ARTICLE

Collagen crosslinking with conventional and accelerated ultraviolet-A irradiation using riboflavin with hydroxypropyl methylcellulose

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Purpose: To evaluate corneal collagen crosslinking (CXL) with conventional and accelerated ultraviolet-A (UVA) irradiation using riboflavin with methylcellulose.

Setting: Department of Ophthalmology, Oslo University Hospital, Oslo, Norway.

Design: Prospective randomized case series.

Methods: Patients with keratoconus were randomized to have CXL using conventional 3 mW/cm^2 UVA irradiation for 30 minutes (CXL30) or accelerated 9 mW/cm^2 UVA irradiation for 10 minutes (CXL10). In both groups, a solution of riboflavin 0.1% with hydroxypropyl methylcellulose 1.1% (methylcellulose–riboflavin) was used. The endothelial cell density (ECD), visual acuity, and tomography were measured at baseline and after 12 months. Anterior segment optical coherence tomography and in vivo confocal microscopy (IVCM) were performed after 1 month.

Results: The study comprised 40 patients (40 eyes). A complete absence of keratocytes in all eyes at 100 μm depths was found on IVCM. At 300 μm, 400 μm, and preendothelial levels, the differences were 83.3% versus 31.3% ($P = .02$), 64.7% versus 20.0% ($P = .01$), and 42.1% versus 5.9% ($P = .02$) in the CXL30 and CXL10 groups. No statistically significant differences were found in the change in visual acuity or maximum keratometry between the groups after 12 months. There was no relationship between the depth of keratocyte absence and the ECD change after 12 months.

Conclusions: Marked deep structural changes with an absence of keratocytes occurred when CXL was used with conventional or accelerated UVA irradiation; however, the changes were more pronounced with the use of conventional UVA irradiation. The use of methylcellulose–riboflavin might explain the deep alterations and raises a long-term safety concern.

J Cataract Refract Surg 2017; 43:511–517 © 2017 ASCRS and ESCRS

The principles that are used at present for the corneal collagen crosslinking (CXL) technique were developed by researchers at the Dresden Technical University in the late 1990s.[1] This strengthening technology halts the progression of keratoconus by increasing corneal rigidity through stiffening of the anterior corneal stroma. At present, CXL is a well-established treatment for progressive keratectasia, and in our department, we have reduced the number of keratoplasties performed for keratoconus by half since we introduced this procedure in 2007.[2]

The ultraviolet-A (UVA) energy dose and exposure time have been thoroughly examined. In accelerated CXL, the UVA power is increased and the exposure time is accordingly reduced, which is an advantage over conventional treatment because it shortens the treatment time.

The use of riboflavin with dextran (dextran–riboflavin) has been the gold standard in CXL procedures. Because of its hyperosmolar effect, it causes thinning of the cornea during the procedure and hence has a potential to harm the endothelium.[3,4] Hydroxypropyl methylcellulose is a well-known agent in ophthalmology, mainly for use as a lubricant, and it has also been shown to be tolerable for the endothelium.[5,6] Recently, riboflavin with hydroxypropyl methylcellulose (methylcellulose–riboflavin) was introduced as a solution that prevents thinning of the cornea during the CXL procedure. Studies[7,8] have

confirmed the beneficial use of methylcellulose–riboflavin for maintaining corneal thickness.

Accelerated CXL protocols using dextran–riboflavin seem to give a more shallow demarcation line than conventional CXL.[9,10] However, the use of methylcellulose–riboflavin in combination with different UVA profiles of accelerated CXL has been shown to give functional outcomes similar to conventional CXL.[11] It has been suggested that the presence of hydroxypropyl methylcellulose might potentiate diffusion of certain molecules into the cornea.[12] It also has been surmised that there might be a higher concentration of riboflavin in corneas after soaking with methylcellulose–riboflavin compared with dextran–riboflavin.[13]

Efficacy and safety studies of diffusion depth with various riboflavin solutions in CXL are important because the depth of the riboflavin diffusion probably influences the CXL depth, and because the stiffening effect of CXL is believed to occur in the anterior corneal stroma, UVA irradiation in the most posterior stroma might be harmful to the endothelium. To our knowledge, there are no studies of the safety aspects of applying methylcellulose–riboflavin during CXL using conventional or accelerated UVA irradiation.

The aim of the study was to evaluate the safety of CXL with conventional irradiation and with accelerated irradiation using methylcellulose–riboflavin. We especially wanted to assess the structural differences in the cornea by measuring the depth of demarcation lines and the absence of keratocytes throughout the cornea after these treatment protocols were performed.

PATIENTS AND METHODS

Study Group and Protocol

This clinical trial enrolled patients with keratoconus. The study protocol was approved by the local Data Protection Office as well as the Regional Committee for Medical and Health Research Ethics. All patients provided written consent before participating in the trial. The study was registered at ClinicalTrials.gov.[A]

The patients were randomized (1:1) into 2 groups. One group (CXL30) had conventional UVA (3 mW/cm^2 for 30 minutes). The other group (CXL10) had accelerated UVA (9 mW/cm^2 for 10 minutes). A corneal thickness of 360 μm or less measured by Scheimpflug imaging (Pentacam HR, Oculus Optikgeräte GmbH) was an exclusion criterion. Other exclusion criteria were central corneal scars, chemical burns, serious corneal infection or ocular surface disease, and pregnancy or lactation during treatment. Patients were to discontinue contact lens use from 1 week before baseline.

Clinical signs of progressive keratoconus over the past 3 to 12 months were set as inclusion criteria and had to include 1 or more of the following: an increase of minimum 1.0 diopter (D) in the maximum keratometry (K) reading (using Scheimpflug imaging), an increase in corneal cylinder of minimum 1.0 D, or a minimum decrease in spherical equivalent of 0.5 D. Ultrasonic pachymetry (Corneo-Cage Plus, Sonogage, Inc.) was performed during the CXL procedure. Endothelial cell density (ECD) was measured preoperatively and after 12 months. Anterior segment optical coherence tomography (AS-OCT) (Nidek RS-3000, Nidek Co., Ltd.) and in vivo confocal microscopy (IVCM) (Confoscan 4, Nidek Technologies Srl) were performed 1 month postoperatively.

Surgical Technique

The CXL procedure was performed according to the modified protocol published by Wollensak et al.[1] in 2003. The epithelium was mechanically removed, riboflavin 0.1% with hydroxypropyl methylcellulose 1.1% (Mediocross M, Medio-Haus-Medizinprodukte GmbH) was then applied for 20 minutes to saturate the cornea via a funnel on the corneal surface containing the riboflavin solution. The corneal thickness was measured using ultrasonic pachymetry preoperatively, after 20 minutes of riboflavin soaking, 10 minutes after UVA irradiation in both groups, and also after 20 minutes and 30 minutes of UVA irradiation in the CXL30 group. The corneas were irradiated with UVA light focused on the center of the cornea, either with UVA light at an irradiance of 3mW/cm^2 (UV-X 1000, IROC Innocross AG) for 30 minutes (CXL30 group) or with UVA light at an irradiance of 9 mW/cm^2 (UV-X 2000, IROC Innocross AG) for 10 minutes (CXL10 group). During UVA irradiation, riboflavin 0.1% with hydroxypropyl methylcellulose 1.1% (methylcellulose–riboflavin) was applied every 2 minutes. A proxymetacaine hydrochloride 0.5% drops solution was applied for pain relief during the procedure. A bandage soft contact lens was used for 4 to 7 days.

Postoperative Regimen

After the CXL procedure, the patients were prescribed dexamethasone natrium phosphate 0.1%–chloramphenicol 0.5% eyedrops (Spersadex med kloramfenikol), diclofenac sodium eyedrops (Voltaren Ophtha), artificial tears (OculacB), and analgesics.

Follow-up Examination

Optical Coherence Tomography: Demarcation Line The AS-OCT analysis was performed 1 month after CXL. The images were analyzed using the corneal radial mode. Images in the 0- to 180-degree meridian were used for depth measurements. First, the demarcation line was interpreted and analyzed according to its visibility. The visibility of the demarcation line was defined as visible when it was sharply defined and unclear when it was not visible or too unclear for depth measurement to be performed. Second, when the demarcation line was visible, the depth of the line was measured in the center of the cornea using the measurement toolbars of the AS-OCT software.

In Vivo Confocal Microscopy Image Analysis: Keratocyte Presence Evaluation In vivo confocal microscopy using a ×40 magnification lens with the Z-ring attachment was applied for contact confocal microscopy analysis. Oxybuprocaine hydrochloride 0.4% was applied on the cornea before each examination, and a carbomer 0.2% gel (Viscotears) was used as lubricant between the lens and corneal surface. Three consecutive image sequences were taken, and approximately 100 images tangential to the corneal surface were automatically captured during each sequence. The images were captured from the posterior to the anterior part of the cornea. Because of the large number of images, the sequences were not always complete. The first complete series of images was used for analysis. If none of the 3 examination sequences was complete, the patient was excluded from the evaluation.

Quantitative keratocyte analysis was performed using IVCM software. The method is based on a procedure described by Jordan et al.[14] The posterior part of the endothelial layer was defined as the first image with visible endothelial cells. Similarly, the anterior part of the epithelial layer was determined when poorly defined epithelial cells were identified. If the images of the anterior border were not present, the depth was defined from the posterior border. A 1000 μm^2 frame of each stromal image was used for analysis. Counting was performed only when keratocyte nuclei were in focus and all edges were within the image frame. The frame area was altered from that used in the study by Jordan et al.[14] because they used different confocal microscopy technology (Heidelberg Retina Tomograph II Rostock Corneal Module, Heidelberg Engineering, Inc.).

The presence or absence of keratocytes were evaluated in the anterior stroma defined as the first clear stromal image immediately posterior to Bowman layer (sub-Bowman layer), and then

at 100 μm, 200 μm, 300 μm, and 400 μm. The most posterior image was defined as the first clear stromal image immediately anterior to the endothelium (preendothelial layer).

In Vivo Confocal Microscopy Image Analysis: Demarcation Line
The IVCM demarcation line was measured using a method previously described.[15] In brief, the principles of the first clear posterior and anterior stromal image were defined in the same way that the presence of keratocytes were evaluated. The depth of the demarcation line was defined as the distance from the anterior border of the epithelial layer until the first clear image with signs of keratocyte disappearance and poorly defined nuclei.

Endothelial Cell Density The ECD was measured using the automated endothelial analysis tool on the Navis platform of the IVCM software. An area showing a minimum of 35 clear endothelial cells was marked, and automatic cell counting was performed in the selected area.

Visual Acuity and Corneal Tomography
Uncorrected distance visual acuity, corrected distance visual acuity (CDVA), and maximum K were measured at baseline and after 12 months. Visual acuity was converted to logMAR notation for statistical analysis.

Statistical Analysis
Sample characteristics are presented as means ± SD or proportions. Differences in continuous variables between the 2 groups were tested with an independent-samples t test. The chi-square test for contingency tables or the Fisher exact test was used to detect associations between categorical independent variables. To assess changes in continuous variables between baseline and 12 months, a paired-samples t test was used. To evaluate the correlation between the depth of treatment and the change in ECD at 12 months, the Pearson correlation was used. The effect was quantified as the mean between group differences with its 95% confidence interval (CI). A P value of 0.05 was selected as the threshold of statistical significance. Statistical analysis was performed using SPSS software (version 21, International Business Machines Corp.).

RESULTS
Change in Intraoperative Central Corneal Thickness
The study comprised 40 patients (40 eyes). The mean central corneal thickness (CCT) before epithelial removal was 516 ± 36 μm (range 434 to 584 μm) in the CXL30 group
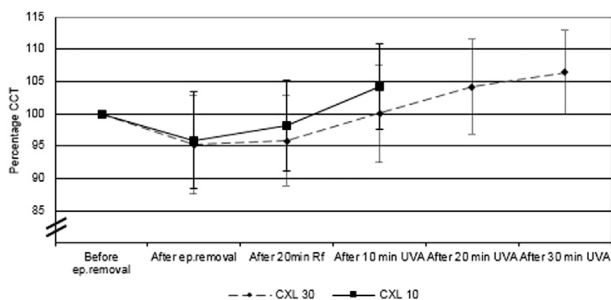
and 487 ± 53 μm (range 397 to 591 μm) in the CXL10 group. Figure 1 shows the change in intraoperative percentage thickness. There was a statistically significant increase in CCT from after the epithelial removal until the end of UVA irradiation in both groups ($P < .001$). There was no statistically significant difference in change in CCT between the 2 groups ($P = .135$).
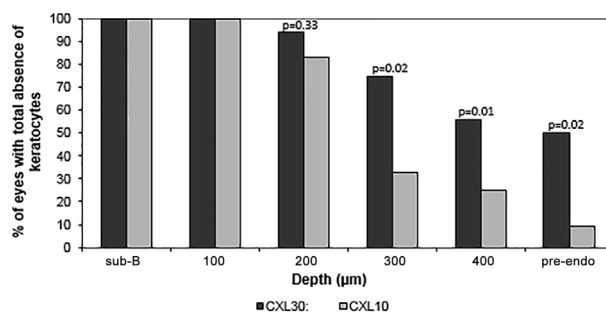
In Vivo Confocal Microscopy Image Analysis
Evaluation of Keratocyte Presence One eye in the CXL30 group and 3 eyes in the CXL10 group were excluded because of suboptimum image quality at 1 month. Figure 2 shows the percentage of eyes with a total absence of keratocytes at different corneal depths. There was a significantly higher percentage of eyes with a total absence of keratocytes deeper than 200 μm in the CXL30 group than in the CXL10 group.

Depth of Demarcation Line Two eyes in each group were excluded because of suboptimum measurements. The mean IVCM demarcation line was 442 ± 80 μm (range 278 to 594 μm) in the CXL30 group and 317 ± 122.5 μm (range 179 to 553 μm) in the CXL10 group ($P = .043$).

Optical Coherence Tomography: Evaluation of Demarcation Line
Two eyes in the CXL30 group and 1 eye in the CXL10 group were excluded because of suboptimum measurements. The demarcation line after 1 month was visible in 5 of 18 eyes in the CXL30 group and in 6 of 19 eyes in the CXL10 group. In the remaining 26 eyes, the demarcation line was too unclear or not visible. In the eyes with a visible demarcation line, the mean depth of the line was 296 ± 54 μm (range 245 to 345 μm) in the CXL30 group and 160 ± 41 μm (115 to 210 μm) in the CXL10 group. However, no statistical analysis could be performed because of the low number of eyes.

Figure 1. Change of intraoperative CCT during CXL with conventional and accelerated UVA irradiation (CCT = central corneal thickness; CXL10 = corneal collagen crosslinking using 9mW/cm² ultraviolet-A irradiation for 10 minutes; CXL30 = corneal collagen crosslinking using 3mW/cm² ultraviolet-A irradiation for 30 minutes; Ep = epithelial; Rf = riboflavin; UVA = ultraviolet-A irradiation).

Figure 2. Percentage of eyes with total absence of keratocytes on IVCM images at different stromal depths. The P values indicate the difference in percentage of eyes with total absence of keratocytes at different stromal depths (CXL10 = corneal collagen crosslinking using 9mW/cm² ultraviolet-A irradiation for 10 minutes; CXL30 = corneal collagen crosslinking using 3mW/cm² ultraviolet-A irradiation for 30 minutes; sub-B = sub-Bowman stromal depth, pre-endo = preendothelial stromal depth).

Comparison of Depth of Demarcation Line on Optical Coherence Tomography and In Vivo Confocal Microscopy

Of the 5 eyes in the CXL30 group and the 6 eyes in the CXL10 group with a visible demarcation line on AS-OCT, 9 eyes had optimum IVCM depth measurements with a mean depth of the demarcation line on AS-OCT of 222 ± 86 μm (range 115 to 345 μm) and on IVCM of 287 ± 108 μm (range 179 to 481 μm). In the remaining eyes in which the demarcation line was unclear or was not visible, making depth measurements unreliable to perform on AS-OCT, the mean demarcation line was 405 ± 111 μm (range 182 to 594 μm) on IVCM.

Endothelial Cell Density

Preoperative ECD measurements were optimum in 20 eyes in the CXL30 group and in 17 eyes in the CXL10 group, and in 18 eyes and 15 eyes, respectively, after 12 months of follow-up. The mean baseline ECD was 2796 ± 364 cells/mm^2 (range 2192 to 3400 cells/mm^2) in the CXL30 group and 2677 ± 271 cells/mm^2 (range 2229 to 3105 cells/mm^2) in the CXL10 group ($P = .275$). There was no statistically significant change in ECD in either group at 12 months (CXL30: -112 ± 302 cells/mm^2, $P = .132$; CXL10: -54 ± 245 cells/mm^2, $P = .410$). There was no statistically significant difference in the ECD change between the 2 groups ($P = .550$).

Depth of Demarcation Line on In Vivo Confocal Microscopy Versus Change in Endothelial Cell Density

An optimum 1-month IVCM and a 12-month ECD analysis were performed in 30 eyes. Of these eyes, 21 had an IVCM demarcation line of 60% or deeper than the total CCT (15 eyes in the CXL30 group; 6 eyes in the CXL10 group). Of the 9 eyes with an IVCM demarcation line shallower than 60%, 1 was in CXL30 group and 8 were in CXL10 group.

There was no statistically significant difference in endothelial cell loss after 12 months in eyes with a demarcation line deeper (-89.6 ± 298.3 cells/mm^2) or shallower (-79.8 ± 264.9 cells/mm^2) than 60% ($P = .933$). There was no correlation between the depths of demarcation lines and change in ECD after 12 months ($P = .423$).

Visual and Topographic Outcomes

Table 1 shows the outcomes after CXL. Both groups had a statistically significant improvement in CDVA compared with baseline; however, there was no statistically significant difference in change between the groups. There was a statistically significant reduction in the maximum K value in the CXL30 group; however, there was no statistically significant difference in the maximum K value change between the groups.

DISCUSSION

In the present study, 40 patients with progressive keratoconus were randomly assigned to CXL with conventional or accelerated UVA irradiation, and methylcellulose–riboflavin was applied in both treatment modalities. This riboflavin mixture has been shown to stabilize CCT during CXL, and it has been favorably compared with dextran–riboflavin.[7,8] Although our intraoperative CCT results are in accordance with findings in these studies, we found a deeper IVCM demarcation line and a marked deep keratocyte loss after CXL with accelerated UVA irradiation and CXL with conventional UVA irradiation compared with similar CXL studies that used dextran–riboflavin. Both findings raise safety concerns about the use of methylcellulose–riboflavin in CXL treatment.

Corneal CXL with accelerated UVA irradiation is a relatively new modification of the original CXL protocol that has been shown to give a shallower OCT demarcation line compared with conventional CXL using dextran–riboflavin.[10] Using a modified accelerated CXL protocol with a higher UVA irradiation (9mW/cm^2 for 14 minutes) and dextran–riboflavin, Kymionis et al.[16] found a depth of OCT demarcation line similar to that achieved with conventional treatment. Tomita et al.[11]

Table 1. Outcomes between the 2 groups at baseline and 12 months after CXL.

Parameter	CXL30 (n = 16)		CXL10 (n = 17)		
	Mean ± SD	Within-Group P Value	Mean ± SD	Within-Group P Value	P Value
Preop					
UDVA (LogMAR)	0.47 ± 0.39	—	0.74 ± 0.49	—	.09
CDVA (LogMAR)	0.15 ± 0.23	—	0.26 ± 0.19	—	.14
Kmax (D)	57.6 ± 9.3	—	58.4 ± 4.4	—	.75
Postop					
UDVA (LogMAR)	0.41 ± 0.36	—	0.58 ± 0.41	—	.20
CDVA (LogMAR)	0.04 ± 0.16	—	0.17 ± 0.19	—	.04
Kmax (D)	56.2 ± 8.4	—	57.9 ± 4.7	—	.47
Change: preop to 12 mo postop					
UDVA (LogMAR)	−0.06 ± 0.20	.24	−0.16 ± 0.25	.02	.22
CDVA (LogMAR)	−0.11 ± 0.14	.007	−0.09 ± 0.08	<.001	.53
Kmax (D)	−1.4 ± 1.5	.002	−0.5 ± 1.7	.22	.11

CDVA = corrected distance visual acuity; CXL10 = corneal collagen crosslinking using 9mW/cm^2 ultraviolet-A irradiation for 10 minutes; CXL30 = corneal collagen crosslinking using 3mW/cm^2 ultraviolet-A irradiation for 30 minutes; Kmax = maximum keratometry; UDVA = uncorrected distance visual acuity

found only a slightly, albeit not statistically significant, deeper OCT demarcation line using conventional CXL with dextran–riboflavin than using accelerated CXL with methylcellulose–riboflavin. We found that only a minor part of the corneas in both groups had a visible OCT demarcation line. In eyes with an unclear OCT demarcation line, the mean depth of IVCM demarcation line was as deep as 405 μm. Brittingham et al.[17] found that only a small percentage of the eyes receiving accelerated CXL with dextran–riboflavin had a visible OCT demarcation line. They speculated that the lack of an OCT demarcation line could be explained by the limited diffusion rate of dextran–riboflavin in the shorter treatment of accelerated CXL, and hence, a shallow cross-linked stroma depth. Kymionis et al.[18] found a correlation between the depth of OCT and an IVCM demarcation line at approximately 300 μm after conventional CXL with dextran–riboflavin. A similar correlation between OCT and IVCM demarcation line depth, although slightly shallower, was found in a study by Ozgurhan et al.,[9] which used an accelerated CXL profile with 2 soaking times with dextran–riboflavin. Both groups in our study received methylcellulose–riboflavin, which might have a faster diffusion rate than dextran.[12,13] In comparing our IVCM findings with the results by Brittingham et al.[17] (in that study the demarcation line was analyzed by OCT), one might hypothesize that the lack of an OCT demarcation line could be a result of the shallow treatment in their study and of the deep treatment in our study. However, because Brittingham et al. did not perform IVCM, a definite conclusion on this matter is not justified.

In vivo confocal microscopy keratocyte densitometry is a useful and an illustrative measurement for quantifying the damaging and remodeling process of the corneal stroma during and after CXL treatment. Mazzotta et al.[19] used IVCM and found a statistically significant reduction in anterior stromal keratocytes with both accelerated CXL and conventional CXL and a repopulation back to baseline levels 12 months after CXL treatment with both procedures. Using IVCM, Jordan et al.[14] found a complete absence of keratocyte nuclei in 86% of the eyes in the sub-Bowman stroma and no significant change in keratocyte density in the preendothelial stroma 1 month after conventional CXL with dextran–riboflavin. In our study, we found a complete absence of keratocyte nuclei in all eyes in both groups at the sub-Bowman depth and 100 μm stromal depth. Furthermore, in both groups, we found a considerably higher number of eyes with a total absence of keratocytes as deep as the preendothelial stroma, with a statistically significant larger percentage of total absence in the CXL30 group than in the CXL10 group. Because of these profound changes, we determined that the presence or absence of keratocytes was a more relevant presentation of data than keratocyte densitometry analysis.

We found a deeper IVCM demarcation line in both groups (440 μm in the CXL30 group; 315 μm in the CXL10 group) compared with that in the study by Kymionis et al.,[18] who found an IVCM demarcation line at an approximate depth of 300 μm. One might speculate that the difference in depth between their study and our study is related to a more potent diffusion capacity of hydroxypropyl methylcellulose compared with dextran, leading to a deeper CXL effect. In vitro studies with dextran–riboflavin have shown distribution only in the anterior stroma, at an approximate depth of 200 μm, and a change in application time and concentration had a limited effect on the depth distribution.[20] It is assumed that the most important biochemical effect in CXL is in the anterior stroma[21] and that riboflavin has a protective role in inhibiting deeper UVA exposure and damage.[22] Hydroxypropyl methylcellulose probably facilitates diffusion of riboflavin into the cornea. This might imply that it is necessary to decrease the riboflavin soaking time. In recent CXL studies with methylcellulose–riboflavin, the soaking time was set to 10 to 15 minutes based on a finite-element model describing the transport of riboflavin with dextran 20% and UVA absorption in the corneal stroma.[11] In addition, the authors did not apply riboflavin during UVA irradiation. Distilled water was used instead of riboflavin if the cornea was thinner than 400 μm. For Mediocross M, the recommended soaking time was 20 minutes when our study was performed. Since our last included patient was treated, the manufacturer's recommended soaking time has been changed to 10 minutes. However, riboflavin application every 2 minutes during UVA irradiation is still recommended. Few studies have assessed the diffusion of methylcellulose–riboflavin into the cornea during CXL procedures. This means there is a lack of knowledge concerning the possible effect on the corneal structure based on the length of the application time of this solution during CXL.

From this perspective, our IVCM findings raise concerns about the safety issues related to the use of methylcellulose–riboflavin with different CXL profiles and a potential risk for toxicity to the corneal endothelium, in particular in thin corneas. Because of the longer irradiation and riboflavin application time required when using CXL with conventional UVA irradiation, our findings highlight concern about this treatment profile, in particular when it includes the use of methylcellulose–riboflavin. However, we also found marked deep structural changes in some of the corneas in the accelerated CXL group, thus raising concerns about this CXL profile.

The 2 different UVA irradiation devices in our study differ in exposure time and effect. In addition, the UV-X 2000 used in the CXL10 group has 7 beams distributed in a ring, whereas the UV-X 1000 used in the CXL30 group has 1 ring in the center in addition to the 6 surrounding beams. The UV-X 2000 has been found

to give more similar CXL depth between the center and periphery than the UV-X 1000.[23] This discrepancy might be because of the different beam profiles and might influence the total UVA energy in the center of the cornea where our depth measurements were performed. Therefore, different beam profiles can, at least theoretically, also explain some of the differences in the IVCM demarcation depths between the CXL10 group and CXL30 group in our study.

Riboflavin in CXL acts as a photosensitizer for the induction of crosslinking and as a protective agent absorbing UVA irradiation. The manufacturer's instructions on the use of hydroxypropyl methylcellulose 1.1% include checking the anterior chamber to ensure the riboflavin has completely penetrated the cornea. However, one might speculate that because of the higher diffusion capacity of methylcellulose–riboflavin compared with dextran–riboflavin, the complete saturation of the cornea might be a safety concern, especially for the endothelium. Thus far, our 1-year results have not shown any reduction of ECD in either of the treatment groups, and there has been no increase in endothelial cell loss in eyes with the deepest keratocyte loss. In addition, after 1 year, there were statistically significant improvements in the CDVA in both groups with no differences in change between the 2 groups. However, these are relatively short-term results, and it remains to be explored whether these deep structural findings will have a negative effect on the cornea, including the endothelium, in the long term.

Although our study has the strength of being prospective and randomized to CXL with either conventional or accelerated UVA irradiation, the main limitation is the relatively small number of patients included. Even so, we believe we can justifiably suggest that methylcellulose–riboflavin seems safer to use in accelerated CXL profiles because they have shorter irradiation time than conventional treatment. The shorter soaking time of 10 minutes now recommended by the manufacturer also seems reasonable. The results of this study suggest that changes in the application time of methylcellulose–riboflavin might lead to a CXL depth that has efficacy and safety consequences; therefore, an evaluation of such potential consequences would help optimize the CXL treatment in terms of the best UVA irradiation power and duration of UVA treatment. Since the introduction of the Dresden protocol, many different UVA irradiation profiles and riboflavin solutions have been tested. However, there is still lack of evidence and consensus regarding the best CXL protocol in terms of efficacy and safety.

In conclusion, our IVCM findings of deep keratocyte loss to the level of the Descemet membrane raises safety concerns regarding the potency of methylcellulose–riboflavin in CXL treatment, especially if soaking and irradiation time is prolonged as it is when CXL is performed with conventional UVA irradiation. Further, both in vitro and well-controlled prospective long-term clinical trials are required to determine the best concentration level and application time of methylcellulose–riboflavin in CXL treatment.

WHAT WAS KNOWN
- The traditional riboflavin solution used with CXL treatment is dextran, which leads to thinning of the cornea during the procedure.
- The recent introduction of riboflavin with hydroxypropyl methylcellulose has resulted in stabilization of the corneal thickness during CXL treatment.
- The diffusion rate of methylcellulose–riboflavin is believed to be higher than that of dextran–riboflavin, although little is known about how the diffusion depth might influence the efficacy and safety of CXL.

WHAT THIS PAPER ADDS
- By using a solution of riboflavin with hydroxypropyl methylcellulose in CXL with conventional UVA irradiation and CXL with accelerated UVA irradiation, marked keratocyte loss as deep as in the preendothelial stroma occurred in a large number of eyes, in particular after CXL with conventional UVA irradiation. The reason might be the fast diffusion rate of hydroxypropyl methylcellulose.
- The diffusion depth of riboflavin using different solutions should be carefully evaluated to optimize the CXL protocol.

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