Keratoconus is a progressive corneal degeneration that exhibits corneal thinning and bilateral conical protrusion. It can present as moderate irregular astigmatism or a more severe visual impairment.1 Keratoconus can lead to biomechanical alterations; however, the pathogenesis has not been completely understood yet.

Corneal cross-linking (CXL) was first performed in 2003 by Wollensak et al.2 and has become the standard treatment of keratoconus.3 Currently, this treatment option for improvement of corneal biochemical properties is the only treatment that addresses the pathophysiology of keratoconus4 and it has been widely used for stabilizing progressive keratoconus.

In the original CXL treatment strategy, known as the Dresden protocol,2 0.1% riboflavin–20% dextran solution (riboflavin-dextran) is applied after removal of corneal epithelium followed by an exposure of ultraviolet-A (UV-A) irradiation at 3 mW/cm² for 30 minutes. Alternative treatment protocols including modifications to UV-A irradiation, such as a change in exposure time (10 min) and intensity (9 mW/cm²), known as “accelerated CXL,” have been subsequently introduced.

The original Dresden protocol, in which riboflavin-dextran solution is used, has been shown to affect intraoperative corneal thinning.5 Therefore, different riboflavin solutions are used. Interestingly, both in vivo and in vitro studies have demonstrated that CXL is not a good

ABSTRACT

PURPOSE: To determine 2-year efficacy of accelerated corneal cross-linking [CXL] in keratoconus treatment using standard riboflavin-dextran or hypotonic riboflavin solutions.

METHODS: Patients undergoing accelerated CXL [epithelium-off 10 minutes, 9 mW/cm² protocol] with standard riboflavin solution (48 eyes of 48 patients) or hypotonic riboflavin solution (43 eyes of 43 patients) were included and followed up for 2 years. Thinnest corneal thickness [TCT], maximum keratometry, and visual acuity were measured and changes from baseline to postoperative 6, 12, and 24 months were compared between the two groups.

RESULTS: The preoperative mean TCT with intact epithelium was 472.0 ± 23.9 and 427.5 ± 22.3 µm in the standard riboflavin and hypotonic riboflavin groups, respectively [P < .001]. The decreases in the mean TCT values from baseline to postoperative 6 months were similar between the standard riboflavin (from 472 to 436 µm) and hypotonic riboflavin (from 427 to 394 µm) groups. This suggested that the hypotonic riboflavin solution was comparable with the standard riboflavin solution in preserving corneal thickness in keratoconus. There were no significant differences between the study groups regarding the postoperative changes in maximum keratometry or visual acuity.

CONCLUSIONS: The efficacy of accelerated CXL with hypotonic riboflavin solution was comparable to that with the standard riboflavin solution in reducing keratoconus progression in a 2-year follow-up period.

method for corneal thickness of less than 400 µm. Alternatively, there are other procedures available for thin corneas, such as transepithelial CXL, CXL based on customized pachymetry-guided epithelial debridement that maintains the epithelium in thinner areas of the cornea, and iatrogenic corneal swelling in advance of CXL. In another technique, developed by Hafezi et al., the cornea was swollen with hypotonic riboflavin (no dextran). UV-A treatment was performed when the corneal thickness was at least 400 µm. Jacob et al. developed a contact lens-assisted CXL technique for corneal thicknesses of less than 400 µm after removal of epithelium. In this technique, a contact lens is soaked in riboflavin solution and placed on the cornea before CXL to artificially increase the corneal thickness. The corneal thickness of 400 µm has become a gold-standard threshold for obtaining low complication rates after CXL.

Corneal stromal swelling can be achieved with a low colloid osmotic pressure solution. With the use of this method, the deepithelialized cornea can swell up to twice its normal thickness via the hydrophilic absorbent ability of the stromal proteoglycans. This method has been used to increase corneal thickness before CXL. However, preoperative application has only a short-term effect; it does not last until the end of treatment and is reversed by iso-osmolar riboflavin application during irradiation.

In their study, Wollensak and Spörl obtained a similar biomechanical effect of CXL using isotonic or hypotonic riboflavin solutions in post-mortem porcine eyes. Because of the localization of the maximum CXL effect in the anterior 200 µm of the cornea, the efficacy of CXL with hypotonic riboflavin solution was not affected by swelling due to the Lambert–Beer law (I = I_0 \times e^{-d/p}, d = corneal thickness; p = absorption coefficient). Owing to the exponentially reduced UV-A irradiance passing through the absorbing medium and resistance of the anterior cornea to hydration due to its structural features, CXL could be performed after corneal swelling at a minimum of 15% in that area. Moreover, the anterior cornea was not affected by the hypotonic swelling because of its structural features, allowing anterior curvature and refractive power to be preserved even in the edematous state.

Efficacy of epithelium-off CXL with standard riboflavin solution has been shown in several reports with adequate follow-up periods. However, clinical data regarding the comparison of hypotonic riboflavin with CXL with standard riboflavin with CXL are lacking. To our knowledge, there are no studies that compare long-term visual acuity and topographic changes after accelerated corneal CXL using standard riboflavin or hypotonic riboflavin.

In the current study, we aimed to compare accelerated CXL procedures using standard riboflavin and hypotonic riboflavin solutions in patients with progressive keratoconus and to determine long-term efficacy of accelerated CXL with these two riboflavin solutions.

**PATIENTS AND METHODS**

Patients with progressive keratoconus were included in this prospective study and followed up for 24 months. Progression was defined based on at least one of the following parameters: an increase of at least 1.00 diopter (D) in the maximum keratometry (K_max) reading, an increase of at least 0.50 D in manifest refraction spherical equivalent, or an increase of at least 1.00 D in manifest cylinder in the past 6 months.

Patients who were younger than 18 years; who had chemical burns, previous anterior segment surgery, ocular surface problems, corneal scars, severe corneal infections, or systemic connective tissue disease; or who were pregnant or lactating were excluded. Patients with rigid contact lenses were asked to remove their lenses at least 3 weeks prior to the evaluation to prevent warpage and to obtain reliable keratometry values. Uncorrected (UDVA) and corrected (CDVA) distance visual acuity, biomicroscopic findings, and topographic findings (Pentacam HR; Oculus Optikgeräte GmbH, Wetzlar, Germany) were evaluated preoperatively and at postoperative 6, 12, and 24 months. Both UDVA and CDVA values were collected and analyzed as the logarithm of the minimum angle of resolution (logMAR) values.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethical Board of Kayseri Training and Research Hospital. Before undergoing CXL, written informed consents were obtained from all patients.

**SURGICAL TECHNIQUE**

Table 1 outlines the CXL methods. Topical anesthesia was applied to all patients and the corneal epithelium was mechanically removed over the central 9 mm. If the thinnest corneal thickness (TCT) was less than 400 µm, hypotonic riboflavin solution (0.1% in sterile water, Medio Cross hypotonic; Medio Cross, Peschke Trade GmbH, Huernenberg, Switzerland) was administered following ultrasonic pachymetry; these patients were referred to as the hypotonic riboflavin group. Administration of hypotonic riboflavin was repeated until TCT of 400 µm or greater was achieved. On the other hand, if the TCT was 400 µm or greater, standard riboflavin solution (0.1% in 20.0% dextran T500 solution; Medio Cross) was administered at 2-minute intervals for 30 minutes; these patients were referred...
to as the standard group. The cornea was then exposed to UV-A 370-nm light (Apollon Cross-linking System; BNM Inc., Meran Tıp, Turkey) at an irradiance of 9 mW/cm² for 10 minutes. During this time, hypotonic riboflavin solution for the hypotonic riboflavin group and riboflavin-dextran solution for the standard riboflavin group were applied to maintain corneal saturation with riboflavin. At the end of the surgery, the cornea was irrigated with cold water and a bandage contact lens was placed on the eye.

After the surgery, all patients received antibiotic therapy. The bandage contact lens was removed following complete epithelial healing, which was generally after 3 to 5 days. Topical steroids were also prescribed to prevent impairment of epithelial healing and to avoid infection on complete epithelial closure.

**STATISTICAL ANALYSIS**

All statistical analyses were performed using the IBM SPSS Statistics for Windows software (version 21.0; IBM Corporation, Armonk, NY). Normality of the groups was evaluated by the Kolmogorov–Smirnov test. Decimal visual acuity was converted to logMAR units. All values were expressed as mean ± standard deviation. The Student’s t test for parametric variables and the Mann–Whitney U test for non-parametric variables were used to compare the parameters between the study groups. For the analyses of postoperative changes from the baseline and changes in postoperative outcomes during the follow-up periods, the paired two-tailed Student’s t test for parametric variables and the Wilcoxon signed-rank test for non-parametric variables were used. A P value of less than .05 was considered statistically significant.

**RESULTS**

In the current study, 91 patients with keratoconus who underwent accelerated CXL were evaluated. Of these patients, 48 (30 men and 18 women) were in the standard riboflavin group and 43 (20 men and 23 women) were in the hypotonic riboflavin group. There were no significant differences between the groups in terms of age and sex. The baseline demographic and clinical characteristics of the study groups are shown in Table 2.

**CORNEAL THICKNESS**

The mean baseline TCT value of the standard riboflavin group (472.04 ± 23.94 µm) was significantly higher than that of the hypotonic riboflavin group (427.53 ± 22.33 µm; P < .001) (Table 2). The mean TCT values of the study groups from baseline to all postoperative follow-up periods are demonstrated in Figure 1.

In the standard riboflavin group, the mean TCT values at postoperative 6, 12, and 24 months were significantly lower than that at baseline (P < .001 for each). On the other hand, mean TCT values at both postoperative 12 and 24 months were significantly higher than that at postoperative 6 months (P < .001 and P = .002, respectively); however, there was no significant difference between the values at postoperative 12 and 24 months (P = .790).

In the hypotonic riboflavin group, the mean TCT values at postoperative 6, 12, and 24 months were also significantly lower than that at baseline (P < .001, P < .001, and P = .003, respectively). However, the decreases in the mean TCT values at postoperative 12 and 24 months were not significantly different from those at postoperative 6 months (P = .829 and .476, respectively). There was also no significant difference between the mean TCT values at postoperative 12 and 24 months (P = .989).

When the standard and hypotonic riboflavin groups were compared according to the changes in the mean TCT values from baseline, no significant difference was obtained at postoperative 6 months (P = .68); however, the changes at postoperative 12 and 24 months were significant (P = .04 and .02, respectively; Table A, available in the online version of this article).

In the standard riboflavin group, postoperative corneal thickness showed thinning at 6 months with recovery over a 2-year period; however, the hypotonic riboflavin group showed thinning at 6 months without recovery over 2 years.

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**TABLE 1 CXL Methods**

<table>
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<th>Parameter</th>
<th>Variables</th>
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<td>Treatment target</td>
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<tr>
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<td>Iso-osmolar or hypotonic</td>
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<td>Light source</td>
<td>Apollon Cross-linking System; Meran Tıp, BNM Inc., Turkey</td>
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</tr>
<tr>
<td>Protocol abbreviation in manuscript</td>
<td>CXL</td>
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CXL = corneal cross-linking
The mean preoperative $K_{\text{max}}$ value of the standard riboflavin group ($53.92 \pm 4.43$ D) was significantly lower than that of the hypotonic riboflavin group ($56.29 \pm 5.06$ D; $P = .019$) (Table 2). The mean $K_{\text{max}}$ values determined by the rotating Scheimpflug corneal topography in the study groups from baseline to postoperative follow-up periods are demonstrated in Figure 2.

In the standard riboflavin group, although there was a slight increase from baseline in the mean $K_{\text{max}}$ value at postoperative 6 months, a slight decrease was observed at postoperative 12 months; however, the differences were not statistically significant ($P = .097$ and .528, respectively). Indeed, there was a significant decrease in the $K_{\text{max}}$ value at postoperative 24 months ($P = .040$).

The hypotonic riboflavin group also showed a slight increase from baseline in the mean $K_{\text{max}}$ value at postoperative 6 months and a slight decrease at postoperative 12 months; however, the differences were again not significant ($P = .468$ and .07, respectively). On the other hand, the decrease at postoperative 24 months was significant ($P = .002$).

There were no significant differences between the study groups in terms of the changes in $K_{\text{max}}$ values from baseline to postoperative 6, 12, and 24 months ($P = .59$, .26, and .31, respectively), although an improvement in the $K_{\text{max}}$ value after CXL was obtained during a 2-year follow-up period in both study groups (Table A).

**UDVA**

There was no significant difference in the mean UDVA (logMAR) values between the study groups at baseline ($P = .13$; Table 2). Figure 3A shows the mean UDVA values in the study groups from baseline to the postoperative follow-up periods. The decreases in the mean UDVA from baseline to postoperative 24 months were significant in both the standard and hypotonic riboflavin groups ($P = .004$ and $P < .001$, respectively).

Although the mean UDVA values were improved in both study groups at the end of the 2-year follow-up period, there were no significant differences between the study groups in terms of the change in the mean UDVA values from baseline to postoperative 6, 12, and 24 months ($P = .67$, .33, and .25, respectively; Table A).

**CDVA**

At baseline, there was no significant difference in the mean CDVA (logMAR) values between the study groups ($P = .07$; Table 2). Figure 3B shows the mean CDVA values in the study groups from the baseline to the postoperative follow-up periods. Although the
mean UDVA values were improved in the standard and hypotonic riboflavin groups (0.14 ± 0.16 and 0.22 ± 0.28 logMAR, respectively) at the end of the 2-year follow-up period \( (P < .001 \) and \( P = .006 \), respectively), no significant differences were observed between the study groups in terms of the changes in UDVA values from baseline to postoperative 6, 12, and 24 months \( (P = .65, .83, \) and \( .23, \) respectively; Table A).

None of the patients developed complications after CXL. Normal healing was observed in all corneas and all corneas were transparent without any detectable scarring in the stroma 2 years after the procedure.

**DISCUSSION**

Several reports have documented the safety and efficacy of CXL. In particular, Wittig-Silva et al.\(^{24}\) demonstrated that 94 patients gained improved flattening in comparison with control subjects after CXL in a 36-month follow-up period. In another study, Viswanathan and Males\(^{25}\) showed that there was a decrease in \( K_{\text{max}} \) by 0.96 D in 51 eyes. Our study revealed that CXL with UV-A-riboflavin was effective in stabilizing keratoconus and recovering visual acuity in both the standard and hypotonic riboflavin groups. Furthermore, in both study groups, there were significant reductions in the \( K_{\text{max}} \) values (by -1.14 ± 2.57 D in the standard group and -1.97 ± 1.86 D in the hypotonic riboflavin group), as well as improvement in vision at postoperative 24 months with respect to the values at baseline.

CXL is the first treatment modality to halt keratoconus progression. Ex vivo studies have demonstrated increased stromal stress-strain measurements, enzymatic digestion resistance, thermal damage, and hydration.\(^{26-30}\) Several prospective clinical studies have also emphasized the efficacy of CXL in the treatment of keratoconus.\(^{16-20}\)

O’Brart et al.\(^{16}\) showed the stabilization of keratoconus in 30 eyes of 30 patients who had undergone CXL, with significant reductions in topographic parameters in 4 to 6 years compared with the reductions in the 1-year follow-up. These results suggest that CXL increases the stability of keratoconus and reduces its...
progression. Raiskup et al.\textsuperscript{17} studied 34 eyes of 24 patients with progressive keratoconus (with more than 10 consecutive years of follow-up) and showed that CXL could provide long-term disease stabilization, significant reduction in $K_{\text{max}}$ value, and improvement in CDVA (by 0.14 logMAR) over time. Similarly, Caporossi et al.\textsuperscript{19} reported stabilization of keratoconus in 44 eyes after 4 years of follow-up and determined that there was a visual improvement and a reduction in keratometry. Poli et al.\textsuperscript{18} reported that CXL reduced the progression of corneal ectasia and improved CDVA and keratometry in 36 eyes of 25 patients in a 6-year follow-up period. Vinciguerra et al.\textsuperscript{20} also reported that CXL appeared to be effective in improving UDVA and CDVA in 28 eyes with progressive keratoconus by significantly reducing the average corneal apex keratometry at 2 years postoperatively. These findings were also compatible with our results, which showed significant improvements in UDVA (0.22 ± 0.37 logMAR), CDVA (0.14 ± 0.16 logMAR), and $K_{\text{max}}$ (1.14 ± 2.57 D) in 48 eyes of patients with keratoconus undergoing CXL with standard riboflavin solution in a 2-year follow-up period.

The changes in clinical results after hypotonic riboflavin with CXL are minimal compared with the changes obtained by standard riboflavin with CXL. However, Raiskup and Spoerl\textsuperscript{21} showed that CXL with hypotonic riboflavin solution was well tolerated at 1 year, with no visual acuity loss or scarring. They also reported a stable $K_{\text{max}}$ value of 65.00 D in 32 patients with keratoconus; however, they did not evaluate postoperative corneal thickness. In a study from China with a follow-up duration of 1 year, $K_{\text{max}}$ was reduced by 3.04 ± 0.75 D following CXL in corneas thinner than 400 µm ($n = 31$ in the hypotonic riboflavin group).\textsuperscript{22} In a study from Turkey, the combination of accelerated CXL and hypotonic riboflavin led to a reduction in corneal thickness from 405 ± 20 µm (without epithelium) to 380 ± 34 µm in the group with thin corneas.\textsuperscript{19} In addition, $K_{\text{max}}$ was reduced by 2.50 D and significant improvements in UDVA and CDVA were observed at 6 months.\textsuperscript{19} In our study, accelerated CXL combined with the application of hypotonic riboflavin solution in the group with thin corneas resulted in a decrease in the mean TCT from 427.53 ± 22.33 to 388.69 ± 42.63 µm, a decrease in $K_{\text{max}}$ by 1.97 ± 1.86 D, and significant improvements in UDVA (0.36 ± 0.37 logMAR) and CDVA (0.22 ± 0.28 logMAR) in a 2-year follow-up period.

In the current study, we applied the same accelerated CXL procedure for our patients in both the standard riboflavin and hypotonic riboflavin groups and obtained effective results in the stabilization of keratoconus over a 2-year follow-up period. Our center is a tertiary health care center where CXL is mostly performed for the treatment of keratoconus. We generally prefer to use the accelerated CXL protocol because it has a shorter application time than conventional CXL (Dresden protocol).

In the literature, there are studies reporting similar efficacies for both accelerated and conventional CXL procedures in the stabilization of keratoconus.\textsuperscript{31–33} Price et al.\textsuperscript{31} performed an electronic survey to assess the satisfaction of 448 patients with keratoconus who had epithelium-off CXL, which they had undergone in five clinical trials, after a median of 3.5 postoperative years. Of the patients, 403 underwent conventional CXL and 45 underwent accelerated CXL (with 9 mW/cm\textsuperscript{2} for 10 minutes). Overall, perceived efficacy was found to be similar between the conventional and accelerated groups (88% and 87%, $P = .78$). In another study, Cummings et al.\textsuperscript{32} used accelerated CXL (with 9 mW/cm\textsuperscript{2} for 10 minutes) in 36 eyes of 34 patients with keratoconus and standard CXL in 66 eyes of 53 patients with keratoconus and found similar efficacy for both methods; they obtained significant improvement in CDVA with both methods at 12 months postoperatively. Sadoughi et al.\textsuperscript{33} also found no significant change in refractive, visual, keratometric, and aberrometric values after a 12-month follow-up period between accelerated (with 9 mW/cm\textsuperscript{2} for 10 minutes) and conventional CXL procedures that were randomly performed in 15 patients with bilateral progressive keratoconus.

Ng et al.\textsuperscript{34} included 26 eyes with progressive keratoconus, of which 14 underwent conventional CXL and 12 underwent accelerated CXL (with 9 mW/cm\textsuperscript{2} for 10 minutes). They obtained significant improvements in CDVA and $K_{\text{max}}$ after conventional CXL compared to accelerated CXL; however, both methods were found to be effective in stabilizing keratoconus progression. On the other hand, in the experimental study by Dias et al.\textsuperscript{35} 40 porcine corneas were divided into four equal groups as control, Dresden, accelerated (with 30 mW/cm\textsuperscript{2} for 3 minutes), and genipin CXL. Although corneal stiffness in the anterior stromal region significantly increased in the Dresden and genipin groups compared with the accelerated group, no significant change in corneal elasticity or viscosity was obtained in any study groups at a stromal depth of 200 µm. Thus, Dias et al.\textsuperscript{35} concluded that all CXL protocols could only affect the anterior stroma.

To the best of our knowledge, this is the first study reporting the long-term clinical outcomes of accelerated CXL using hypotonic riboflavin. In the current study, we also compared the long-term results of hy-
potonic riboflavin with accelerated CXL with those of standard riboflavin with accelerated CXL. We found the efficacy of both methods was comparable in halting the progression of keratoconus in a 2-year follow-up period. In addition, reduction in the mean TCT value was significantly higher in the hypotonotic riboflavin group than in the standard riboflavin group at postoperative 12 and 24 months.

The current study presented encouraging long-term results of hypotonotic riboflavin with CXL that were comparable to those of the standard riboflavin with CXL. Accelerated CXL using hypotonotic riboflavin solution may be a promising method for treating thin corneas by providing high visual and topographic efficacy.

AUTHOR CONTRIBUTIONS

Study concept and design (SA, DMU); data collection (SA, DMU, ZD, AAD); analysis and interpretation of data (SA); writing the manuscript (SA); critical revision of the manuscript (SA, DMU, ZD, AAD); statistical expertise (SA); supervision (SA)

REFERENCES


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<th>Hypotonic Riboflavin Group (n = 43)</th>
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<td>24 months</td>
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<td>Change in CDVA (logMAR)</td>
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<td>24 months</td>
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<td>.23</td>
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TCT = thinnest corneal thickness; $K_{max}$ = maximum keratometry; D = diopters; UDVA = uncorrected distance visual acuity; CDVA = corrected distance visual acuity

*Values are presented as mean ± standard deviation.*