Corneal Crosslinking for the Treatment of Keratitis

This corneal strengthening procedure also has antimicrobial effects.

BY KARIM MAKDOUMI, MD; JES MORTENSEN, MD; AND SVEN CRAFOORD, MD, PhD

Since the introduction of corneal collagen crosslinking (CXL), this therapeutic procedure has been developed as an option for the management of keratoconus and other corneal ectatic conditions. A number of publications have assessed the safety and clinical efficacy of the procedure in the treatment of these conditions. Such studies seem to signify that CXL is a treatment that decreases the need for corneal transplantation.1,2 It is the only known procedure that targets the main concern in corneal ectasia—the weakening of stromal tissue.

Utilizing riboflavin as a photosensitizer, with a standard concentration of 0.1%, as described in the original protocol by Wollensak et al,3 combined with application of ultraviolet-A (UV-A) light at a wavelength of 365 or 370 nm, reactive oxygen species (ROS) are produced in corneal tissue. These ROS cause covalent bonds to form between collagen fibrils, which result in an increase in tissue rigidity.

The complication rate after CXL therapy is low. The most commonly described events postoperatively include stromal haze (a small minority develops permanent haze) and microbial keratitis.4,5 In most reported cases of infection, a bandage contact lens was applied after the procedure, and the descriptions of several of these subjects indicate the use of inadequate lens wear hygiene.

ANTIMICROBIAL EFFECTS

Photoactivation of riboflavin induces oxidative stress; elucidation of the antimicrobial properties of this process began in the 1960s. Since then, the concept of combining riboflavin with UV-A light has been explored methodically. The principle was introduced into a device (Mirasol; Navigant Biotechnologies, Inc, Fort Collins, Colorado) used in transfusion medicine for the reduction of potential contaminants in blood products.6 Efficient elimination of a number of pathogens by this method has been demonstrated, and bactericidal effects of CXL that cannot be explained by the disinfecting consequence of the UV-A light alone have been confirmed.7 The photochemical interaction in CXL can also potentiate antifungal therapeutic drugs, which further supports the potential use of the procedure as an adjuvant to antibiotics in keratitis.8 The concentration of riboflavin needed for antimicrobial interaction is comparatively low, suggesting that the stromal concentration of riboflavin achieved in a clinical situation should be sufficient to produce a clinical effect.9

The first treatment of corneal ulcers by means of riboflavin–UV-A photosensitization was published in 2000,10 and thereafter several case reports have documented a response to CXL therapy in complicated infectious keratitis. The first clinical implementation of this model in Scandinavia was, to the best of our knowledge, in 2007, when one of us (Dr. Mortensen) treated a refractory case of microbial keratitis after a long duration of...
topical therapy. Rapid improvement in symptoms and subsequent healing of the epithelium indicated that the treatment was a highly potent technique for the management of corneal infections.

Since then, at the Örebro University Hospital in Sweden, CXL has been evaluated as an adjuvant treatment in severe therapy-resistant cases of microbial keratitis and corneal melting, with remarkable results in our estimation. Several highly advanced infections have been managed with this technique (Figures 1 through 3), some of which were included in a published retrospective case series.

In our experience, the procedure elicits a rapid response, with a reduction of the inflammatory intensity associated with the infection. In addition to this, there is, in most cases, a marked subjective improvement in symptoms, often within 1 day postoperative. This differs notably from the postoperative pain described by patients receiving CXL treatment for keratoconus. The duration of epithelial healing has varied considerably, which may be explained by the heterogeneity of the conditions treated, including eyes with other corneal pathologies. Observations from the management of keratitis in our department indicate that signs of epithelial healing can be observed at an early stage after treatment.

EXPERIMENTAL, CLINICAL RESEARCH

Because of the positive clinical results observed, we have initiated experimental research projects at the Clinical Research Centre of Örebro University Hospital to evaluate the antimicrobial action of the photochemical interaction in CXL. These experiments have demonstrated that several bacteria can be eliminated using the combination of UV-A and riboflavin and that it is not the action of UV-A alone that eliminates the pathogens but more likely the oxidative stress induced by the ROS that are generated by the illumination of riboflavin.

In March 2009, a clinical nonrandomized pilot study was launched at our clinic and the Department of Ophthalmology at Ryhov County Hospital, Jönköping, Sweden. In this study, 16 patients received CXL as primary therapy for suspected bacterial keratitis. Of these patients, only two required antibiotics, signifying that the action of CXL seems to be an effective complement to antibiotics in the management of corneal infections because healing in microbial keratitis could be achieved by CXL single-handedly in the majority of cases. These data should be confirmed by larger randomized trials to establish a clear and evidence-based effect regarding the healing time and complication frequency rate with this technique in comparison with customary antibiotic treatment; however, in nonresponsive cases of corneal infections, we believe that CXL can be considered as a treatment option.

The mode of action, employing the singlet oxygen molecule to induce oxidative stress, is comparable to that of photodynamic therapy (PDT); however, in CXL the antimicrobial action is believed to be more specific due to the intercalation properties of riboflavin into the RNA and DNA of pathogens, leading to more extensive damage to microorganisms. Based on use of the same mechanism for eradication of pathogens in transfusion medicine, riboflavin–UV-A irradiation for the induction of ROS in microbes may be an efficient strategy upon encountering antibiotic-resistant bacteria in keratitis or treatment-refractory corneal infections, as the pharmacologic principle differs considerably from customary antibiotics.

It is plausible that other mechanisms are protective when CXL is used as a treatment for keratitis, based on
the observation that CXL significantly elevates corneal resistance against collagenase degradation in vitro.14
Because collagen-degrading enzymes are active components in the process of corneal melting, CXL may be suitable for the arrest of this severe complication of corneal infection.

The reduction of inflammatory intensity and rapid alleviation of symptoms that we have consistently observed after CXL could be a sign of an effect on ocular immune response, which also might be of vital importance in corneal melting, through modulation of the action of the stromal immune cells. The riboflavin–UV-A interaction is known to induce inhibition of white blood cell activity and has been proposed as a possible means for prevention of transfusion-associated graft-versus-host disease,15 supporting the hypothesis of an immunoregulatory effect.

**SURGICAL TECHNIQUE**

The surgical technique we use when applying CXL as a photochemical therapy is highly similar to the protocol of standard CXL for keratoconus and ectasia. No alterations have been made to the UV-A illumination settings, exposure time, or dosage. The concentration of riboflavin has not been modified, as the vitamin is highly important for the treatment effect. The isotonic preparation is instilled for at least 20 minutes, usually at an interval of every 2 minutes.

In thinned corneas caused by melting, we have decided to take the risk of potential endothelial damage, as the imminent alternative would be the possibility of emergent corneal transplant. Clinical complications regarding endothelial function have not been seen to date.

Because an epithelial defect is present in all treated eyes, only removal of the loose epithelium surrounding
the ulcer is conducted. This is accomplished by wiping with a sterile swab prior to riboflavin administration. A complete corneal abrasion is not performed, but this could be considered to facilitate the diffusion of riboflavin into the stroma.

Monitoring of riboflavin uptake is performed through slit-lamp microscopy and is confirmed by the observation of a slightly yellowish cornea and a yellowish flare in the anterior chamber before light exposure. Because uptake is confirmed in this manner, complete epithelial removal is not considered essential for the desired effect.

In some complicated cases, the treating physician may decide to combine the procedure with a human amniotic membrane transplant to further assist corneal healing, either directly following CXL or after the stabilization of the infectious intensity. This has been successful in a few advanced microbial ulcers, but we feel it could potentially be counterproductive, as a thicker amniotic membrane could make evaluation of corneal status more difficult and also may reduce the penetration of antibiotics into the stromal tissue.

Repetition of the UV-A–riboflavin procedure after a primary response has been described in management of nonresponsive Acanthamoeba keratitis. We have no data regarding this protocol, and it is not a method that has been applied at our clinic to this date due to the unknown consequences of applying doubled doses of oxidative stress and UV-A irradiation to the corneal tissues within a limited time. It is nevertheless possible that repetition of CXL could augment the antimicrobial and clinical effects. It is also a concern that too extensive corneal apoptosis or other UV-A or ROS-associated damage might be provoked. Therefore, this concept should be carefully assessed before it is implemented in practice.

CONCLUSION

Treating microbial keratitis photochemically using CXL appears to be a promising new method of managing complicated and nonresponsive corneal ulcers and corneal melting. The increasing alarm that has been raised regarding the detection and spread of multiple antibiotic-resistant pathogens makes the utilization of ROS more interesting as a potential antimicrobial tool in infections.

Given that CXL seemingly employs multiple mechanisms in interfering with the infectious and inflammatory response, only one of which is typically targeted by antibiotics, interest in further evaluation of CXL as an adjuvant treatment option for keratitis should be elevated. There are substantial potential gains to be reaped from implementing a one-time photochemical irradiation, possibly additionally improving the clinical management of corneal infections, decreasing complication rates linked with these conditions, and facilitating treatment of nonbacterial pathogens.

Karim Makdoumi, MD, is a Specialist Ophthalmologist in the Department of Ophthalmology, Örebro University Hospital, Sweden. Dr. Makdoumi states that he has no financial interest in the products or companies mentioned. He may be reached at email: karim.makdoumi@orebroll.se.

Jes Mortensen, MD, is a Consultant Ophthalmologist in the Department of Ophthalmology, Örebro University Hospital, and at Ryhov County Hospital, both in Sweden. Dr. Mortensen states that he has no financial interest in the products or companies mentioned. He may be reached at email: jes.mortensen@orebroll.se.

Sven Crafoord, MD, PhD, is a Consultant Ophthalmologist and Associate Professor in the Department of Ophthalmology, Örebro University Hospital, Sweden. Dr. Crafoord states that he has no financial interest in the products or companies mentioned. He may be reached at email: sven.crafoor@orebroll.se.