Update on Corneal Crosslinking

Our study indicates that epithelial removal is not a fundamental component.

BY ROBERTO PINELLI, MD; TAREK EL BELTAGI, MD; AND ANTONIO LECCISOTTI, MD

The term *crosslinking* indicates a medical intervention; it was originally used in dentistry and orthopedics. Theo Seiler, MD, PhD, of Switzerland, was the first to suggest applying this principle to ophthalmology, more specifically crosslinking corneal collagen fibers.

After researching this idea, Professor Seiler and his colleagues studied the use of riboflavin (vitamin B2) and ultraviolet-A (UVA) irradiation, noting that the combination strengthened the corneal stroma. This effect was obtained by creating new bonds between the collagen fibers—where unstable riboflavin molecules produced these bonds after irradiation with UVA. This early research proved an effective treatment for keratoconus; however, one problem was standardizing the parameters of the treatment, including riboflavin concentration and penetration, UV fluence, and time of exposure. Standardization was necessary to render the treatment safe and effective.

Additional studies defined the treatment's main parameters: (1) riboflavin concentration at 0.1%, (2) UVA irradiation of 370 nm fluence at 3mW/cm^2, (3) up to 30 minutes of exposure, (4) diameter of the treatment from 7 to 9 mm, and (5) a minimum corneal thickness of 400 µm to avoid damaging endothelial cells.

CURRENT STATUS

The corneal collagen crosslinking with riboflavin (C3-R) treatment initially required epithelial debridement to improve riboflavin penetration in the stroma; however, our research indicates that the treatment may be performed with or without deepithelialization. There are opinions regarding epithelial debridement. We must remember that most complications associated with this procedure, such as infections, slow healing, and subepithelial haze, occur because of deepithelialization. Epithelial healing in keratoconic corneas is, indeed, much slower than in healthy corneas. In some eyes, it may take several weeks after C3-R. Some surgeons argue that leaving the epithelium on the stroma is less efficacious because it slows the penetration of the riboflavin into the stroma; however, our experiences demonstrate the opposite.

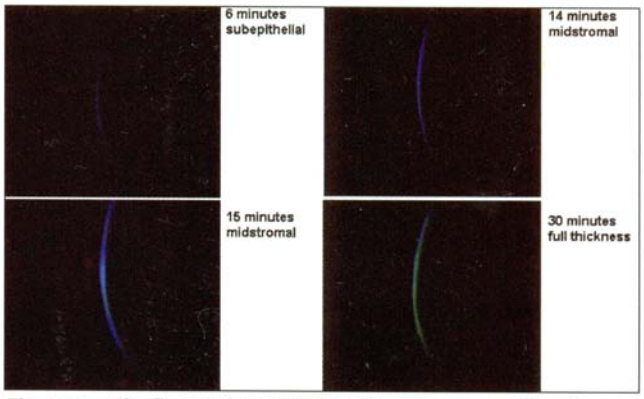

Figure 1. Riboflavin absorption via fluoroscopy without epithelial debridement.

Recently, we conducted a pilot study using fluoroscopy to observe the absorption of riboflavin in the absence of epithelial debridement.[1] Riboflavin 0.1% (KeraCure; Priavision, Menlo Park, California) was applied to the cornea via a saturated Merocel sponge and left on the eye for 5 minutes before the start of UVA light administration. We repeated riboflavin applications every 3 minutes. After 6 minutes, the riboflavin penetrated under the epithelium; after 14 minutes, it penetrated the middle of the stroma; and after 30 minutes, we observed full diffusion (Figure 1). Our research demonstrated that during C3-R treatments, leaving the epithelium intact does not significantly limit the penetration of riboflavin.

We also conducted a comparative study for the results of C3-R with and without deepithelialization.[1] A total of 10 patients with keratoconus were enrolled. Preoperatively, keratometry (K) values ranged from 45.00 to 49.00 D, pachymetry from 432 to 463 µm, UCVA from 0.1 to 0.3, and BCVA from 0.4 to 0.7. All patients were treated monocularly, half using C3-R without deepithelialization and the other half using C3-R with deepithelialization.

At 6 months, we observed similar clinical aspects typical of

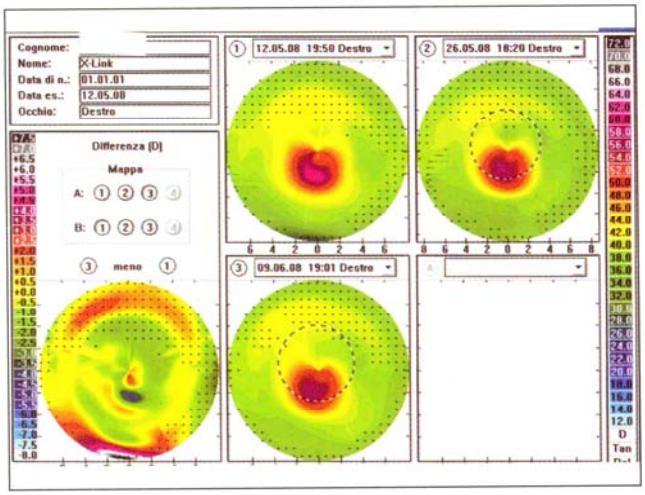

Figure 2. Changes of central curvature after corneal collagen crosslinking without deepithelialization. Top center: preoperative. Top right: 1 week after treatment, showing initial improvement. Bottom center: 3 weeks after treatment, further improvement. Bottom left: differential map, showing a cone flattening of 4.00 D.

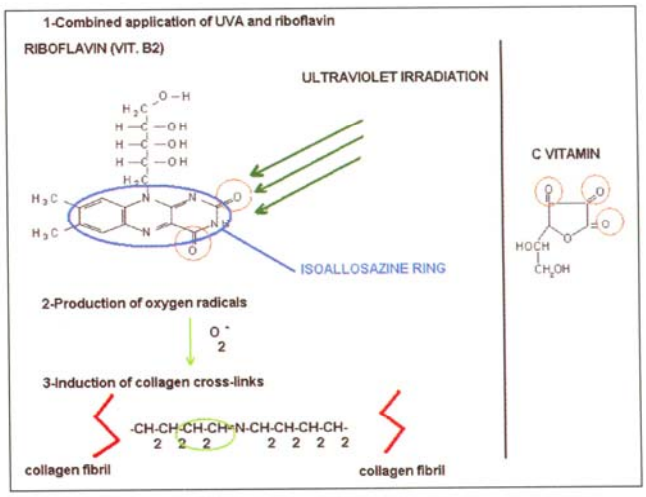

Figure 3. Mechanism of C3-R and vitamin C (right). Oxygen links (red), broken with UVA irradiation are responsible for collagen crosslinking. Riboflavin has two oxygen links; vitamin C has three.

the treatment in both groups, including a mean decrease in K readings, spherical equivalent, and root mean square error; a gain of lines in UCVA and BCVA; an increase in pachymetry; and no endothelial cells loss.

Postoperatively, patients were asked to fill out a questionnaire about the procedure. In the deepithelialization group, patients were more apt to describe discomfort and a lower total satisfaction rate; however, both groups reported the following visual symptoms: improved night vision, less glare, decreased photophobia, and improved quality of vision. Based on response to our findings, we believe epithelial debridement is not a fundamental component of a C3-R treatment.

At the Second International Corneal Crosslinking Congress, in Zurich, Switzerland, Dr. Pinelli reported results and characteristics of our C3-R treatment protocol:[2]

- No epithelial debridement;
- Two drops of proparacaine 0.5% every 5 minutes for 15 minutes;
- A 5-minute presoak with riboflavin solution (0.1% riboflavin-5-phosphate and dextran);
- Up to 30 minutes of exposure to UVA light (370 nm fluence at 3mW/cm^2) to the central 7 mm of the cornea (with the speculum in place); and
- UVA light combined with reapplication of riboflavin solution every 3 minutes.

The penetration of riboflavin through intact epithelium can be enhanced by substances that increase its permeability, such as ethylenediaminetetraacetic acid (EDTA)[3] and topical gentamicin. Currently, Professor Leccisotti uses an industrial preparation of topical gentamicin (Ribomicin eyedrops; Farmigea, SPA, Pisa, Italy) to pretreat the cornea for 3 hours. He then follows with application of a topical anesthetic (oxybuprocaine) for 30 minutes, and finally instills riboflavin and irradiates with UVA. Professor Leccisotti has encouraging follow-up results at 6 months: mean BCVA improvement, 0.15, where BCVA was unchanged in 21 eyes, improved in 11 eyes, and worsened in one eye by one Snellen line; and mean curvature improvement, 1.30 D (Figure 2). Endothelial safety was tested by specular microscopy, and cell density was unchanged at 1 and 6 months. UVA penetration is—as expected—under the threshold of endothelial damage.

Dr. Pinelli and colleagues have patented a riboflavin formula (0.1% plus tensioactive) that is currently under investigation in rabbits eyes. We believe that transepithelial procedures will be a new frontier for the treatment of keratoconus. There is a firm belief that several refractive treatments to cure keratoconus, including C3-R, may coexist in the near future.

DIRECTIONS

Although ophthalmologists are still debating whether to remove or keep the epithelium intact before C3-R treatment, we prefer to avoid deepithelialization and its associated discomfort, especially until a scientific method or new technology in vivo will demonstrate the opposite.

In our opinion, the C3-R treatment of the future will be a less invasive, painless technique that does not require deepithelialization. A bilateral option may also be psychologically easier and more accepted by our patients. Thus far, C3-R treatments are effective, and results and follow-up are encouraging.

TAKE-HOME MESSAGE

• Characteristics of the authors' C3-R treatment protocol include:

No epithelial debridement;

Two drops of proparacaine 0.5% every 5 minutes for 15 minutes;

A 5-minute presoak with riboflavin solution (0.1% riboflavin-5-phosphate and dextran);

Up to 30 minutes of exposure to UVA light (370 nm fluence at 3mW/cm^2) to the central 7 mm of the cornea (with the speculum in place); and

UVA light combined with reapplication of riboflavin solution every 3 minutes.

In the future, new active principles will also improve the efficacy of the treatment. Currently, we are studying the effects of combining vitamin C (ascorbic acid) with riboflavin.

Vitamin C is a cofactor for several enzymes involved in the biosynthesis of collagen, of which the best known are proline and lysine hydroxylase. These enzymes are responsible for the capacity of UVA to be absorbed by the vitreous body. They may also detox the formation of organic radicals, for example, by ionizing radiation in vivo.

Vitamin C presents structural analogies with riboflavin (Figure 3) and its capability to absorb UVA.

We are developing a new treatment, C3-C, and conducting comparative studies in vitro between riboflavin and vitamin C crosslinking. Further investigation is necessary to develop C3-C. ■

Tarek El Beltagi, MD, is a Professor of Ophthalmology at the Research Institute of Ophthalmology, Cairo, Egypt. Dr. Beltagi states that he has no financial interest in the products or companies mentioned. He may be reached at e-mail: tarekelbeltagi@yahoo.com.

Antonio Leccisotti, MD, PhD, is a Visiting Professor at the School of Biomedical Sciences, University of Ulster, Coleraine, UK, and Director of the Ophthalmic Department, Casa di Cura Rugani, Siena, Italy. Professor Leccisotti states that he has no financial interest in the products or companies mentioned. He may be reached at e-mail: leccisotti@libero.it.

Roberto Pinelli, MD, is the Scientific Director of the ILMO, Brescia, Italy. Dr. Pinelli states that he has no financial interest in the producs or companies mentioned He may be reached at e-mail: pinelli@ilmo.it.

1. Pinelli R. Corneal collagen cross-linking with riboflavin (C3-R) treatment opens new frontiers for keratoconus and corneal ectasia. *Eyeworld.* 2007;5:34-40.
2. Pinelli R. The Italian Refractive Surgery Society (SICR) results using C3-R. Paper presented at the: Second International Congress of Corneal Cross Linking (CCL); Zurich; December 7-10, 2006.
3. Nakamura T, et al. Electrophysiological characterization of tight junctional pathway of rabbit cornea treated with ophthalmic ingredients. *Biol Pharm Bull.* 2007;30:2360-2364.