LABORATORY SCIENCE

Quantitative analysis of corneal stromal riboflavin concentration without epithelial removal

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Purpose: To compare the corneal stromal riboflavin concentration and distribution using 2 transepithelial corneal crosslinking (CXL) systems.

Setting: Absorption Systems, San Diego, California, USA.

Design: Experimental study.

Methods: The stromal riboflavin concentration of 2 transepithelial CXL systems was compared in rabbit eyes in vivo. The systems were the CXLO (Group 1) and Paracel/Vibex Xtra, containing TRIS and ethylenediaminetetraacetic acid and an isotonic solution of riboflavin 0.25%, (Group 2). Manufacturers' Instructions For Use were followed. The intensity of riboflavin fluorescence by slitlamp observation 10, 15, and 20 minutes after instillation was graded on a scale of 0 to 5. The animals were humanely killed and the corneal stromal samples analyzed with liquid chromatography and mass spectrometry.

Results: The mean riboflavin fluorescence intensity grades in Group 1 (4 eyes) were 3.8, 4.8, and 4.8 at 10, 15, and 20 minutes, respectively. The mean grades in Group 2 (3 eyes) were 2.0, 2.3, and 2.0, respectively. The riboflavin distribution was uniform in Group 1 but not in Group 2. The mean riboflavin concentration by liquid chromatography and mass spectrometry was 27.0 μg/g stromal tissue in Group 1 and 6.7 μg/g in Group 2. A stromal riboflavin concentration theoretically adequate for CXL, 15 μg/g, was achieved in all eyes in Group 1 and no eyes in Group 2. Slitlamp grading correlated well with liquid chromatography and mass spectrometry concentration (R^2 = 0.940).

Conclusions: The system used in Group 1 produced corneal riboflavin concentrations that were theoretically adequate for effective transepithelial CXL (≥15 μg/g), while the system in Group 2 did not. Slitlamp grading successfully estimated the corneal riboflavin concentration and can be used to ensure an adequate concentration of riboflavin in the cornea for transepithelial CXL.

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Stiffening of the cornea by crosslinking collagen and other corneal stromal molecules was first reported in animals by Spoerl et al. in 1998[1] and in humans by Wollensak et al. in 2003.[2] This original Dresden protocol requires the mechanical removal of the corneal epithelium because the intact epithelium prevents passage of topically applied riboflavin into the stroma.[2–4] Epithelial debridement creates prolonged pain, delays visual recovery, and can lead to a variety of complications.[5–7]

Strategies used to saturate the corneal stroma with riboflavin through an intact epithelium have included mechanical disruption of the epithelium,[8,9] increased exposure time,[10] the addition of excipients (eg, benzalkonium chloride, vitamin E),[11–14] and the use of iontophoresis[15–18] or ultrasound[19] to transport riboflavin across the epithelium. These strategies have increased the absorption of topical riboflavin but have not produced stromal concentrations equal to those obtained with epithelial debridement.[9–17,19]

The use of transepithelial riboflavin formulations, commercially available outside the United States, in patients with keratoconus produced encouraging early clinical results followed by reports of keratoconus progression between 1 year and 2 years after treatment.[6,18,20,21] Overall, the effectiveness of transepithelial techniques and products has been disappointing.[22]

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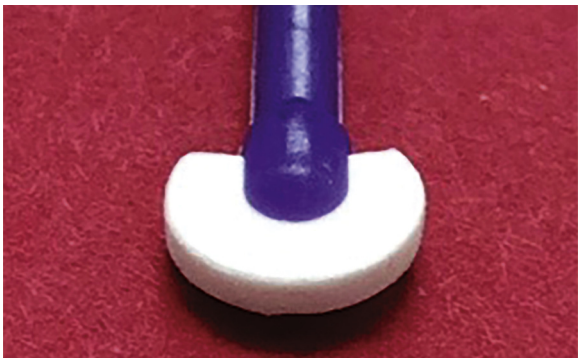

Figure 1. Proprietary sterile delivery device before hydration.

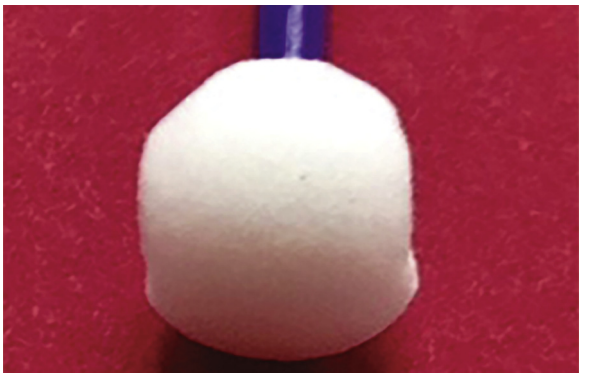

Figure 2. Proprietary device after hydration with no sharp edges to induce epithelial disruption.

We compared the transepithelial penetration of riboflavin into the corneal stroma using a new transepithelial corneal crosslinking (CXL) system and a commercially available transepithelial CXL system in vivo in rabbit eyes.

MATERIALS AND METHODS

Animals

Seven New Zealand White rabbits (Western Oregon Rabbit Co.) weighing 3.02 to 4.34 kg were housed under a 12/12 hour light/dark cycle with food and water provided ad libitum. All experimental protocols complied with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research and the U.S. Department of Agriculture Animal Welfare Act and Public Health Service approved Animal Welfare Assurance (A4282-01). The study protocol was approved by an Institutional Animal Care and Use Committee (Absorption Systems, Inc., San Diego, California, USA).

Surgical Technique

Seven animals with normal corneas and anterior ocular segments on slitlamp examination were divided into 2 groups. After an intramuscular injection of ketamine (30 mg/kg) and xylazine (5 mg/kg), 1 drop of topical proparacaine hydrochloride (0.5%) was instilled into 1 eye of each animal.

Animals in Group 1 were treated with a commercially available transepithelial CXL system (Paracel/Vibex Xtra, Avedro, Inc.) according to the manufacturer's Instructions For Use.[A] This system consists of 2 solutions, a riboflavin 0.25% solution containing TRIS and ethylenediaminetetraacetic acid (EDTA) (Paracel) and an isotonic solution of riboflavin 0.25% (Vibex Xtra) used sequentially. One drop of the solution containing TRIS and EDTA was applied to the cornea every 90 seconds for approximately 4 minutes. The cornea was rinsed with the riboflavin 0.25%, and 1 drop of riboflavin 0.25% was then applied every 90 seconds for 6 minutes, for a total riboflavin exposure time of 10 minutes.

Group 2 animals were treated with the test transepithelial riboflavin system (CXLO, CXL Ophthalmics LLC). This system consisted of (1) a new transepithelial riboflavin formulation without dextran and with a sodium iodide excipient in which the riboflavin concentration, pH, and osmolarity were designed to enhance absorption and (2) 2 sterile proprietary applicators designed to maximize contact between the riboflavin solution and the corneal surface.[23–25] First, eyes were gently brushed with minimal pressure in a circular motion over the entire cornea for 30 to 40 seconds with a patent-pending specially-designed sterile applicator that had been fully saturated with proparacaine. This applicator (Figures 1 and 2) is constructed of a nonabrasive porous material with patent-pending shapes, pore size, flexibility, and hydration properties specifically designed to enhance penetration of the new transepithelial riboflavin solution into the corneal stroma without disrupting the corneal epithelium (Figures 3 and 4). Unlike previously described techniques and devices,[8,9] this device does not use the principle of inducing epithelial disruption to improve epithelial permeability. The intact nature of the epithelium was confirmed on slitlamp examination by an independent laboratory researcher (G.G.G.). Next, a sponge-like loading device, shaped to conform to the cornea's curvature (including steep, cone-shaped corneas) and maximize contact with the cornea was saturated with the test transepithelial riboflavin solution and placed over the entire cornea (Figure 5). Continuous exposure to the new transepithelial riboflavin solution was ensured by the addition of 1 to 2 drops of the solution to the sponge every 1 to 3 minutes for 10 minutes.

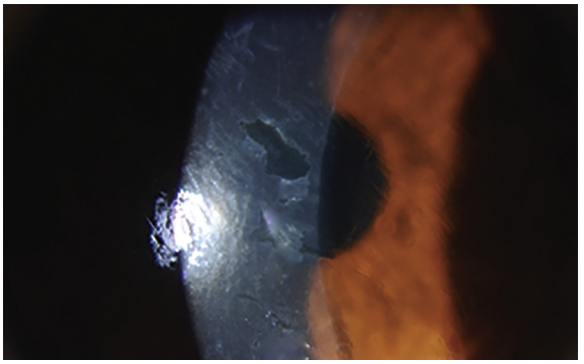

Figure 3. Epithelial defects typical of those induced by standard ophthalmic sponges with sharp edges in institutional review board–approved human studies.

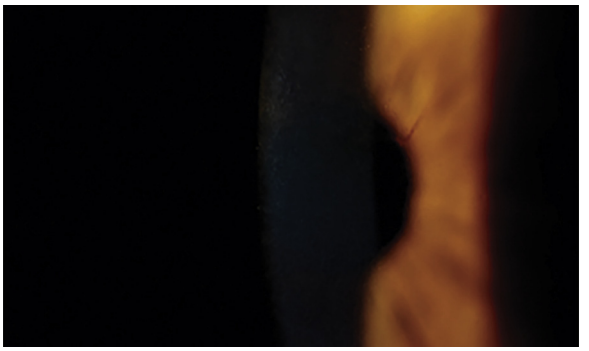

Figure 4. Undisrupted epithelium after treatment with the proprietary device shown in Figures 1 and 2. Human eye treated in an institutional review board–approved clinical trial.