Treatment Effect and Corneal Light Scattering With 2 Corneal Cross-linking Protocols A Randomized Clinical Trial

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IMPORTANCE We describe and evaluate a complementary method to indirectly quantify the treatment effect of corneal cross-linking (CXL). Additional methods to indirectly quantify the treatment effect of CXL are needed.

OBJECTIVE To assess the spatial distribution and the time course of the increased corneal densitometry (corneal light backscatter) seen after CXL with riboflavin and UV-A irradiation.

DESIGN, SETTING, AND PARTICIPANTS Open-label randomized clinical trial of 43 patients (60 eyes) who were 18 to 28 years of age and had progressive keratoconus and a plan to be treated with CXL at Umeå University Hospital, Umeå, Sweden. The patients were randomized to receive conventional CXL (n = 30) using the Dresden protocol or CXL with mechanical compression of the cornea using a flat rigid contact lens sutured to the cornea during the treatment (CRXL) (n = 30). All participants were followed up during a 6-month period from October 13, 2009, through May 31, 2012.

INTERVENTIONS Corneal cross-linking according to the Dresden protocol or CRXL.

MAIN OUTCOMES AND MEASURES Change in corneal densitometry after CXL and CRXL for keratoconus.

RESULTS Of the original 60 eyes included, 4 had incomplete data. A densitometry increase was seen after both treatments that was deeper and more pronounced in the CXL group (difference between the groups at 1 month in the center layer, zone 0-2 mm, 5.02 grayscale units [GSU], 95% CI, 2.92-7.12 GSU; P < .001). This increase diminished with time but was still noticeable at 6 months (difference between the groups at 6 months in the center layer, zone 0-2 mm, 3.47 GSU; 95% CI, 1.72-5.23 GSU; P < .001) and was proportional to the reduction in corneal steepness (R = −0.45 and −0.56 for CXL and CRXL, respectively).

CONCLUSIONS AND RELEVANCE The degree of corneal light backscatter relates to the reduction in corneal steepness after cross-linking and may become a relevant complement to other methods in evaluating the cross-linking effect, for example, when comparing different treatment regimens.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT02425150

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Keratoconus (KC) is a noninflammatory progressive corneal degeneration that causes corneal thinning and ectasia with myopia and irregular astigmatism, affecting visual quality. The refractive errors can be corrected with eyeglasses or rigid contact lenses and, in the late stages, with keratoplasty, but none of these interventions have any effect on the underlying mechanisms that cause the disease. Corneal cross-linking (CXL) with riboflavin and UV-A photoactivation is a safe and efficient method to halt the progression of KC by increasing the biomechanical stability of the cornea with formation of covalent bonds (cross-links) in the corneal stroma.

Increased corneal biomechanical stability has been demonstrated in vitro after CXL treatment in several studies. In a previous study, we demonstrated an increase in biomechanical stability, also in vivo, using applanation resonance tomometry (ART; BioResonator Good Eye) technology. In vitro studies have shown that significant stiffening of the corneal tissue occurs chiefly in the anterior part of the stroma. In the same area where the cross-linking occurs, CXL also induces keratocyte apoptosis up to a depth of 300 to 350 μm. A lacunar edema follows during the healing process after the keratocyte apoptosis, leading to superficial light scattering that is visible as a reversible superficial corneal haze weeks to months after the treatment. Scheimpflug imaging has shown to be a potential tool for monitoring the cross-linking effect after the CXL method by measuring the stromal reflectivity.

With the widespread use of CXL in KC today and the ongoing development of the method, there is a growing need for tools to evaluate the treatment effects in vivo in individual cases and in studies comparing different treatment regimens. This study involves patients treated with standard CXL and cross-linking with mechanical compression of the cornea using a flat rigid contact lens sutured to the cornea during the treatment, which we termed corneal reshaping and cross-linking (CRXL). Given the spatial relationship between corneal haze and cross-linking and the fact that both appear to be related to the degree of irradiation, we hypothesize that quantification of the light scattering may be an indirect way to quantify the treatment effect of CXL as a complement to biomechanical and structural evaluations. The aim of this study was to assess the spatial distribution and time course of corneal densitometry changes after CXL and CRXL and their relationship to biomechanical and structural changes due to the treatments.

Methods

In this open-label randomized clinical trial, patients with KC and a plan to be treated with CXL were recruited from the Department of Ophthalmology, Umeå University Hospital, Sweden, from October 13, 2009, through May 31, 2012 (NCT02425150). The full study protocol can be found in the trial protocol in Supplement 1.

The study comprised 60 eyes of 43 patients with KC (39 men and 4 women) who were 18 to 28 years old. The group sizes were determined with a power analysis; the study design allows for detection of a difference in spherical equivalent of 1.9 diopters (D) between treatments and 0.8 D between time points (α = 0.05, with an 80% chance of detecting a between-group difference). After inclusion, the patients were randomized to receive either conventional CXL (n = 30) using the Dresden protocol or CRXL (n = 30). Randomization was conducted using a computer-generated list of random numbers and the patients were randomized using a list of unique random numbers between 1 and 60. They were included in running numbers according to the list; an even number was treated with CXL and an odd number with CRXL.

The study was approved by the Regional Ethical Review Board in Umeå, Sweden, and was performed in accordance with the Declaration of Helsinki. All patients were provided both oral and written information about the procedures before they gave their written consent to participate.

The inclusion criteria were patients with progressive KC who had a plan to be treated with CXL. In 43 eyes, the progression was documented by repeated Scheimpflug tomographic measurements with increasing keratometry readings and/or thinning of the cornea, and in 17 eyes, the progression was documented by increasing corneal steepness and astigmatism on keratometry and decreasing best spectacle-corrected visual acuity. The KC diagnosis was based on the Amsler-Krumeich grading and the total deviation KC quantification value from the Belin-Ambrosio enhanced ectasia measurements of the Pentacam HR (Oculus, Inc). In addition, an altered red reflex and/or an irregular cornea, which is seen as distortion of the keratometric mires, was required for diagnosis. Inclusion also required a minimum corneal thickness of 400 μm at the thinnest point after epithelial removal. In borderline cases, ultrasonic pachymetry (the ultrasonic pachymeter featured in the ORA [Ocular Response Analyzer], software version 1.02; Reichert, Inc) was also performed after epithelial removal, and hypo-osmotic riboflavin was used when deemed necessary (in 1 patient). Finally, the included patients had no ocular abnormalities except KC, no previous ocular surgery, and no cognitive insufficiency that interfered with informed consent.

The exclusion criteria were being younger than 18 years or older than 28 years, any corneal abnormalities except KC, previous ocular surgery, or cognitive insufficiency.

The conventional CXL treatments at the baseline visit involved mechanical removal of the central 9-mm corneal epithelium after topical anesthesia with tetracaine, repeated topical application of riboflavin, 0.1% (Ricrolin), every 3
minutes for 30 minutes, and UV-A irradiation for 30 minutes using a solid-state UV-A illuminator (Caporossi/Baiocchi/Mazzotta X Linker; CSO). During UV-A irradiation, riboflavin was given every 5 minutes. The diameter of the irradiation area was 8 mm, the delivered energy was 3 mW/cm², and the shutter was set to an 8-mm irradiation area.16 In the CRXL group, a semiscleral rigid Boston XO contact lens (Nordic Lenses, Inc; diameter, 12.5 mm; back surface curvature, 11.0 mm; Dk value [oxygen diffusivity and solubility], 100.0) was included in the 1- and 6-month analyses. CXL indicates corneal cross-linking; CRXL, corneal reshaping and cross-linking.

The changes in densitometry from baseline and the quotient of this densitometry increase was more persistent in the central anterior part of the cornea in the CXL group compared with the peripheral and the central zones at different depths were determined for each cornea at 1 and 6 months after treatment.

The changes in densitometry were analyzed for correlations with changes in maximum corneal curvature, minimum corneal thickness, mean keratometry, logMAR for best spectacle-corrected visual acuity, spherical equivalent, and for the CXL group, corneal hysteresis measured with ORA and with ARTr technology.10,21-24

Paired or unpaired 2-sided t tests were used for statistical comparisons, as appropriate. Correlations were assessed using Pearson correlations. P < .05 was considered statistically significant.

Results

The study CONSORT diagram is shown in Figure 1. One patient developed a keratitis after CXL but the infection was subsequently successfully treated and he could complete the study.

No differences were detected between the treatment groups at baseline (Table 1 and Table 2). An increase in corneal densitometry was seen in essentially all zones and layers at 1 month in both treatment groups (Table 2), apart from the peripheral 10- to 12-mm zones. The densitometry increase was larger in the CXL-treated corneas (change from baseline in central layer, zone 0-2 mm, 7.2 GSU for CXL and 2.2 GSU for CRXL).

At 6 months, a regression of this densitometry increase was seen in both groups but the increase was generally still present at 6 months (change from baseline in center layer, zone 0-2 mm, 4.0 GSU for CXL and 0.5 GSU for CRXL) (Table 2). The densitometry increase was more persistent in the central anterior part of the cornea in the CXL group compared with the

Figure 1. CONSORT Flow Diagram

Table 1. Overview of Baseline Data for Each Treatment Group

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CXL</th>
<th>CRXL</th>
</tr>
</thead>
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<tr>
<td>Eyes, No.</td>
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<td>30</td>
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<tr>
<td>Sex</td>
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<td>3</td>
</tr>
<tr>
<td>Male</td>
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<td>27</td>
</tr>
<tr>
<td>Smoker</td>
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<td>7</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
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<td></td>
</tr>
<tr>
<td>[range], y</td>
<td>23.7 (3.1)</td>
<td>23.4 (3.0)</td>
</tr>
<tr>
<td>Sphere, D</td>
<td>−0.3 (2.7)</td>
<td>−0.5 (2.5)</td>
</tr>
<tr>
<td>Cylinder, D</td>
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<td>−2.9 (2.2)</td>
</tr>
<tr>
<td>Spherical equivalent, D</td>
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<td>−1.9 (2.5)</td>
</tr>
<tr>
<td>BSCVA, logMAR</td>
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<td>0.23 (0.29)</td>
</tr>
<tr>
<td>Kmax, D</td>
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<td>53.9 (5.4)</td>
</tr>
<tr>
<td>Kmean, D</td>
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<td>46.7 (3.5)</td>
</tr>
<tr>
<td>Rmin, μm</td>
<td>6.4 (0.6)</td>
<td>6.3 (0.6)</td>
</tr>
<tr>
<td>CTmin, μm</td>
<td>480 (40)</td>
<td>479 (30)</td>
</tr>
</tbody>
</table>

Abbreviations: BSCVA, best spectacle–corrected visual acuity; CRXL, corneal reshaping and cross-linking; CTmin, minimum corneal thickness; CXL, corneal cross-linking; D, diopters; Kmax, maximum corneal curvature; Kmean, mean keratometry; Rmin, minimum radius of curvature.

The number of eyes assessed, enrolled, randomized to the 2 interventions, and included in the 1- and 6-month analyses. CXL indicates corneal cross-linking; CRXL, corneal reshaping and cross-linking.
CRXL group (mean [SD] regression from 1 to 6 months, −2.7 [7.1] vs −5.9 [4.8] GSU). Furthermore, the increase in densitometry was larger at 1 and 6 months in the deeper layers of the central zones of the CXL compared with the CRXL group (eg, difference between the groups at 1 month in the posterior 60 μm, zone 0-2 mm, 2.61 GSU; 95% CI, 1.03-4.19; P < .001; difference between the groups at 6 months in the posterior 60 μm, zone 0-2 mm, 1.12 GSU; 95% CI, 0.24-2.00; P = .01).

Generally, in both treatment groups, the densitometry decrease at 1 month was more pronounced in the central cornea and leveled off toward the periphery (Table 2). This gradient, however, was less marked in the CRXL corneas. At 6 months, a difference in densitometry increase between the central and peripheral cornea could no longer be detected in the CRXL group, whereas it persisted in the anterior and central layers of the CXL group.

Interestingly, the increase in central corneal densitometry at 6 months showed a correlation with the reduction in maximum corneal curvature at the same time point (R = −0.31 for the entire material) (Figure 2), but if the 2 treatments were
Figure 2. Correlation Between Corneal Densitometry and Maximum Corneal Curvature

The differences in corneal densitometry plotted against the difference in maximum corneal curvature at 6 months for patients who received corneal cross-linking (CXL) or corneal reshaping and cross-linking (CRXL). A linear model, $y = -2.19x + 6.49 (R^2 = 0.21)$, was found for the CXL group. The corresponding model for the CRXL group was $y = -0.99x + 0.66 (R^2 = 0.31)$. 

Discussion

The corneal light backscatter, measured with corneal densitometry, increases after cross-linking; here we show that this increase relates to the degree of posttreatment corneal flattening, measured as a reduction in maximum corneal curvature. Our impression is that the demarcation line often seen after CXL is part of what we measured as a densitometry increase because it occurs at a similar depth and has a similar time course as the densitometry increase assessed in this study. Furthermore, the densitometry increase coincides with the area where the biomechanical stability is believed to increase after CXL, which adds to the potential relevance of densitometry increase as an indicator of treatment effect.

As one might have expected, the increase in densitometry measured by the Pentacam HR software in this study shows a strong correlation to the increased light scattering that was measured manually in a previous study ($R = 0.90$; eFigure in Supplement 2). Because this new method measures essentially the same way, but considerably faster, it means that automated densitometry has the potential to become clinically useful. Today, CXL treatments generally adhere to a few standardized treatment protocols, but which regimen is preferable is still being debated. Supplementary ways to quantify the effect of CXL can aid in developing optimized treatment regimens for the benefit of ophthalmologists and patients with KC.

As we previously reported, the refractive and structural effects of CRXL are inferior to those of CXL in some respects. Accordingly, we see here that the increase in corneal densitometry is less pronounced in the CRXL group. One possible explanation to the inferior treatment effect with CRXL could be that the rigid contact lens blocks the oxygen from reaching the stroma, a well-known feature of rigid contact lenses. Such a phenomenon is compatible with the inferior treatment effect seen in accelerated CXL in some studies in which oxygen depletion has been suggested as an underlying mechanism. Furthermore, in this study, the increased densitometry goes deeper in the cornea in CXL and has a more prolonged time course compared with CRXL. This finding resembles the occurrence of a deeper demarcation line in conventional cross-linking that was reported by Kymionis et al and could have a similar explanation, that is, a higher oxygen tension in the corneal stroma during treatment. Alternative explanations could be that the UV effect or riboflavin concentration may be lower in CRXL compared with conventional CXL, but regardless, such differences will ultimately result in a reduced generation of oxygen free radicals and a reduced cross-linking effect. In this study, differences in corneal light backscatter between the 2 treatments can be demonstrated on the group level, but the method is still too imprecise to quantify the treatment effect in individual cases, something that would require further refinement of the method.

Although the increase in densitometry is generally lower after CRXL, the peripheral cornea shows more densitometry increase relative to the central cornea after this treatment. The reason may be that the rigid contact lens flattens the cornea during treatment, which may render a more uniform illumination pattern in CRXL than in CXL, in which the cornea is more steep and protruding.
In the extreme periphery of the cornea, the densitometry increase is variable between measurements and these findings are more difficult to interpret. Our impression is that this difficulty may owe to a measurement artifact because more or less of the opaque limbal tissue may have been included in the measurements, which increases the intermeasurement variability. Had it not been for this artifact, densitometry of the untreated peripheral cornea might have been used as a calibration reference; this method is, unfortunately, not possible.

The corneal densitometry increase differs in different parts of the cornea and between the 2 treatment groups and appears to be related to the cross-linking treatment effect. This difference is evident when CXL- and CRXL-treated corneas are compared, and the previously published differences in treatment effect between these 2 treatments reflect in a less pronounced corneal densitometry increase in the CRXL group. These differences are large enough to be clinically relevant, and mapping of corneal densitometry changes can be a supplementary way to monitor the effects of CXL, both locally in each cornea and for comparison of the overall effects of different treatment regimens.

A weak point in the study design is the lack of masking, but given the nature of the 2 treatments with a sutured contact lens in CRXL, masking was deemed impossible. However, the densitometry and keratometry values are automatically generated by the Pentacam HR software, which reduces possible bias. Another possible confounder in all KC research is the high variability in KC severity between the patients. Including more participants in a study or matching of study participants between the treatments can be ways to handle this factor. The use of strict age limits was an attempt to make the material more homogeneous. The contact lens in our model needs to compress the cornea to change the corneal shape but this compression will inevitably also change the precorneal environment regarding the riboflavin film and the corneal oxygenation. These factors likely influenced our result, giving less cross-linking effect in CRXL.

Conclusions

Our findings suggest that corneal densitometry may be related to the treatment effect of CXL and has the potential to become a useful complement to other forms of evaluation of cross-linking treatment effects, including assessment of future treatment regimens.

ARTICLE INFORMATION
Submitted for Publication: February 9, 2015; final revision received July 2, 2015; accepted July 4, 2015.

Author Contributions: Drs Hallberg and Behndig had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: All authors.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Rehnman, Lindén, Behndig.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: All authors.
Obtained funding: Rehnman, Lindén, Behndig.
Administrative, technical, or material support: Rehnman, Lindén, Behndig.
Study supervision: Lindén, Hallberg, Behndig.
Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.
Funding/Support: This study was supported in part by unrestricted grants from the Kronprinsessan Margaretas Arbetssnämnd (Crown Princess Margarita’s Working Committee) Fund, Ögonfonden, Västerbotten County Council (Avtalet för Läkare Forskning), Stiftelsen J. C. Kæmpes Minnes Stipendiefond, and the European Regional Development Fund.
Role of the Funder/Sponsor: The funding sources had no role in the design or conduct of the study; the collection, management, analysis, and interpretation of the data; the preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication.

REFERENCES


