Taylor & Francis
Taylor & Francis Group

Cutaneous and Ocular Toxicology

ISSN: 1556-9527 (Print) 1556-9535 (Online) Journal homepage: https://www.tandfonline.com/loi/icot20

Transient corneal endothelial changes following accelerated collagen cross-linking for the treatment of progressive keratoconus

Abdullah Kürşat Cingü, Esin Sogutlu-Sari, Yasin Çınar, Muhammed Şahin, Fatih Mehmet Türkçü, Harun Yüksel, Alparslan Şahin & İhsan Çaça

To cite this article: Abdullah Kürşat Cingü, Esin Sogutlu-Sari, Yasin Çınar, Muhammed Şahin, Fatih Mehmet Türkçü, Harun Yüksel, Alparslan Şahin & İhsan Çaça (2014) Transient corneal endothelial changes following accelerated collagen cross-linking for the treatment of progressive keratoconus, Cutaneous and Ocular Toxicology, 33:2, 127-131, DOI: 10.3109/15569527.2013.812107

To link to this article: https://doi.org/10.3109/15569527.2013.812107

Cutaneous and Ocular Toxicology

http://informahealthcare.com/cot
ISSN: 1556-9527 (print), 1556-9535 (electronic)

Cutan Ocul Toxicol, 2014; 33(2): 127–131
 DOI: 10.3109/15569527.2013.812107

informa
healthcare

RESEARCH ARTICLE

Transient corneal endothelial changes following accelerated collagen cross-linking for the treatment of progressive keratoconus

Abdullah Kürşat Cingü[1], Esin Sogutlu-Sari[2], Yasin Çınar[1], Muhammed Şahin[1], Fatih Mehmet Türkçü[1], Harun Yüksel[1], Alparslan Şahin[1], and İhsan Çaça[1]

[1]Department of Ophthalmology, School of Medicine, Dicle University, Diyarbakır, Turkey and [2]Department of Ophthalmology, School of Medicine, Balikesir University, Balikesir, Turkey

Abstract

Purpose: To evaluate the corneal endothelial changes following accelerated collagen cross-linking (CXL) for the treatment of progressive keratoconus.
Methods: Thirty-six consecutive progressive keratoconus patients who received accelerated CXL treatment were enrolled in the study. Following de-epithelization, isoosmolar 0.1% riboflavin solution without dextran was instilled every 3 min throughout the 30 min of soaking time before the 5 min of 18 mW/cm^2 UVA irradiation and every 2 min during the UVA irradiation. Corneal specular microscopy was performed on both treated and fellow eyes of each patient preoperatively, in the first week, and in the first, third and sixth month postoperatively.
Results: There were significant differences in endothelial cell density (ECD), percentages of hexagonality (6A) and coefficient of variation of endothelial cell area (CV) in the first week and first month postoperatively in the treated eyes when compared to their preoperative values and also to the first week and first month ECD, 6A and CV values of the non-operative eyes. ECD returned to the preoperative values at sixth month whereas 6A and CV returned to the preoperative values at third month.
Conclusion: Our results suggested that there may be transient changes in human corneal endothelium following accelerated UVA/riboflavin CXL. Resolution of these changes during the follow-up may indicate a safe recovery. However, the treatment guidelines for accelerated CXL including irradiance level and soaking time should be clearly established to minimize the toxic effects of the treatment.

Keywords

Accelerated CXL, corneal endothelium, riboflavin

History

Received 16 May 2013
Revised 30 May 2013
Accepted 2 June 2013
Published online 16 July 2013

Introduction

Collagen cross-linking (CXL) based on the combined ultraviolet-A (UVA) light and riboflavin use is relatively a new method to increase covalent bonds between collagen fibers in the anterior corneal stroma[1]. The therapeutic effect of CXL for progressive keratoconus (KC) was firstly demonstrated by Wollensak et al.[2] in 2003, since then favorable short-term and long-term results have been reported[3,4].

Because UVA irradiation may cause damage in keratocytes, corneal endothelial cells, crystalline lens and retina, safe treatment parameters and inclusion criteria had been clearly described in rabbit experiments. For this issue, the treatment was set to the anterior 250 or 350 micron (μ) of the corneal stroma[5,6]. Despite the use of standard protocols with high safety profile, there are a few reports of complications after CXL including permanent corneal haze, diffuse lamellar keratitis and herpetic keratitis[7–9]. In recent years, CXL-induced corneal endothelial damage has been increasingly reported in both case reports and case series with KC. Hence, there have been concerns about the CXLs' relative safe side effect profile, especially on the endothelium.

The effect of CXL depends on the total energy doses, so the same therapeutic effect can be theoretically obtained by increasing the intensity and decreasing the time of irradiation. For this reason, second-generation CXL devices have been developed to increase the intensity and optimized beam shaping to accelerate the treatment. In the literature, there are a few reports about the safety of accelerated protocols. In this study, we aimed to report transient corneal endothelial changes following accelerated CXL for the treatment of KC.

Patients and methods

This study was a prospective, fellow eye controlled interventional case series. Patients diagnosed with KC at a university hospital between January 2012 and December 2012 were enrolled. The diagnosis of KC was based on the corneal topography and clinical findings of slit-lamp examination including corneal thinning, conical protrusion of the apical cornea, Fleisher's ring and Vogt's striae. Inclusion criteria

Address for correspondence: Abdullah Kürşat Cingü, Department of Ophthalmology, School of Medicine, Dicle University, Diyarbakır, Turkey. E-mail: kursatcingu@yahoo.com

were presence of progressive KC which was clinically demonstrated in the past 12 months by the manifest refraction and topographical examination (0.75 diopters (D) increase in the cone apex keratometry or 0.75 D alteration in the spherical equivalent (SE) refraction)[10], without history of any ocular surgery, absence of corneal scarring, avoidance of contact lens for 1 month before and 6 month after the treatment and having at least 400 µm of central corneal thickness (CCT). Patients who were lost to follow-up and had an evidence of Fuch's endothelial dystrophy were excluded. All patients were instructed about the advantages and disadvantages of the procedure. Patient informed consent was obtained from all participants in accordance with the Declaration of Helsinki, and ethics approval was obtained from Local Ethical Committee. In patients less than 18 years of age, written informed consent was obtained from their parents.

All patients were treated with UVA/riboflavin CXL under aseptic conditions by using proparacaine 0.5% (Alcaine; Alcon Pharmaceuticals, Hünenberg, Switzerland) eye drops. Periocular skin was scrubbed with 10% povidone-iodine (Betadine; Purdue Pharma, LP, Stamford, CT). After draping, an eyelid speculum was inserted and 5% povidone-iodine was instilled onto the ocular surface.

Before de-epithelization, ultrasonic pachimetry (UP) readings were obtained. The sterilized probe was placed at the center of the cornea, considering the pupil center by the investigator, and 10 consecutive measurements were taken. By using a blunt spatula, an 8.0 mm diameter of corneal epithelium was removed. The isoosmolar 0.1% riboflavin solution without dextran (Mediocross M isotonic, Medio-Haus Medizinprodukte GmbH, Kiel, Germany) was instilled every 3 min for 30 min. Ten repeated UP measurements were performed again from the center of the cornea to record the mean CCT of de-epithelized cornea in all eyes before the UVA irradiation. After checking the presence of surface irradiance of 18 mW/cm^2 by using a UVA meter, UVA irradiation was applied for 5 min at an 8 mm treatment zone by using UVA system device (CCL-VARIO; Peschke Meditrade GmbH, Huenenberg, Switzerland) at 5 cm working distance. The isoosmolar 0.1% riboflavin solution without dextran was applied every 2 min during the irradiation. At the end of the procedure, soft contact bandage lens (Lotrafilcon B; Air Optix; Ciba Vision, Duluth, GA; 14.0 mm in diameter, 8.6 base curvatures, Dk = 140 barrers) was placed. Postoperatively, moxifloxacin hydrochloride 0.5% (Vigamox, Alcon, TX) eye drops three times a day for one week and preservative free artificial tears four times a day for one month were prescribed. Additionally, Fluorometholone 0.1% (FML, Allergan, CA) eye drops were also prescribed four times a day and were tapered for the next three weeks.

All patients were examined preoperatively and at 1st week, 1st, 3rd and 6th month after the treatment including best spectacle corrected visual acuity (BSCVA), slit-lamp biomicroscopic examination, corneal topographic analyses, CCT measurement with UP and status of the corneal endothelium with specular microscope. The endothelium was photographed in both treated and fellow eyes using a Konan Noncon Robo SP 6000 noncontact specular microscope (Konan Medical Inc, Hyogo, Japan). Images were taken and evaluated by the same observer (AKC). For each examination, three measurements of endothelial cell density (ECD), percentages of hexagonality (6A) and coefficient of variation of endothelial cell area (CV) were taken and the two closest measurements were averaged.

In the statistical analysis, SPSS statistical software package version 15.0 for Windows (SPSS, Chicago, IL) was used. Because the data follows Gaussians distribution, repetitive measurements of the eyes were analyzed with paired samples *t*-test, and operative and non-operative eyes were compared with student's *t*-test. The data was described as mean ± standard deviation. A p value of less than 0.05 was considered statistically significant.

Results

Thirty-six eyes of 36 consecutive patients (15 [41.7%] male, 21 [58.3%] female) underwent accelerated CXL with riboflavin treatment. The mean patient age was 18.63 ± 4.53 years (11–32). All patients completed 6 months follow-up after treatment. No intraoperative complication was observed at the time of CXL treatment. Stromal haze was seen in all eyes at 1st month and resolved spontaneously during follow-up.

The mean CCT was 423 ± 34.0 µm after deepithelization and 423 ± 28.4 µm at the end of soaking time and just before the UVA irradiation ($p = 0.9$).

Visual and refractive results are demonstrated in Table 1. Although there was slight decrease in spherical equivalent (SE), mean keratometry (Km), maximum keratometry (Kmax), and keratometric astigmatism (Kast), only BSCVA ($p = 0.03$) showed significant improvement at 6th month visit when compared to preoperative values. There was slight but not significant deterioration in the non-operative eyes at the end of the 6 month by means of SE, Km, Kmax, Kast and BSCVA.

Corneal endothelial and pachymetric changes are demonstrated in Table 2. In treated eyes, ECD values were significantly decreased at 1st week, at 1st month and at 3rd month of follow-up ($p = 0.006$, $p < 0.001$, and $p = 0.014$, respectively). The mean percentages of the hexagonality significantly decreased and CV significantly increased at 1st week and at 1st month postoperatively when compared with the preoperative measurements ($p = 0.03$ and $p = 0.03$; $p = 0.006$ and $p = 0.001$, respectively) whereas turned toward preoperative values at 3rd month ($p = 0.43$ and $p = 0.22$, respectively). There were also significant differences between the treated and fellow eyes in ECD, hexagonality and CV at 1st week and 1st month ($p < 0.05$). At 6 months, there was no significant difference in terms of any of the endothelial measurements in treated eyes when compared with their preoperative values or between the measurements of treated and fellow-eyes ($p > 0.05$). Regarding the corneal pachymetric values, CCT was significantly increased at 1 week postoperatively in treated eyes ($p < 0.001$), whereas the same trend was not observed in the remaining follow-up period ($p > 0.05$). Besides, there was no significant difference between the treated and the fellow-eyes in CCT in any of the follow-up periods ($p > 0.05$). Figure 1 demonstrates the changes in ECD, hexagonality, pachymetry and CV of an eye

Table 1. Comparisons of the spherical equivalent, visual acuity, mean keratometry, maximum keratometry and keratometric astigmatism at preoperative and postoperative 6th month visits of the operative eyes, and at baseline and 6th month visits of the non-operative eyes.

	Preoperative		6th month			
	Mean (SD)					
	Operative eyes*	Non-operative eyes**	Operative eyes*	Non-operative eyes**	p*	p**
SE	**−6.83 (3.72)**	−3.36 (2.07)	**−6.71 (3.58)**	−3.45 (2.34)	**0.7**	0.6
BCVA (logMAR)	**0.57 (0.33)**	0.30 (0.36)	**0.45 (0.37)**	0.35 (0.43)	**0.03**	0.2
Km	**52.27 (4.21)**	50.2 (5.68)	**52.23 (4.20)**	50.7 (6.89)	**0.7**	0.3
Kmax	**60.36 (6.37)**	57.2 (8.31)	**59.96 (6.42)**	58.6 (12.6)	**0.1**	0.2
Kast	**5.01 (2.06)**	3.99 (2.66)	**4.81 (1.81)**	4.28 (2.47)	**0.1**	0.3

p* represents the significance for the pairwise comparisons between preoperative measurements and post-operative values in the operative eyes.

p** represents the significance for the pairwise comparisons between baseline and 6th month visit measurements in the non-operative eyes.

SD: Standard deviation, SE: Spherical equivalent, BCVA: Best corrected visual acuity, Km: Mean keratometry, Kmax: Maximum keratometry, Kast: Keratometric astigmatism, *: Operative eyes, **: Non-operative eyes.

Table 2. Comparisons of the central endothelial cell density, coefficient of variation of cell size, percentage of hexagonal cells and corneal pachymetry of the operative and non-operative eyes.

		Mean ± SD			
		CXL (+) eyes	Fellow eyes	p*	p†
Cell density	Preoperative	2738 ± 229.3	2807 ± 184.6	0.19	
	Postop 1st week	2532 ± 172.2	2856 ± 244.2	<0.001	0.006
	Postop 1st month	2512 ± 240.2	2766 ± 230.4	<0.001	<0.001
	Postop 3rd month	2545 ± 293.6	2805 ± 275.5	0.001	0.014
	Postop 6th month	2718 ± 174.9	2773 ± 261.9	0.31	0.36
Coefficient of variation	Preoperative	44.2 ± 6.6	44.6 ± 10.4	0.89	
	Postop 1st week	49.6 ± 8.0	42.6 ± 6.6	0.001	0.006
	Postop 1st month	51.9 ± 11.8	45.3 ± 12.5	0.03	0.001
	Postop 3rd month	50.9 ± 15.8	43.2 ± 7.8	0.05	0.22
	Postop 6th month	45.7 ± 8.3	43.3 ± 8.6	0.28	0.25
Hexagonality	Preoperative	45.4 ± 8.05	44.9 ± 9.36	0.79	
	Postop 1st week	38.6 ± 7.99	47.6 ± 7.58	<0.001	0.003
	Postop 1st month	39.4 ± 8.05	46.6 ± 10.72	0.006	0.003
	Postop 3rd month	42.7 ± 9.39	47.2 ± 8.96	0.06	0.43
	Postop 6th month	43.7 ± 7.38	47.1 ± 10.4	0.13	0.32
Ultrasound pachymetry	Preoperative	454 ± 44.2	460 ± 55.2	0.62	
	Postop 1st week	491 ± 51.0	464 ± 68.8	0.07	<0.001
	Postop 1st month	462 ± 48.9	466 ± 63.9	0.77	0.19
	Postop 3rd month	462 ± 45.1	466 ± 65.6	0.79	0.08
	Postop 6th month	455 ± 44.1	467 ± 50.3	0.36	0.82

CXL: Collagen cross-linking, SD: Standard deviation, p*: Significance of the comparisons between operative and non-operative eyes of the patients, p†: Significance of the pairwise comparisons between preoperative measurements and post-operative values in the operative eyes.

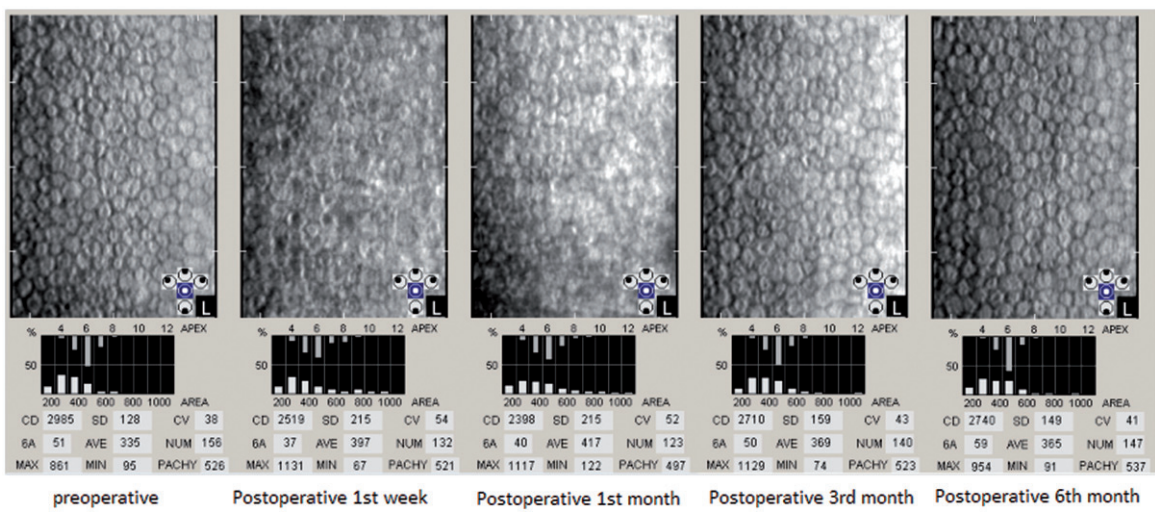

Figure 1. Specular microscopic examinations of an eye treated with accelerated UVA/riboflavin CXL during the six-month follow-up, demonstrating the alterations in endothelial cell density, percentages of hexagonality, coefficient of variation of endothelial cell area and pachymetric measurements.

treated with accelerated UVA/riboflavin CXL during the six-month follow-up period.

Discussion

Ultraviolet-A irradiation has a well-known cytotoxic and pro-_apoptotic potential in human cells. It causes the formation of free radicals such as singlet oxygen, superoxide and hydrogen peroxide species in endothelial cells, which can consequently result in apoptosis[5]. In human corneas, the cytotoxic level of endothelial UVA irradiance has been described as 0.35 mW/cm^2, that is, approximately twice the UVA reaches the corneal endothelium in standard protocol (0.18 mW/cm^2). Experimental studies demonstrated that the combined use of UVA irradiation with riboflavin can decrease the toxic effects of the treatment by 10 times compared with the use of UVA alone[11].

Corneal endothelial changes after the standard CXL with UVA and riboflavin in progressive KC were previously evaluated in different investigations. However, the acute effects of accelerated protocol on the corneal endothelium are still unknown and have not been reported previously in the literature. In this study, we firstly demonstrated transient endothelial changes after the accelerated CXL procedure.

Goldich et al.[12] showed stable ECD in 14 keratoconic eyes during the early and the late periods following the standard UVA/riboflavin CXL treatment. Similar stability of ECD was reported in other preliminary results of randomized clinical trial[1]. In accordance with these studies, in this study there was no significant change in corneal endothelium at postoperative 6th month when compared with preoperative levels in the treated eyes. Regarding the early visits (1st week, 1st and 3rd months) the mean ECD was significantly lower in the UVA/riboflavin CXL-treated eyes than their preoperative values and also lower than that of untreated fellow eyes. Therefore, the observed early corneal endothelial changes following accelerated UVA/riboflavin CXL had spontaneously resolved at routine follow-up and turned to the preoperative values at 6th month on specular microscopy.

Recently, safety of the accelerated CXL was evaluated with KC and laser *in situ* keratomileusis patients in a meeting paper[13]. In this study, the authors did not report any changes in the corneal endothelium at one week and one month postoperatively. The procedure included 20 min of soak time with 0.1% riboflavin followed by UV-A exposure of 30 mW/cm^2 for 3 min. In a different manner, in this study we instilled 0.1% riboflavin for 30 min and used UV-A exposure of 18 mW/cm^2 for 5 min. We followed the standard protocol defined by the manufacturer in which riboflavin application was 30 min, to allow the photosensitizing agent to penetrate into the deeper stromal layers and enhance the treatment. However, this report may be biased by its small and heterogeneous study population.

Although corneal thickness below 400 μm was considered the most important risk factor for the endothelial damage, Gokhale[14] reported a case of severe corneal endothelial changes in a cornea thicker than 400 μm before surgery. More recently, Sharma et al.[15] presented the case series of CXL-induced persistent corneal edema which required penetrating keratoplasty. Various possibilities were speculated about the endothelial toxicity of standard irradiation protocol, including inadvertent delivery of excessive energy, unpredictable intra-operative corneal thinning, inaccurate pachymetry measurements, history of acute hydrops and pre-existing endothelial dystrophy[15]. Endothelial toxicity may occur even if all measurements are rechecked and the guidelines are tightly followed. In a previous study, we showed that isoosmolar 0.1% riboflavin solution without dextran did not cause corneal thinning and slightly increased the CCT during the 30 min soaking time[16]. Similarly, we did not see further thinning following de-epithelization during the riboflavin instillation and none of the eyes had CCT under 400 μm before UV irradiation.

Corneal microstructure in KC has previously been evaluated by using *in vivo* confocal microscopy and several changes were observed in all corneal layers. Matsuda et al.[17] and Laing et al.[18] demonstrated elongation of endothelial cells toward the cone apex in KC as a result of a stretching mechanism. Mocan et al.[19] suggested that there may be a decrease in ECD in moderate-to-severe KC patients even in the absence of contact lens use. Accordingly, Ucakhan et al.[20] prospectively evaluated 48 keratoconic eyes and observed some changes in both density and the morphology of endothelial cells that become more prominent with the increasing grades of the disease. They proposed that in KC, unstable endothelium becomes susceptible to damage, and thus endothelial damage following CXL may be related to an indirect response of this unstable endothelium against UVA irradiation and consequent oxidative stress.

Cell density alone is not the most sensitive measure of endothelial health. Several authors have suggested that polymegathism (determined by the CV) and polymorphism (determined by percentage of hexagonal cells) are sensitive measurements of the endothelium under stress. As the CV increases and the percentage of hexagonality decreases, there would be a less stable thermodynamic relationship between the individual and the neighbor endothelial cells[21]. In this study, the mean percentage of hexagonality significantly decreased and accordingly the mean CV significantly increased at 1st week and at 1st month following the CXL treatment in the operating eyes. Similarly, the mean percentage of hexagonality and the mean CV were also significantly different between the treated and untreated fellow-eyes at 1st week and at 1st month. However, the observed morphological changes had spontaneously resolved during routine postoperative follow-up period.

It is difficult to speculate about the pathophysiologic features of the observed transient endothelial changes in our case series. Recently, Touboul D et al.[22] compared corneal healing following conventional and accelerated CXL and they reported that apoptotic changes in keratocytes particularly in the anterior stroma were more prominent in the accelerated CXL eyes. In their accelerated protocol 0.1% riboflavin was instilled for 10 min. They hypothesized that the more intense anterior affected zone following accelerated CXL may be due to the shorter riboflavin soaking time which is defined by the manufacturers of accelerated CXL used in their study. Even though they did not find a significant difference among the groups by means of ECD following the conventional and accelerated protocols, there was 500 cell/mm^2 decrease in

ECD at first postoperative month in their accelerated CXL group. However, their 3rd month and 6th month ECD results in accelerated CXL group were similar to that of preoperative levels. In this study, we found a similar but this time significant trend in the postoperative variation of ECD during the follow-up. In addition to this, Cui et al.[23] reported that riboflavin concentration also affects the penetration velocity into the stroma. In this study, we instilled isoosmolar 0.1% riboflavin solution without dextran for 30 min by the recommendation of the manufacturer and possibly reached a more intense intrastromal riboflavin concentration in deeper stroma before the UVA irradiation. Although same total energy dose is used in both accelerated and conventional protocols, intensity of the irradiance is six times higher with accelerated CXL. Transient endothelial changes observed in this study may be due to higher energy delivery into the deeper stromal layers both with the effects of increased riboflavin saturation and with the more intense UVA irradiance.

There are some limitations that need to be mentioned regarding this study. First, the peripheral corneal endothelium could not be evaluated which may be essential in determining the extent of endothelial cell damage and the contribution of peripheral cell migration in the recovery of normal central endothelial cell appearance. Second, a larger sample size and longer follow-up could have strengthened our conclusion and provided further insight into the potential long-term consequences of our findings. Using anterior segment OCT and confocal microscopy would have been more beneficial in demonstrating the depth of the treatment zone and the endothelium. Lastly, mild corneal edema and ongoing healing process in the corneal epithelium may interfere with the specular microscopic measurements particularly at the first postoperative week. Nevertheless, we presented a similar trend in the all endothelial parameters at the first postoperative month also.

In conclusion, our results demonstrated that transient changes in human corneal endothelium may occur following accelerated UVA/riboflavin CXL. Although resolution of these changes during the follow-up may indicate a safe recovery, future consequences are not well known and have not become evident yet. It is always a concern that both photochemically induced free radicals and UVA irradiation itself have the potential to damage the intraocular structures including endothelium. Evaluation of the endothelial cell safety with regards to CXL is essential as it is gradually becoming the standard treatment option for progressive KC worldwide. We strongly recommend that the treatment guidelines for accelerated CXL including irradiance level and soaking time should be clearly established to minimize the toxic effects of the treatment. Further studies are needed to determine this issue. Therefore, corneal specialists need to be aware of the risks of CXL treatment and should document the endothelial parameters of the patients before and after the CXL treatment both for medical and legal issues.

Declaration of interest and Source of Funding

None of the authors have any financial interests to disclose. There is no founding resource for this study.

References

1. Wittig-Silva C, Whiting M, Lamoureux E, et al. A randomized controlled trial of corneal collagen cross-linking in progressive keratoconus: preliminary results. J Refract Surg 2008;24: S720–S725.
2. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-a-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620–627.
3. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. J Cataract Refract Surg 2008;34:796–801.
4. Wollensak G, Sporl E, Seiler T. Treatment of keratoconus by collagen cross linking. Ophthalmologe 2003;100:44–49.
5. Spoerl E, Mrochen M, Sliney D, et al. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007;26:385–389.
6. Wollensak G, Sporl E, Reber F, et al. Corneal endothelial cytotoxicity of riboflavin/UVA treatment *in vitro*. Ophthalmic Res 2003;35:324–328.
7. Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. J Refract Surg 2009;25:S824–S828.
8. Kymionis GD, Portaliou DM, Bouzoukis DI, et al. Herpetic keratitis with iritis after corneal crosslinking with riboflavin and ultraviolet A for keratoconus. J Cataract Refract Surg 2007;33: 1982–1984.
9. Wollensak G, Hammer T, Herrmann CI. Haze or calcific band keratopathy after crosslinking treatment? Ophthalmologe 2008; 105:864–865
10. O'Brart DP, Chan E, Samaras K, et al. A randomised, prospective study to investigate the efficacy of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linkage to halt the progression of keratoconus. Br J Ophthalmol 2011;95:1519–1524.
11. Kolli S, Aslanides IM. Safety and efficacy of collagen cross-linking for the treatment of keratoconus. Expert Opin Drug Saf 2010;9:949–957.
12. Goldich Y, Marcovich AL, Barkana Y, et al. Safety of corneal collagen cross-linking with UV-A and riboflavin in progressive keratoconus. Cornea 2010;29:409–411.
13. Matalia H JA, Kumar M, Shetty R. Is accelerated corneal collagen cross-linking a safe procedure? A pilot study. ESCRS 2012, free paper 2012.
14. Gokhale NS. Corneal endothelial damage after collagen cross-linking treatment. Cornea 2011;30:1495–1498.
15. Sharma A, Nottage JM, Mirchia K, et al. Persistent corneal edema after collagen cross-linking for keratoconus. Am J Ophthalmol 2012;154:922–926.
16. Cınar Y, Cingu AK, Sahin A, et al. Intraoperative corneal thickness measurements during corneal collagen cross-linking with isotonic riboflavin solution without dextran in corneal ectasia. Cutan Ocul Toxicol 2013 May 21. [Epub ahead of print]. doi:10.3109/15569527.2013.793700.
17. Matsuda M, Suda T, Manabe R. Quantitative analysis of endothelial mosaic pattern changes in anterior keratoconus. Am J Ophthalmol 1984;98:43–49.
18. Laing RA, Sandstrom MM, Berrospi AR, Leibowitz HM. The human corneal endothelium in keratoconus: a specular microscopic study. Arch Ophthalmol 1979;97:1867–1869.
19. Mocan MC, Yilmaz PT, Irkec M, Orhan M. In vivo confocal microscopy for the evaluation of corneal microstructure in keratoconus. Curr Eye Res 2008;33:933–939.
20. Ucakhan OO, Kanpolat A, Ylmaz N, Ozkan M. *In vivo* confocal microscopy findings in keratoconus. Eye Contact Lens 2006;32: 183–191.
21. Benetz BA, Yee R, Bidros M, Lass J. Imaging techniques of cornea: specular microscopy. In: Krachmer JH, Mannis MJ, Holland EJ, eds. Cornea. 3rd ed. New York: Mosby Elsevier; 2011:14:177–204.
22. Touboul D, Efron N, Smadja D, et al. Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus. J Refract Surg 2012;28:769–776.
23. Cui L, Huxlin KR, Xu L, et al. High-resolution, noninvasive, two-photon fluorescence measurement of molecular concentrations in corneal tissue. Invest Ophthalmol Vis Sci 2011;52:2556–2564.