Corneal Crosslinking in Progressive Keratoconus: Comparison of Dextran-Based and Hydroxypropyl Methylcellulose - Based Riboflavin Solutions

Differences in demarcation line depth and 1 yaear outcomes

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Keratoconus is a progressive ectatic disorder characterised by irregular myopic astigmatism and loss of visual acuity due to corneal thinning. Corneal collagen crosslinking is the treatment recognised to stop the progression of keratoconus. Our study evaluates and compares visibility and depth of the stromal demarcation line after corneal collagen crosslinking using anterior segment optical coherence tomography between two groups:crosslinking with dextran-based and hydroxypropyl methylcellulose-based riboflavin solutions. Our work proved a better visibility and a deeper demarcation line when we used HPMC-based riboflavin. Also the study revealed that HPMC-based riboflavin is associated with better biomechanical outcomes than dextran-based riboflavin.

Keywords.keratoconus, crosslinking, riboflavin, demarcation line, corneal biomechanics

Keratoconus is a progressive ectatic disorder characterised by irregular myopic astigmatism and loss of visual acuity due to corneal thinning and steepening[1].

In 2003, Wollensak et al, introduced for the first time the procedure of corneal crosslinking in order to halt the progression of keratoconus[2]. This procedure uses ultraviolet A light and riboflavin (vitamin B2) in order to increase corneal rigidity and biochemical stability by creating new covalent bounds between corneal collagen molecules, fibres and microfibrils[3]. Riboflavin ($C_{17}H_{20}N_4O_6$) (fig1.) represents the coenzyme of flavoenzymes which play an important role as cofactor in enzymes and as cromophores in blue-light sensitive biological photoreceptors[4]. The standard protocol involves using 0,1%riboflavin with 20% dextran T500 with an iradiation of 3.0mW/cm2, while maintaing a constant energy of 5.4J/cm2 for 30 min. In this procedure dextran represents the use source to crosslinking of collagen fibrilles in cornea. Furthermore, it provides the viscosity of the solution [5].

Dextran (H(C6H10O5)xOH) (fig.2) is a bacterial polysaccharide composed of (1→6) linked α-D-glucopyranosyl residues, and it has three hydroxyl groups per anhydroglucose unit. Upon light exposure, a higher degree of substitution results in higher crosslinking density which in turn decreases the degree of swelling and the water content[6].Besides different protocols, in order to facilitate diffusion through the corneal epithelium, a number of modified riboflavin formulations have been developed [7]. Even if the use of riboflavin with dextran has been the gold standard in CXL procedures, it has a hyperosmolar effect, determining thinning of the cornea during the procedure and thus having a potential harmful effect for the endothelium[8,9].Therefore riboflavin with hydroxypropylmetylcelluose was introduced as a solution that prevents thinning of the cornea during the CXL procedure. It is also known in ophthalmology for the use as a lubricant and has been shown to be tolerable for the

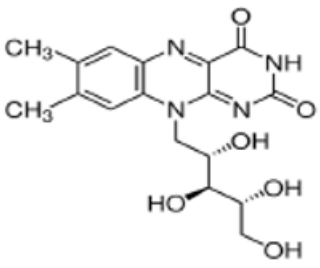

Fig. 1. Riboflavin [4]

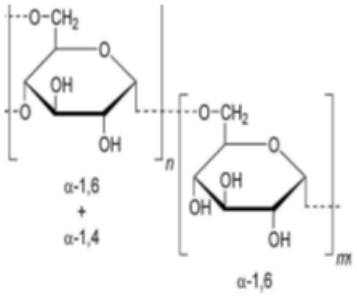

Fig. 2. Dextran [6]

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endothelium [10,11]. There are studies that reported there might be a higher concentration of riboflavin in corneas after soaking with methylcellulose riboflavin compared with dextran riboflavin [5,10,12].

Hydroxypropyl methyl cellulose ($CH_2CH(OH)CH_3$) (fig.3) is propylene glycol ether of methyl cellulose, hydroxypropyl and methyl combine with anhydrous glucose ring by ether bond [13]. Methylcellulose has a low surface tension and contact angle, which increases coating ability, having a lack of elasticity, which makes it more 'viscoadherent' than viscoelastic [13,14].

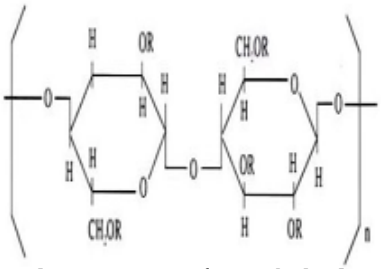

Fig. 3. HPMC [13]

The transition of crosslinked to non-crosslinked tissue is detected as a demarcation hyperreflective line within the corneal stroma (fig.4), possibly representing the effectiveness of the CXL treatment. Studies have shown that the demarcation line is biomicroscopically detectable in slit lamp examination as early as 2 weeks after treatment and it can be equally detected by confocal microscopy and anterior segment optical coherence tomography (AS-OCT) up to a depth of aproximately 300µm[15-18].

In a previous study, Malhotra et al. compared the demarcation line depth after contact lens-assisted CXL using dextran-based and hydroxypropyl methylcellulose based riboflavin solutions. They reported the results 6 months after procedure, showing that HPMC-based riboflavin is associated with a deeper demarcation line, but they didn't evaluate the biomechanical changes to determine whether a deeper demarcation line is associated with better biomechanical outcomes[19].

To our knowledge, to date, there has been no comparison of riboflavin formulations to evaluate whether they determine different biomechanical outcomes. Taking into account the data avaiable untill now, this study was performed to investigate the visibility and depth of the stromal DL after standard corneal collagen crosslinking using AS-OCT. It also assessed whether riboflavin solutions of similar concentration (0,1%), but different carrier agents, such as dextran 20% or HPMC 1.1% determine different corneal biomechanical results.

Experimental part

Materials and methods

This prospective study evaluated 60 eyes from 46 patients with progressive keratoconus who underwent CXL between March 2016 and March 2017 in Oftaclinic Center, Bucharest. Of these, 30 eyes were treated using dextran riboflavin and 30 eyes were treated with HPMC-riboflavin. The treatment protocols of CXL were randomly selected and all patients were followed for at least 12 months. As keratoconus is characterised by binocular asymmetry, when both eyes of keratoconus patients were treated with CXL, the CXL protocol using the same riboflavin was selected. Informed consent was obtained from all study participants before the initiation of CXL treatment.

Inclusion criteria were as follows: progressive keratoconus, no previous ocular surgery, corneal thickness of 400µm or more. Keratoconus was considered to be progressive if, during a 12 months follow-up, there was an increase in simulated maximum keratometry by at least 1D, based on corneal topography, a deterioration of visual acuity(loss of at least 1 Snellen line) or an increase in astigmatism by at least 1.0D, with subjective deterioration in vision. Exclusion criteria included corneal thickness of less than 400 µm at the thinnest point, central or paracentral corneal opacities, history of herpetic keratitis or recurrent infections, any corneal endothelial pathology, pregnancy, concomitant autoimmune diseases. All procedures were performed by a single surgeon. Cases were classified into four stages based on corneal power, astigmatism and corneal thickness according to the classification of Amsler-Krumeich [19].

As parameters we evaluated demarcation line depth, visual acuity, cornea dioptric powers, manifest refraction, pachymetry as well as corneal hysteresis, corneal resistance factor and keratoconus match index.

Each patient underwent preoperatively and at 1 month, 3 month, 6 month and 12 month postoperatively, a complete ophthalmologic examination , including: measurement of manifest refraction, uncorrected and of best corrected visual acuity, slit lamp and fundus examination, corneal topography with Topcon CA- 200F Corneal Analyser(Topcon Medical Systems) , ultrasound pachymetry (Alcon® OcuScan® RxP Ophthalmic Ultrasound System), anterior segment imaging using optical coherence tomography(OCT, Optical coherence tomography 3D OCT 2000 series), corneal biomechanics examination (Ocular Response Analyzer, Reichert, New York).

All patients were treated with UVA-riboflavin CXL in the operating room under sterile conditions and topical anesthesia with oxybuprocaine hydrochloride 0.4% (Benoxi, Unimed Pharma Ltd). Collagen crosslinking was performed according to the classical methodology, with corneal epithelial mechanical debridement in a 9.0 mm diameter area. The epithelial tissue was removed with a blunt spatula to ensure penetration of riboflavin in the corneal stroma. Normo-osmolar riboflavin solution 0.1 % in dextran 500 20%(Pesche D) or in HPMC 1.1% (Pesche M) was applied to the cornea for 30 min every 3 min. After that , the cornea was exposed to UVA 365 nm light for 30 min at an irradiance of 3.0mW/cm^2 . During the 30 min of irradiation the riboflavin administration was continued every 5 min. At the end of surgery, eye drops were instilled in the form of combination of antibiotic (moxifloxacin) and

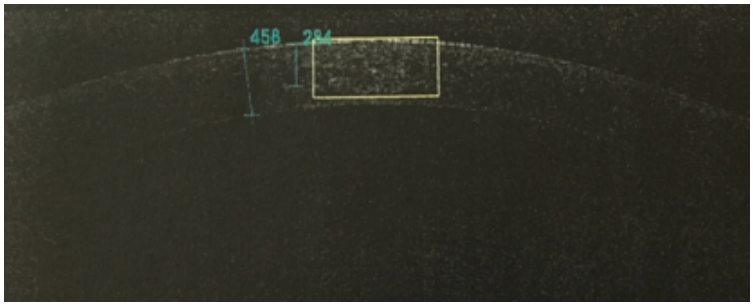

Fig. 4 Representative demarcation line seen in one patient treated with HPMC riboflavin

nonsteroidal anti-inflammatory drops (pranoprofen 1mg/mL) and a bandage soft contact lens was applied until corneal epithelium healing was completed. Postoperatively the patients were followed at day one, one week, one month, three months, six months and one year.

Statistical analysis

Statistical analysis was performed using statistical software -SPSS statistics, version 20. The Shapiro-Wilk test was used to check for a normal distribution of quantitative data, appropriate for small sample sizes of fewer than 50 participants. Postoperative changes were evaluated using a paired t-test. If the data were not distributed normally, the Wilcoxon test was performed. An independent sample t-test was performed to analyze the difference in outcomes between the two groups, while the Mann-Whitney test was performed when data were not distributed normally. Continuous variables are presented as mean+/-standard deviation. A p value <0.05 was considered statistically significant.

Anterior segment OCT. Anterior segment OCT using the Spectralis anterior segment module was performed preoperatively and then 1 month after the CXL procedure. We measured the DLD centrally to determine the absolute depth and related to the total central corneal thickness to determine the depth/CCT ratio.

Results and discussions

In the present study, a total of 60 eyes were analyzed, 30 in the dextran-based riboflavin group and 30 in the HPMC-based group. At baseline, there were no significant differences between the two groups that were analyzed, in terms of their age, UDVA, CDVA, MRSE, astigmatism, Kmax, Kmin, pachymetry or corneal biomechanics parameters (CH, CRF, KMI). The baseline parameters are summarized in table 1. The results 1 year after crosslinking are shown in table 2.

Demarcation line depth

Of the eyes studied, 26 of 30 (86.7%) showed a clear demarcation line on OCT evaluation 1 month after CXL using dextran-based riboflavin. We observed a higher occurence rate of the demarcation line in 28 of 30(93.3%), using HPMC-based riboflavin($p<0.05$) (fig.5). Retrospective analysis of the DLD as measured by AS-OCT showed a deeper localization of the demarcation line after CXL using HPMC based riboflavin group compared with the dextran based group (HPMC: mean: 316.96±22.53 ; dextran: mean 267.54±20.34, $p=0.0001$) (fig.6). A similar ratio was obtained for the DLD in relation to the total corneal thickness of the HPMC-based versus dextran-based group(66.9±5.25% versus 60.4±4.95%, $p=0.0001$) (fig.7)

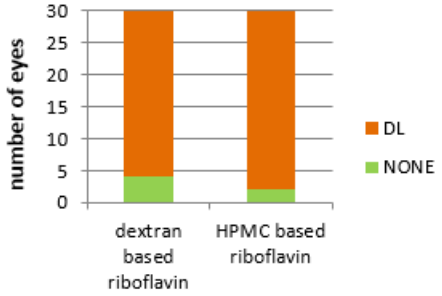

Fig. 5. Showing increased occurence of DL 1 month aftre CXL using dextran-based compared with HPMC-based riboflavin

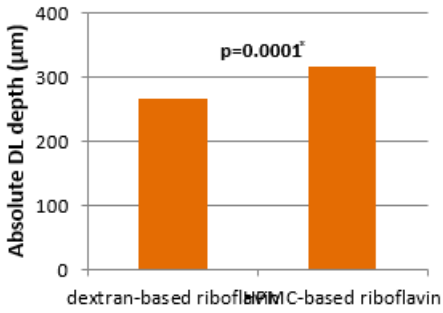

Fig. 6. showing increased occurence of DL 1 month after CXL using dextran-based compared with HPMC-based riboflavin

Parameter	Dextran Group (30 eyes)	HPMC Group (30 eyes)	p-value
Age	23.53±4.76	25.47±6.91	0.548
Sex (M:F)	22:8	26:4	
UDVA(SDE)	0.23±0.20	0.25±0.23	0.851
CDVA(SDE)	0.48±0.17	0.51±0.13	0.265
MRSE	-3.78±2.48	-3.34±2.91	0.530
Astigmatism	-3.77±1.74	-4.26±3.02	0.959
K max	51.59±4.35	52.40±4.65	0.383
K min	47.03±3.7	46.48±3.68	0.412
Pachymetry	450.20±37.96	468.93±40.95	0.071
CH	8.36±1.29	8.99±1.36	0.73
CRF	7.02±1.08	7.64±1.71	0.126
KMI	0.220±0.26	0.268±0.32	0.528

Table 1
BASELINE CHARACTERISTICS OF PATIENTS IN THE TWO GROUPS

Parameter Postop	Dextran group(n=30 eyes)		HPMC group(n=30eyes)		P value
	Mean±SD	Within-group p value	Mean±SD	Within-group p value	
UDVA(SDE)	0.30±0.22	0.007*	0.34±0.27	0.002*	0.66
CDVA(SDE)	0.62±0.21	0.001*	0.65±0.17	0.0001*	0.62
MRSE	-3.24±2.51	0.003*	-2.92±2.41	0.006*	0.62
Astigmatism	-3.04±1.87	0.006*	-3.74±2.39	0.005*	0.65
Kmax	50.57±4.25	0.002*	51.27±4.79	0.0001*	0.6
Kmin	46.81±3.3	0.276	46.21±3.64	0.044*	0.359
Pachymetry	464.9±38.9	0.0001*	487±39.03	0.001*	0.032*
CH	8.51±1.22	0.0001*	9.25±1.56	0.027*	0.047*
CRF	7.26±1.15	0.039*	8.04±1.6	0.0001*	0.041*
KMI	0.314±0.24	0.038*	0.467±0.32	0.0001*	0.045*

Table 2
COMPARISON OF THE TWO GROUPS AT 12-MONTH FOLLOW-UP

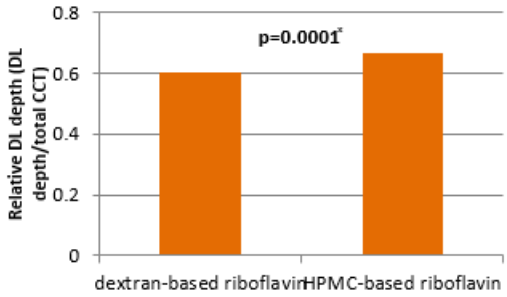

Fig. 7. Showing a significant difference in the relative depth of the DL in relation to CCT 1 month after CXL between-based and HPMC-based riboflavin

The efficacy of CXL, using 3mW/cm^2 ultraviolet A light for 30 min, has been supported by various randomized controlled studies[21]. However, a number of modified riboflavin formulations have been introduced to facilitate diffusion through the corneal stroma[6]. Previous studies have demonstrated the efficacy and safety of standard CXL using dextran based riboflavin or HPMC based riboflavin [21-23]. In the present sudy, a comparative analysis demonstrated the efficacy of standard CXL using dextran-based and HPMC-based riboflavin, in stabilizing keratoconus progression after 12 months of follow-up, although a small case series was used. To the best of our knowledge, no previous studies exist in the literature comparing the biomecanichal outcomes of standard CXL using dextran-based and HPMC-based riboflavin solutions.

Improvements in UCDVA, BCVA, mean spherical equivalent refractive error, mean cylindrical error were observed in the both groups, however not statistically significant between groups.

The effects of CXL mainly present as variations of keratometry over time, as observed on corneal topography. A randomized study of CXL in progressive keratoconus reported that, after 6 months of follow-up, treated eyes with dextran riboflavin experienced a mean reduction of Kmax by 1.83D, and eyes treated with HPMC riboflavin had a mean value of Kmax decreased by 1.48D[19]. In a study by Brittinhgham et al, with a minimum of 12 months follow-up, the mean value of Kmax decreased by 0.76D[16]. The present study findings were similar to these aforementioned results. In the current study, a decrease of the Kmax values was observed at 12 months after the two treatments(dextran group reduced by 1.02; HPMC group reduced by 1.12-with no difference between both groups), which indicated that the corneas became flattened.Therefore, both riboflavin solutions appear to have the same efficency.

When CXL is performed, there is happening a keratocyte damage because of UVA-induced apoptosis and extracellular matrix increases its density. This change in tissue composition appears as a demarcation line, which represents the transition between anterior treated and posterior untreated stroma. The DL is used to reveal the extent of treatment [24]. This study investigated not only the visibility, but also the depth of DL. In our study the occurence rate of the DL was higher in the HPMC group. In the dextran group, a line was visible in 86.7% of the eyes. In the HPMC group a line was visible in 93.3% of the eyes treated. The mean demarcation line depth seen in the dextran group 267.54± 20.34 was comparable to that reported by Jacob et al (252.9±40.8µm) who also used a dextran-based riboflavin of the same concentration (0.1%)[23]. However, using the HPMC 1.1% riboflavin, we noted the mean demarcation line depth to be deeper, at 316.96±22.53.

In addition, the results of our study showed a significant difference between the mean depth values as a percentage of central thickness, revealing a deeper percentage in HPMC group than in dextran group(66.9±5.25% versus 60.4±4.95%, $p<0.05$).

The deeper demarcation line and others structural changes associated with HPMC-based riboflavin versus dextran-based riboflavin could be explained by the chemical difference of the two substances. Dextran carrier has been shown to retard the stromal penetration of riboflavin in comparison with hydroxy-propyl methyl-cellulose, because of its high viscosity. During the crosslinking procedure, the greatest number of crosslinks happen in the zone with the highest riboflavin concentration. Thus, because HPMC tends to be concentrated more in the posterior stroma than dextran, may be the explanation for a deeper demarcation line encountered in the HPMC group[25,26].

The present study has another important outcome which reffers to biomechanical corneal changes.The previuos published data suggested that subjects with low biomecanical corneal properties are candidates for a variety of ocular diseases[27]. Despite the results of other studies which showed no statistically significant difference in CH, CRF after CXL compared with baseline, our study revealed improvements in CH, CRF and KMI in the both groups, beeing statistically significant [28,29]. The changes of these parameters were greater in the HPMC group as compared to the dextran-group, these differences presenting statistical significance. This might be explained by the deeper stromal penetration of riboflavin associated with HPMC and also by the increasing of corneal thickness. Concerning the corneal thickness, our findings of a a statistically significant greater increase after CXL in the HPMC group, could be explained by previous in vivo findings showing that T-dextran has a dehydrating effect [25].

The limitations of the current study include the small number of patients in each group and the short follow-up period. The long-term effects of the two types of riboflavin require further investigation.

In conclusion, the results of our retrospective analysis sugest a superior effectiveness of the HPMC-based riboflavin in comparison to the dextran-based riboflavin with regard to the occurence of the corneal demarcation line and corneal biomechanical changes at 12 months follow-up.

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