures at greatest risk of damage from induced free radicals are the keratocytes and corneal endothelium.

Keratocytes show apoptosis after CXL to a stromal depth of 320 µm,[15] which correlates with the clinically observed demarcation line after CXL (Figure 3).[10] As long as the corneal stroma shows a thickness of 400 µm and the irradiance is 3 mW/cm^2 or less, the endothelium is protected by the riboflavin concentration in the stroma (riboflavin shielding).[6]

Nevertheless, various cases with complications after CXL were reported at the Fourth CXL Congress in Dresden.[7] Complications were either related to epithelial healing after abrasion (ie, infectious keratitis) or to variable degrees of stromal scarring—the latter dissolving after several weeks and even months of topical steroid treatment. Interestingly, only one case of endothelial damage was reported. Here, a cornea too thin to be eligible for the standard treatment protocol was nevertheless treated.

CONCLUSION

New treatment protocols allow the cornea to swell preoperatively. This approach safely broadens the spectrum of CXL indications to corneas that would otherwise be ineligible for treatment due to minimal stromal thickness. Nevertheless, CXL remains a relatively new method with a potential for complications that is not yet fully understood. CXL should, therefore, be performed only by surgeons with knowledge of corneal wound healing, and only when the indication for CXL is clearly documented. ■

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