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Transepithelial corneal collagen cross-linking by iontophoresis of riboflavin

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ABSTRACT.

Purpose: **To evaluate the effectiveness of transepithelial cornea impregnation with riboflavin 0.1% by iontophoresis for collagen cross-linking.**

Material and methods: **Transepithelial collagen cross-linking by iontophoresis of riboflavin was performed in a series of 22 eyes of 19 patients with progressive keratoconus I–II of Amsler classification. The riboflavin solution was administered by iontophoresis for 10 min in total, after which standard surface UVA irradiation (370 nm, 3 mW/cm^2) was performed at a 5-cm distance for 30 min.**

Results: **The riboflavin/UVA treatment resulted in a decrease in the average keratometry level from 46.47 ± 1.03 to 44.12 ± 1.12 D 1 year after the procedure. Corneal astigmatism decreased from 3.44 ± 0.48 to 2.95 ± 0.23 D. Uncorrected distance visual acuity improved from 0.61 ± 0.44 up to 0.48 ± 0.41 (LogMAR). Preoperative and postoperative endothelial cell density remained unchanged at 2765 ± 21.15 cells/mm^2.**

Conclusion: **Transepithelial collagen cross-linking by iontophoresis might become an effective method for riboflavin impregnation of the corneal stroma reducing the duration of the procedure and being more comfortable for the patients. Further long-term studies are necessary to complete the evaluation of the efficacy and risk spectrum of the modified cross-linking technique.**

Key words: cross-linking – iontophoresis – keratoconus – riboflavin

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Introduction

Cross-linking treatment of progressive keratoconus using riboflavin and UVA was introduced successfully by Wollensak et al. from Germany in 2003 and is currently becoming the standard, low-invasive, safe (Romano et al. 2012) treatment for progressive keratoconus (Wollensak 2006). Riboflavin acts as a photosensitizer and enhances UVA absorption increasing the efficacy of the cross-linking process while providing also increased shielding of the deeper ocular structures from excessive UVA (Spörl et al. 2007). Riboflavin is stimulated by UVA light of 370 nm, which corresponds to one of the absorption peaks of riboflavin. Riboflavin is excited into a triplet state at this wavelength and releases highly reactive oxygen species. These oxygen species react with surrounding molecules and, amongst several non-specific interactions, trigger formation of cross-links that consist of intra and interfibrillary covalent bonds (Wollensak 2006). The cornea including the riboflavin film can be considered a two-compartment system, with the riboflavin solution being an integral part of the cross-linking procedure and important in achieving the correct stromal and endothelial UVA irradiance (Spörl et al. 2007; Wollensak et al. 2010).

Several variations of the standard cross-linking procedure have been proposed as its introduction to avoid epithelial debridement and increase the patient's comfort and safety. Some doctors used a cross-hatched grid pattern for epithelial debridement to accelerate postoperative epithelial healing. However, spectrophotometer analysis has revealed that riboflavin absorption is significantly nonhomogeneous with this variation (Bakke et al. 2009). Using 20% alcohol (Bakke et al. 2009; Samaras et al. 2009; Wollensak & Iomdina 2009) or an Amoil brush has also been suggested (Vinciguerra et al. 2009), and superficial removal of 35 μm of the epithelium with an excimer laser has also been tried (Bakke et al. 2009). The efficacy of riboflavin impregnation without epithelial debridement by treating eyes preoperatively with benzalkonium chloride and ethylenediaminetetraacetic acid for 3 hr (Leccisotti & Islam 2010) or by applying

tetracaine-containing anaesthetic eye drops for 30 min and subsequently instilling preoperative riboflavin solution into the eye for another 30 min was described (Chan & Wachler 2007). Kanellopoulos suggested to create at 100 μm deep intrastromal pocket injecting the riboflavin solution into the pocket combined with a higher UVA irradiation of 7 mW/cm^2 for 15 min. Initial results were promising (Kanellopoulos 2009).

Riboflavin is a water soluble, negatively charged, very small molecule with a molecular weight 376.40 g/mol, which makes it a good candidate for iontophoresis. In the present study, we tried to use iontophoresis as a new transepithelial method for stromal riboflavin delivery before cross-linking to reduce the long treatment time of the transepithelial approach and possibly increase its efficiency.

Materials and Methods

Study group

Nineteen patients with progressive keratoconus of grade I–II according to Amsler classification (without stromal scarring) were enrolled in the study.

Progressive keratoconus was defined by the following changes over 1 year: an increase of the steepest K by 1.0 dioptre (D) or more in manifest cylinder, or an increase in 0.5 D or more in manifest spherical equivalent refraction by repeated keratotopography ODP-scan ARK-1000 (Nidek, Aichi, Japan).

All patients signed an informed written consent. The study was approved by the ethics committee of Ufa Eye Research Institute following the Tenets of the Declaration of Helsinki.

At each follow-up, a standard examination was carried out to assess uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), refractometry, keratometry, corneal topography (ODP-scan ARK-1000 Nidek), pachymetry (Visante OCT; Carl Zeiss, Zeiss-Meditec, Jena, Germany), endothelial cell count and confocal microscopy using confocal scanning laser ophthalmoscope (HRT II; Heidelberg Engineering, Dossenheim, Germany). Each follow-up also included a slit-lamp examination.

Surgical technique

Impregnation of the cornea with a riboflavin 0.1% hypotonic solution (Riboflavine-5-monophosphate natrii 0.1%, Chlorbutanol hemihydrate 0.05%, Natrii EDTA 0.03%) was performed using the iontophoreris device 'galvanizator' (Potok-1, Russian Federation, Moscow, Russia; Fig. 1). The passive electrode (anode, made of lead, 6 cm^2) was applied to the inferior part of cervical vertebrae (Fig. 2A). The active electrode (cathode, made from graphite, 0.5 cm length, 0.785 cm^2), a bath tube made of glass or plastic with a capacity of 10–12 ml, was applied to the open eye (Fig. 2B). After the tube was taped to the skin of the orbital margins, it was filled with riboflavin 0.1% (Fig. 2C). During the procedure, there is no pressure on the eyeball, the eye is in direct contact with the riboflavin solution. The current intensity was initially 0.2 mA and was gradually increased to 1.0 mA at an increment rate of 0.2 mA per 10 seconds to find out the individual tolerance and to avoid patients discomfort. The total time that the riboflavin solution was administered by iontophoresis was 10 min. The procedure is completely comfortable for the patient and easy to perform for the doctor. Anaesthesia is unnecessary.

Fig. 1. The control unit of the iontophoresis device and the bath-tube cathode (bottom left).

During the procedure of iontophoresis, the patient was sitting or lying with head elevated (Fig. 2D), depending on the patients' preferential comfort position in terms of complete bath-tube fullness and direct contact of riboflavin solution with eye. The

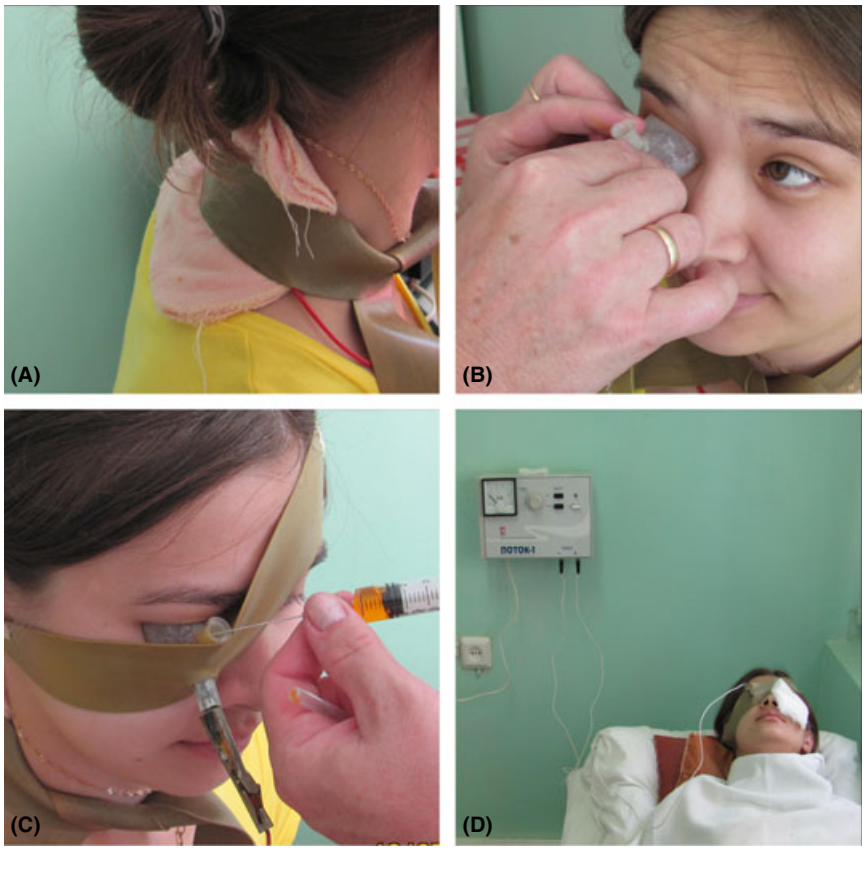

Fig. 2. Iontophoresis procedure. (A) Application of anode to inferior part of cervical vertebrae, (B) application of cathode (bath tube) to the orbital margins, (C) filling of bath tube with riboflavin 0.1%, (D) the patient's position during procedure.

Table 1. Summary of visual and refractive outcomes.

	Mean ± SD					
	Preop	1 week	1 month	3 months	6 months	12 months
UDVA (LogMar)	0.61 ± 0.44	0.52 ± 0.42	0.48 ± 0.38	0.51 ± 0.29	0.49 ± 0.31	0.48 ± 0.41
CDVA (LogMar)	0.34 ± 0.29	0.26 ± 0.25	0.30 ± 0.31	0.29 ± 0.22	0.28 ± 0.28	0.29 ± 0.25
Keratometry (D)						
K1	44.6 ± 1.12	45.06 ± 2.11	44.02 ± 1.19	43.98 ± 1.97	42.38 ± 1.75	42.31 ± 1.87
K2	47.82 ± 2.23	47.12 ± 1.89	46.81 ± 2.03	46.94 ± 2.12	45.78 ± 2.01	45.72 ± 2.13
Av	46.47 ± 1.03	46.98 ± 1.85	46.2 ± 1.99	46.21 ± 1.79	44.19 ± 1.16	44.12 ± 1.12
Astigmatism (D)	3.44 ± 0.48	3.36 ± 0.42	3.47 ± 1.12	3.12 ± 0.95	2.87 ± 0.67	2.95 ± 0.23

UDVA = uncorrected distance visual acuity, CDVA = corrected distance visual acuity, K1 = corneal dioptric power in the flattest meridian for the 3-mm central zone, K2 = corneal dioptric power in the steepest meridian for the 3-mm central zone, Av = mean corneal power in the 3-mm zone, D = Dioptre.
Visual acuity and refractive outcomes are shown.

efficiency of riboflavin penetration into the corneal stroma was checked by slit lamp using a dark blue cobalt filter. An intense yellow glow in the anterior chamber indicated complete impregnation with riboflavin after 10 min of iontophoresis in all patients. Standard surface UVA irradiation (370 nm, 3 mW/cm^2; UFalink, Russian Federation, Ufa Eye Research Institute, Ufa, Russia) was then applied at a 5-cm distance for 30 min. During UVA exposure, hypotonic riboflavin drops were continued every 2 min. Postoperative corticosteroid drops were used for 2 weeks. Postoperative evaluations were at 1 day, 1 week, and 1, 3, 6 and 12 months.

Statistical analysis was performed using StatsDirect software (StatsDirect, Ltd, Cheshire, UK). All data samples were first checked by means of the Smirnov test. When parametric analysis was possible, the Student's *t*-test for paired data was performed for all parameter comparisons between preoperative and postoperative examinations. When parametric analysis was not possible, the Wilcoxon rank-sum test was applied to assess the significance of differences between preoperative and postoperative data, using the same level of significance ($p < 0.05$) in all cases. As the sample size was small, statistical power was limited.

Results

Twenty-two eyes of 19 patients with a mean age of 32.15 ± 9.12 years were included; 15 patients were men (78.95%) and four patients were women (21.05%). According to the Amsler–Krumeich grading system, nine eyes had a keratoconus grade I (40.9%) and 13 eyes had a keratoconus grade II (59.1%).

Postoperatively our patients did not complain of early postoperative pain or increased glare as can be encountered with the standard cross-linking technique.

Table 1 summarizes the visual and refractive outcomes obtained by transepithelial collagen cross-linking by iontophoresis of riboflavin (TCCIR). A slight improvement of one line was observed in UDVA 1 week after TCCIR in all patients, with no significant changes afterwards, but this value did not exceed the statistical significance ($p = 0.23$), For CDVA, no statistically significant changes were found at any time-point during follow-up ($p \geq 0.062$). Keratometry did not change significantly between 1 week and 3 months after surgery; however, at 6 months after surgery, a statistically significant corneal flattening was observed ($p = 0.005$). Anterior corneal astigmatism was also reduced 6 months after procedure, but the change did not reach statistical significance ($p \geq 0.059$).

No significant haze has been found after TCCIR; however, the demarcation line (Seiler & Hafezi 2006; Doors et al. 2009) was observed on OCT images from 1 week to 1 month after treatment at a depth of 200–250 μm in all patients (Fig. 3). During 1–3 months of follow-up, there were no significant changes in pachymetry; however, a slight decrease of 30 μm in corneal thickness within the 2–5 mm zone inferiorly was detected 6 months after TCCIR ($p < 0.05$).

To control the safety of the procedure, endothelial cell density was counted in all patients and corneas were scanned by laser scanning confocal microscope (Fig. 4). Images of the endothelium were acquired with a confocal scanning laser ophthalmoscope (Heidelberg Retina Tomograph III/Rostock Corneal Module; Heidelberg Engineering GmbH). Endothelial cell density was assessed using the software provided by the system.

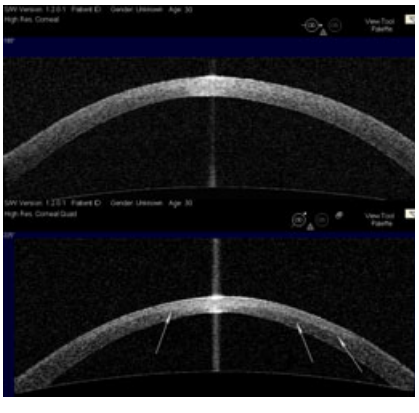

Fig. 3. Demarcation line on OCT images of cornea (top) before and after (bottom) procedure. Arrows show the demarcation line.

Seven days after the procedure, the confocal microscopy showed visible corneal epithelial cells with abnormal hyperreflectivity. The cells borders were unclear; however, the thickness of epithelial cell layer was within normal range. A few sub-basal nerves were detected. The anterior corneal stroma had a 'honeycombed' appearance with reduced number of keratocytes' nuclei with a maximum depth of penetration at about 210–230 μm, measured from the surface of epithelium. At about 6 months postoperatively, the corneal stroma had regained its normal configuration (Mazzotta et al. 2008; Mencucci et al.

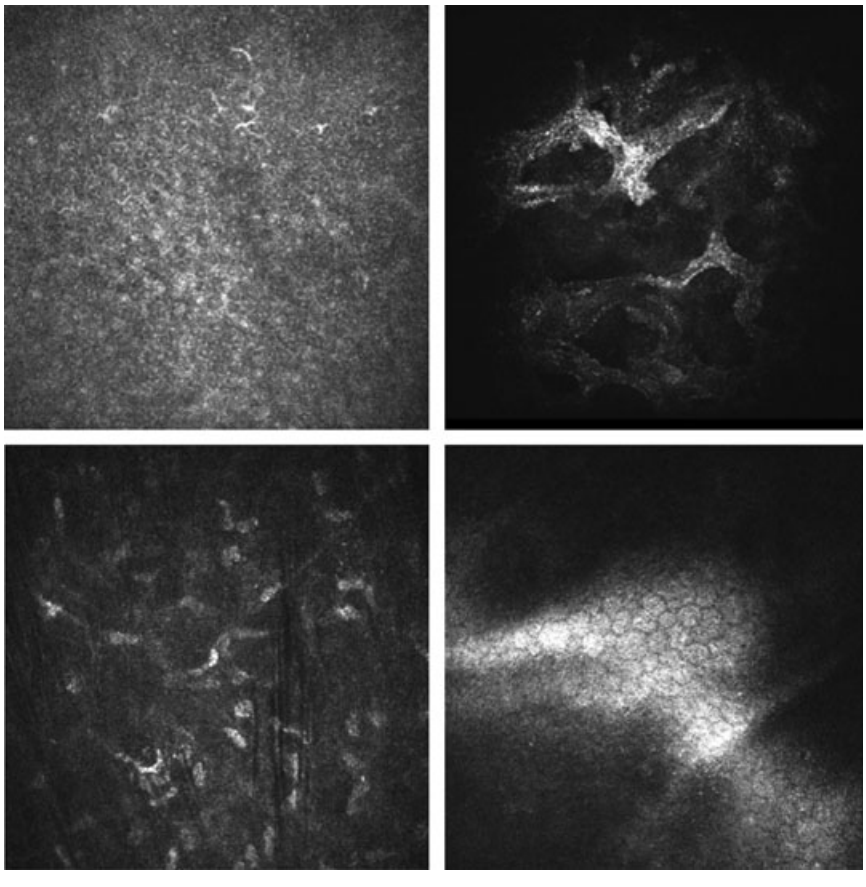

Fig. 4. Confocal microscopy images of the cornea 1 week after transepithelial collagen cross-linking by iontophoresis of riboflavin (TCCIR; ×300) (top left) epithelial cell layer showing abnormal hyperreflectivity and poorly demarcated cell borders, (top right) anterior stroma at depth 140 μm with the honeycombed hyperreflective appearance, (bottom left) intact posterior part of the stroma at depth 290 μm, (bottom right) intact endothelial cells layer.

2010, 2011). No endothelial damage was observed at any time during follow-up period. Preoperative and postoperative endothelial cell density remained unchanged at 2765 ± 21.15 and 2759 ± 73.12 cell/mm^2, respectively.

Discussion

Iontophoresis has been investigated as a technique of drug delivery in ophthalmology since the 1908 (Wirtz 1908). It was found to be effective for transcorneal drug delivery to corneal tissues and the aqueous (Rootman et al. 1988).

The transepithelial approach may reduce early postoperative pain, vision impairment and risk of infection (Rama et al. 2011). In addition, the total time for riboflavin administration by iontophoresis is 10 min, which significantly reduces the procedure's duration compared with the other transepithelial methods (Leccisotti & Islam 2010). Additionally, the irradiation might be further reduced in the future by so-called accelerated cross-linking using an equivalent UVA dose, but a higher irradiance and a shorter irradiation time (Kanellopoulos 2012).

Our study evaluated the visual, refractive and pachymetric outcomes after TCCIR in eyes with corneal ectasia. Our statistical analysis revealed decreased *K*-values at the apex of keratoconus 6 months after the procedure, with a mean change in the keratometry value of 2.3 D. Patients also demonstrated stable visual acuity without statistically significant changes from preoperative values. The exact mechanism and efficacy of the iontophoresis-assisted riboflavin diffusion is not yet fully elucidated. The results of confocal microscopy have demonstrated the safety for the corneal endothelium, but an apoptotic keratocyte effect of this treatment was observed only down to 210–230 μm depth while it is 270–300 μm with standard CXL (Wollensak 2006; Wollensak & Iomdina 2009; Mencucci et al. 2010). This less intense apoptotic effect on the deep keratocyte population might be due to reduced riboflavin supply during the UVA irradiation when the iontophoresis device has been removed and might also possibly lead to a lower overall cross-linking-induced biomechanical stiffening effect. The results of our study have been promising so far, but future studies must show if the effect achieved by the present method will actually be sufficient for the long-term stabilization of the cornea. Future investigations and more experience will show whether unexpected rare side-effects or treatment failures may be possible with this method similar like in the standard cross-linking procedure.

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Conflict of Interest

None of the authors has conflict of interest with the submission.

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