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Comparison of pachymetry values during crosslinking with different riboflavin solutions

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Abstract

Aim: To compare the effect of two different riboflavin solutions we use in the accelerated crosslinking (CXL) process on the pachymetry value.

Materials and Methods: Patients who had accelerated CXL treatment with dextran-free riboflavin solution due to progressive keratoconus were retrospectively screened. Thirty-seven eyes of 27 patients (group 1) treated with Hydroxyl Propyl MethylCellulose (HPMC) and 0.1% riboflavin containing solution and 29 eyes of 23 patients (group 2) treated with D-alpha-tocopheryl polyethylene-glycol 1000 succinate (VE-TPGS) and 0.1% riboflavin containing solution were compared in terms of pachymetry changes during treatment.

Results: In groups 1 and 2, the mean age was 23.92 ± 5.66 and 23.30 ± 4.89, respectively. Mean age, gender, initial keratometry values, mean central corneal thickness (CCT) before ($p = 0.158$) and after epithelial debridement ($p= 0.320$) did not differ significantly between the two groups ($p>0.05$). The CCT measured after riboflavin instillation was 453.22 ± 37.48 µm in the group 1 and 479.34 ± 38.54 µm in the group 2 ($p=0.007$). After instillation of riboflavin, according to the initial CCT, there was an average thinning of 21.57 ± 16 µm in the group 1 and 18.72 ± 22.13 µm thickening in the group 2 ($p<0.001$). According to the CCT measured after epithelial debridement, a thickening of 20.70 ± 21.77 µm in the group 1 and 62.76 ± 21.65 µm in the group 2 was observed ($p<0.001$). The rate of change of CCT after riboflavin instillation of significant differences existed between both groups ($p<0.001$).

Conclusion: VE-TPGS, which is utilized to increase the cornea's permeability to riboflavin tissue during crosslinking treatment, significantly increases the corneal thickness compared to HPMC and provides a safer tissue thickness for ultraviolet A.

Introduction

The cornea is sensitized with a riboflavin solution before being exposed to ultraviolet A (UV-A) light during the Corneal Crosslinking (CXL) process. The purpose of the process is to form new covalent bonds between collagen fibers to strengthen the stability and stiffness of the cornea. [1]. The FDA approved the use of 0.154% riboflavin in a 20% dextran solution to halt the progress of keratoconus (KCN) and ectasia following refractive surgery [2]. However, because dextran-based riboflavin solutions cause corneal stroma thinning during the treatment, riboflavin solutions in different compositions have started to be frequently used. One of the commonly used products for this purpose is the Hydroxypropyl Methyl Cellulose (HPMC) based riboflavin solution [3]. Contrary to dextran, which has a strong affinity for water and causes the cornea to dry up and thin, HPMC is a water-soluble, viscoelastic polymer that has little impact on the hydration and thickness of the cornea. In addition, HPMC-containing riboflavin solutions accelerated the rate of diffusion [2]. In recent years, riboflavin solutions fortified with D-alpha-tocopheryl polyethylene-glycol 1000 succinate (VE-TPGS) have been used for the same purposes. The well-known non-ionic surfactant VE-TPGS is frequently employed as a solvent, emulsifier, and carrier for lipid-based drug delivery formulations [4]. This solution is mostly used in the transepithelial CXL procedure, and studies are evaluating the efficacy of using this way [5-7].

This study compares the impact of these two various riboflavin solutions, which we used in accelerated epi-off CXL, on the pachymetry value.

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Materials and Methods

This study was carried out in the Cornea department of a tertiary hospital. The study protocol was approved by the hospital's Ethic Committee in accordance with the Declaration of Helsinki's principles (University of Health Sciences Bursa Yüksek Ihtisas Training and Research Hospital Clinical Research Ethics Committee, number: 2023/09-11). Retrospective screening was performed in patients with advancing keratoconus who received accelerated CXL treatment. Thirty-seven eyes of 27 patients (group 1) treated with HPMC and 0.1% riboflavin containing solution (MedioCROSS® M; Avedro Inc, USA), and 29 eyes of 23 patients treated with VE-TPGS and 0.1% riboflavin containing solution (Ribofast® IROMED Group, Italy) (group 2) were compared for changes in pachymetry during treatment. We use VE-TPGS riboflavin solution both transepithelially and epi-off. Since our aim in this study was to compare the effectiveness of two riboflavins, patients using epi-off were included in the study. Thus, a more objective comparison was possible. Topography and pachymetry measurements were performed by the same surgeon (ATB). The patients' keratometry values (K1, K2, Kmax) before CXL were recorded from the topography device. Corneal topography of the patients was performed with the Sirius corneal topography device (CSO, Florence, Italy). Pachymetry measurements were made with Pachy-Pen handheld pachymeter (keeler's Accutome, USA). The UV-A device (Peschke trade CCL-Vario Cross-linking system, Switzerland) was used for CXL.

Based on the results of corneal topography and the biomicroscopic examination, keratoconus was diagnosed using the Rabinowitz and McDonnell criteria. The global consensus on KC was used to evaluate the progression of ectasia [8]. Accordingly, progression of keratoconus is defined as a consistent change in at least two of the following parameters: steepening of the anterior corneal surface; steepening of the posterior corneal surface; thinning and/or an increase in the rate of change of corneal thickness from the periphery to the thinnest point.

The exclusion criteria of the patients in the study were as follows: thinnest corneal thickness less than 370 µm, chronic eye disease other than keratoconus, history of herpetic keratitis, history of chronic systemic disease, topical or systemic drug use, history of eye surgery, severe dry eye, contact lens use, pregnancy and breastfeeding.

In line with these criteria, crosslinking treatment was performed on patients whose Keratoconus progressed or was at risk of progression during follow-up. The selection of riboflavin to the patients was made randomly. (Since different riboflavin solutions are taken in our hospital at certain periods, whatever solution we had was used when the patient's operation was planned). The patients' demographic data, preoperative topography data, which riboflavin was used, and intraoperative pachymetry values were obtained retrospectively from the patient files.

After administering topical anesthesia with proparacaine hydrochloride 0.5% eye drops, the periocular area was cleaned with a 10% povidone-iodine solution and then covered with a sterile covering. Before the CXL procedure, the corneal center was measured three times by ultrasonic pachymetry. Using a spatula, the epithelium was removed (epi-off). Additionally, three ultrasonic pachymetry measurements of the corneal center thickness were taken. In group 1, HPMC based riboflavin was applied to the debridement area for 30 minutes at 2-minute intervals. In the group 2, VE-TPGS based riboflavin was dripped for 15 minutes at 30-second intervals. After the transition of riboflavin to the anterior chamber was observed with biomicroscopy, the corneal center was measured again three times with ultrasonic pachymetry. The UV-A gadget was then positioned 5 cm away from the eye. A 370 nm UV-A beam of 9 mW/cm^2 was administered for 10 minutes after the corneal center was the focus of the beam. The averages of these three pachymetry measurements were taken and compared statistically.

Statistical analysis

Utilizing IBM SPSS Statistics 23.0 (IBM Corp., 2015 release), statistical analyses were carried out. For continuous variables, the results are shown as mean±SD. Frequency and percentage were used to describe categorical variables. The Shapiro-Wilk test was used to assess the data to see if it has a normal distribution. Independent samples t-test was used to compare normally distributed data. The percentages of nominal variables were compared using the Pearson Chi-square test. $P<0.05$ was the minimum statistical level of significance.

Results

When comparing the mean age, gender, and baseline keratometry values of the two groups, there was no discernible difference (Table 1). Central corneal thickness (CCT) was 474.78 ± 39.48 µm in group 1 and 460.62 ± 40.65 µm in group 2 before CXL procedure ($p = 0.158$). The two groups did not significantly vary from one another in CCT measured after epithelial debridement (epi-off) ($p= 0.320$). CCT measured after riboflavin instillation was 453.22 ± 37.48 µm in group 1 and 479.34 ± 38.54 µm in group 2 ($p= 0.007$) (Table 1). CCT measured after instillation of riboflavin was 21.57 ± 16 µm thinner in the group 1 and 18.72 ± 22.13 µm thicker in the group 2 compared to the initial CCT ($p<0.001$). According to the CCT measured after epithelial debridement, a thickening of 20.70 ± 21.77 µm in the first group and 62.76 ± 21.65 µm in the second group was observed. The rate of change of CCT after riboflavin instillation of the significant differences existed among both groups ($p<0.001$) (Table 2).

Discussion

In previous studies, riboflavin solutions that are isotonic with and without dextran were compared in terms of their effects on central corneal thickness during crosslinking, similar to our study. In these studies, pachymetry thinning was detected in the dextran-containing group, while the dextran-free group showed a little increase in pachymetry [9-11]. However, in these studies, comparisons were made with riboflavin containing HPMC in groups without dextran. In our study, for the first time, the pachymetry changes of riboflavin containing VE-TGPS and riboflavin containing HPMC were compared during CXL.

The impact of riboflavin solution including VE-TGPS on corneal permeation, as well as its protective function

Table 1. Demographic and topographic data of the patients.

	Group 1 (HPMC) n=27	Group 2 (VE- TPGS) n=23	p*
Age, years (Mean ± SD)	23.92 ± 5.66	23.30 ± 4.89	0.683
Gender. n (%)			
Female	16 (59.3)	14 (60.9)	0.908
Male	11 (40.7)	9 (39.1)	
K1	45.11 ± 2.49	44.93 ±1.91	0.757
K2	48.27 ± 2.54	48.18 ± 2.98	0.894
Kmax	52.79 ± 4.19	53.16 ± 10.09	0.840
Inicial CCT (µm)	474.78 ± 39.48	460.62 ± 40.65	0.158
Epi-off CCT (µm)	432.51 ± 38.24	416.59 ± 38.16	0.320
After riboflavin CCT (µm)	453.22 ± 37.48	479.34 ± 38.54	**0.007**

HPMC: Hydroxypropyl Methyl Cellulose; VE-TPGS: D-alpha-tocopheryl polyethylene-glycol 1000 succinate; K1: flat keratometry; K2: steep keratometry; K max: maximum keratometry; CCT: central corneal thickness. * Independent Sample t-test, Pearson Chi-Square.

Table 2. Pachymeter changes during crosslinking.

	Group 1 (HPMC) n=27	Group 2 (VE- TPGS) n=23	p*
Epi-off CCT – Inicial CCT (µm)	-42.27 ± 16.41	-44.03 ± 5.63	0.582
After riboflavin CCT – Inicial CCT (µm)	-21.57 ± 16	18.72 ± 22.13	**<0.001**
After riboflavin CCT- Epi-off CCT (µm)	20.70 ± 21.77	62.76 ± 21.65	**<0.001**

HPMC: Hydroxypropyl Methyl Cellulose; VE-TPGS: D-alpha-tocopheryl polyethylene-glycol 1000 succinate; CCT: central corneal thickness. * Independent Sample t-test.

against free radicals produced during CXL operations, were assessed both in vitro and in vivo in studies [4,5]. In a recent study, to improve corneal diffusion, in place of the dextran-riboflavin solution, a VE-TPGS-riboflavin solution was employed. The time-dependent corneal accumulation of VE-TPGS or a control riboflavin solution was assessed in this study in both epi-on and epi-off circumstances, and no significant differences were detected. [5]. Caruso et al. described and investigated a revised protocol of UV-A corneal cross-linking named custom-fast corneal cross-linking (CF-CXL), which employs an isotonic solution of riboflavin VE-TPGS, with laboratory and clinical findings [12]. These studies found that using this technique quickly and permanently halted the progression of keratoconus.

In the present study, riboflavin containing HPMC and riboflavin containing VE-TPGS were compared in terms of changes in pachymetry during CXL treatment. Compared to the CCT measured after epithelial debridement, a thickening of 20.70 ± 21.77 µm in the HPMC group and 62.76 ± 21.65 µm within the VE- TPGS group was observed in the CCT measured after riboflavin instillation. Since the thin cornea in keratoconus increases the risk of endothelial damage in the CXL procedure, the increase in pachymetry with riboflavin solutions increases the protection on free radicals formed during CXL and reduces the risk of tissue damage. In current study, HPMC based riboflavin was applied to the debridement area for 30 minutes at 2-minute intervals, VE-TPGS based riboflavin was dripped for 15 minutes at 30-second intervals. Thus, it has been shown that the solution containing VE-TPGS, which had sufficient penetration into the cornea in transepithelial use and short-term application in previous studies, reached sufficient concentration in a shorter time than HPMC in epi-off use. A recent study showed that both riboflavins were successful in slowing the advancement of keratoconus and were safe for endothelium after 12 months. [13]. The riboflavin solution used should be both effective in stopping keratoconus and increase the CCT value during the operation for sufficient effectiveness of UV-A without damaging the endothelium. Considering that keratoconus patients have thin corneas, increasing CCT provides a safer working area. Intraoperative pachymetry measurement is an important measure to evaluate whether the riboflavin we use provides a safe tissue thickness during the procedure.

Both HPMC and VE-TPGS increase riboflavin permeability and concentration in the stoma through different pathways. HPMC works by enhancing UVA absorption, preserving the osmotic pressure of the corneal matrix, and increasing the viscosity of crosslinking solutions [14]. VE-TPGS is well-known as a nonionic surfactant and is effective in improving drug permeation across different biological barriers as a specific riboflavin transporter. Thus, it has been shown to increase the penetration of riboflavin in the corneal stoma [4]. The fact that two different solutions increased corneal thickness at different rates in the current study may be due to these different mechanisms of action.

The current study has limitations because of the small study population. In addition, VE-TPGS solution, which has the possibility to use epi-on, was used as epi-off in this study. Studies investigating the results of using two riboflavins in epi-on are required.

Conclusion

In conclusion, VE-TPGS, which is utilized to improve riboflavin diffusion into the corneal layer during CXL therapy, significantly increases the corneal thickness, shortens the procedure time and provides a safer tissue thickness for ultraviolet A compared to HPMC. To evaluate the effect of the two products on the cornea during and after CXL, more comprehensive and longer-term studies should be conducted.

Conflict of interest

The authors declared that there were no conflicts of interest.

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Ethical approval

Approval was received for this study from the Clinical Research Ethics Committee of the University of Health Sciences Bursa Yüksek Ihtisas Trainig and Research Hospital (number: 2011-KAEK-25 2023/09-11).

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