Quantitative Analysis of Trans-Epithelial Corneal Riboflavin Loading

Roy Rubinfeld, MA, MD,
Jonathan Talamo, MD,
Doyle Stulting, MD, PhD
Affiliations

Dr. Rubinfeld: Re:Vision Private Practice and Department of Ophthalmology, Georgetown University Medical School/Washington Hospital Center, Washington, DC
Dr. Stulting: Stulting Research Center, Woolfson Eye Institute, Atlanta, GA, Professor of Ophthalmology Emeritus, Emory University
Dr. Talamo: Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA
# Financial Interests

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<tr>
<th>R. Rubinfeld</th>
<th>J. Talamo</th>
<th>D. Stulting</th>
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<td>- CurveRight, LLC</td>
<td>- Aura Biosciences</td>
<td>- Alcon Laboratories</td>
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<td>- CXLUSA, LLC</td>
<td>- Cowen and Company</td>
<td>- Allergan</td>
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Background

- Dresden protocol effective, epi removal risks
- Advantages of less invasive transepi CXL
  - Return to preop vision, function POD 1
  - Log scale shift in safety and risk/benefit
  - Treat upon diagnosis
Perforation after Epi-Off CXL
Transepithelial corneal collagen crosslinking for progressive keratoconus: 24-month clinical results

Aldo Caporossi, MD, FRCs, Cosimo Muzzetta, MD, PhD, Anna Lucia Paradiso, MD, Stefano Baiocchi, MD, PhD, Davide Marigliani, MD, Tomaso Caporossi, MD

**PURPOSE:** To assess the clinical results of transepithelial collagen crosslinking (CXL) in patients 16 years and younger with progressive keratoconus under suitable for epithelium-off (epi-off) CXL.

**SETTING:** Department of Ophthalmology, Siena University Hospital, Siena, Italy.

**DESIGN:** Prospective case series.

**METHODS:** The study included 26 eyes (26 patients) treated by transepithelial (epithelium-on) CXL. The mean age was 22 years (range 11 to 29 years), 16 (younger than 18 years; 16 between 16 years and 26 years). Preoperative and postoperative examinations included uncorrected (UDVA) and corrected (CDVA) distance visual acuity, simulated maximum keratometry (K), coma and spherical aberration, and corneal optical coherence tomography optical polychromy. The solution for transepithelial CXL (Microrin®) comprised riboflavin 0.1%, sodium 15.0%, tromethamin (Tris), and ethylendiaminetetraacetic acid. Ultraviolet-A treatment was performed with the Caporossi Baiocchi Muzzetta Linker Vega @ 3 mW/cm².

**RESULTS:** After relative improvement in the first 3 to 6 months, the UDVA and CDVA gradually returned to baseline preoperative values. After 12 months of stability, the simulated maximum K value worsened at 24 months. Coma aberration showed a statistically significant change. Spherical aberration increased at 24 months. Polychromy showed a progressive, statistically significant, decrease at 24 months. Fifty percent of pediatric patients were retrained with epi-off CXL due to significant deterioration of all parameters after 12 months of follow-up.

**CONCLUSIONS:** Functional results after transepithelial CXL showed keratoconus instability, in particular in pediatric patients 18 years old and younger. There was also functional regression in patients between 16 years and 26 years old after 24 months of follow-up.

**Financial Disclosure:** No author has a financial or proprietary interest in any material or method mentioned.

CXL Photochemistry

UVA Light
Energy Source

Riboflavin
Energy Transfer

Oxygen
Rate Limiting Reagent

Amide Aldehyde
Epi-On Success Requires

1. **Stroma**: Reliable, consistent riboflavin loading
2. **Epithelium**: Good UVA transmission, relatively clear of riboflavin, intact, non-edematous,
3. **Oxygen**: Rate limiting reagent if 1,2 present
Clear Epithelium, Loaded Stroma

No Visible Green Color in Epithelium

Well Loaded Green Stroma
Purpose

• Evaluate corneal penetration of riboflavin using a novel, proprietary, patent pending formulation and delivery system
• Compare results to those using a commercially available product
• Correlate slit lamp findings with quantitative measurements of corneal riboflavin concentration
Materials

- *Absorption Systems, Inc.*, San Diego California
- 3-4.4 kg New Zealand white rabbits
- **Group 1**: ParaCel™ q90 sec x 4 min., then VibeX Xtra™ q90 sec x 6 min. (per label)¹
- **Group 2**: Proprietary prep x 30-60 sec. then CXLUSA/CXLO riboflavin² via sponge with drops q1-3 min x 10 min.

¹Trans-epithelial Cross-Linking Kit, Avedro, Inc.
²Proprietary system from CXL Ophthalmics utilized by CXLUSA investigators under a physician-sponsored IRB approved study.
Methods

• Slit lamp photos at baseline
• Riboflavin application
• Masked grading and slit lamp photos at 10, 15, and 20 min. (2X labeling)
• Euthanasia, epithelial removal, and riboflavin assay by liquid chromatography/tandem mass spectrometry (LC-MS/MS) 22-25 min. after application
# Slit Lamp Grading System

<table>
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<tr>
<th>Grade</th>
<th>Findings</th>
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<tbody>
<tr>
<td>I</td>
<td>Mild green tint just visible</td>
</tr>
<tr>
<td>II</td>
<td>Substantial green visible</td>
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<tr>
<td>III</td>
<td>Obvious green color</td>
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<tr>
<td>IV</td>
<td>Bright green appearance</td>
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<tr>
<td>V</td>
<td>Strong, bright green color</td>
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Ex Vivo Rabbit Cornea SL Photos

Paracel™-VibeX Xtra™ At 20 min

CXLUSA Formulation At 10 Min
Ex Vivo Rabbit Cornea SL Photos

Paracel™-VibeX Xtra™
At 20 min

CXLUSA Formulation
At 10 Min
Slit Lamp Grade

Paracel™-VibeX Xtra™

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<tr>
<th>Minutes</th>
<th>10</th>
<th>15</th>
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<td>Slit Lamp Grade</td>
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3 Rabbits

CXLUSA

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<th>15</th>
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<td>Slit Lamp Grade</td>
<td>4</td>
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4 Rabbits
Riboflavin Concentration
(μg/g at 20-25 min.)

Paracel™-VibeX Xtra™

CXLUSA

Rabbit

Riboflavin Conc. μg/g

Rabbit

Riboflavin Conc. μg/g
Correlation Between SLE and LC-MS/MS
15 Min Epi-On Human CXLUSA
Conclusions

- CXLUSA Riboflavin system produces a 4.0-fold greater corneal stromal concentration in the rabbit than commercially available system.
- This correlates well with the 4.3-fold greater concentration of stromal riboflavin produced in the human by the epi-off technique [Gore et al., 2015; IOVS 56:5006] compared to the same commercially available system used in this study.
Conclusions

• Failure of previous epi-on protocols to halt progression of ectatic corneal disease may, in part, be due to inadequate corneal riboflavin concentration

• Slit lamp exam provides a valid estimate of stromal riboflavin concentration that can be used to assure adequate corneal loading prior to UVA exposure
Conclusions

• The CXL technique used by CXLUSA may achieve corneal stiffening similar to that achieved by the classical Dresden epi-off technique without the risk of epithelial removal

• Early 2-year clinical data suggest this is a valid hypothesis
Thank You