ISSN: 2090-9047
Online ISSN: 2536-9466

JOURNAL OF OPHTHALMOLOGY AND RELATED SCIENCES

Corneal Collagen Crosslinking with Riboflavin and Ultraviolet - A Irradiation in the Management of Progressive Ectatic Corneal Disorders. Review Article

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Submitted: 23-6-2017; Revised: 5-7-2017; Accepted: 13-7-2017

ABSTRACT

Corneal crosslinking (CXL) has been proposed as a new modality to stop progression of keratoconus or secondary corneal ectasia, aiming to prevent progressive visual loss and to delay or avoid corneal transplantation. The possibility of strengthening corneal tissue by means of a photochemical reaction of corneal collagen by the combined action of riboflavin and ultraviolet-A irradiation (UVA), radically modified the conservative management of progressive corneal ectasia. This is a review of the state of the art of CXL. The paper describes basic principles, advantages and limitations of different CXL techniques and possible future evolution of the procedure.

Keywords: Keratoconus, ectasia, collagen cross-linking, epithelium-off collagen corneal cross-linking; epithelium-on, transepithelial cross-linking.

Keratoconus is a degenerative disorder distinguished by para-central corneal thinning and secondary ectasia, resulting in irregular astigmatism with impaired vision, ghosting and polyopia. It is typically bilateral but often asymmetrical. Onset typically is at puberty with progression of disease for 10–20 years when it tends to stabilize. It is the commonest corneal dystrophy, affecting about 1 in 1750 individuals. It occurs in all racial groups and equally affects males and females. Its etiology includes genetic, biochemical, and physical factors, with no sole proposed theory elucidating its range of clinical presentation. It usually appears as an isolated condition, but has been associated with a number of ocular and systemic disorders, including, vernal disease, retinitis pigmentosa, blue sclera, atopy, magnesium deficiency, Down's syndrome, Turner

syndrome and connective tissue disorders such as Marfan's syndrome, and pseudoxanthoma elasticum. It has been related to repeated surface ocular trauma associated with hard contact lens wear, allergic eye disease and eye rubbing. Genetic factors are important, up to 10% having a family history of the condition[1-5].

Pellucid marginal corneal degeneration (PMD) is less common than keratoconus. It usually affects the inferior peripheral, rather than paracentral cornea in about 85% of cases and the superior peripheral cornea in 15%. It occurs in a crescentic fashion typically between the 5 and 7 o'clock positions. It often presents with severe against the rule astigmatism typically with good spectacle corrected acuity until the advanced stages of the disease. Topography typically shows a "lobster-claw pattern" but this is not pathognomonic and may also occur in keratoconus and there is overlap between the two conditions, as well as with keratoglobus[6].

Kerat-ectasia after refractive surgery is a rare but often visually devastating complication. As well as following laser-insitu keratomileusis (LASIK)[7], it has been reported after photorefractive keratectomy (PRK)[8,9] and radial keratotomy[10]. Management depends on the severity and the extent of irregular astigmatism. Mild cases are correctable with spectacles and soft toric contact lenses. However, with progressive disease, the cornea becomes more irregular and rigid gas permeable lenses are required[11]. In 15–20% of keratoconic patients, surgery, typically keratoplasty, becomes necessary, as a result of contact lens intolerance, corneal scarring and thinning[12]. None of these interventions, treat the underlying causes of corneal ectasia and its progression. It is only with the advent of corneal (CXL) that we can hope to slow, stop or even to a limited extent reverse keratoconus. CXL process occur physiologically with age via natural enzymatic pathways such as transglutaminase and lysyl oxidase. Photochemical CXL with riboflavin (vitamin B2)/ultraviolet-A (UVA) (370 nm) was developed at the University of Dresden by Spoerl and Seiler[13,14]. The procedure induces physical CXL by Riboflavin absorbing UVA to act as a photosensitizer to produce free radicals (oxygen singlets) that activate the natural lysyl oxidase pathway. By absorbing UVA, the riboflavin also prevents damage to deeper ocular structures, including the endothelium, lens and retina. The precise location of the cross-links at a molecular level is yet undetermined. Hayes et al in an ex-vivo study using X-ray scattering, investigated the hydrodynamic behavior and enzyme digestion. He determined that it was likely that the cross links formed during riboflavin/UVA therapy were occurring predominantly at the collagen fibril surface, rather than within the fibrils themselves, and in the protein network surrounding the collagen[15].

Biomechanical and biochemical considerations Ex-vivo laboratory studies have reported changes in physico-chemical properties of the stroma following CXL. Stress-strain measurements of corneal stromal tissue are significantly increased, both immediately as well as several months following the procedure[16]. These changes principally occur in the anterior 200μm of the stroma where most of the UVA absorption takes place. Increased resistance of stromal tissue to enzymatic digestion has been confirmed after CXL, with a dose response in relation to the UVA intensity[17]. In addition, an increased resistance to matrix

metalloproteinase (MMP), in particular sub-types MMP-1, -2, -9, and -13, degradation of collagen and small leucine-rich proteoglycans following riboflavin/UVA CXL has been found. The protective effect of CXL on collagen and proteoglycans from MMP cleavage is likely to be important in the mechanism of preventing the progression of keratoconus and other corneal ectatic disorders, where an increased activity in collagenases has been established[18]. Other biophysical and biomechanical alterations include, an increase in collagen fiber diameter in rabbit corneas, and a significant reduction in the hydration behavior of the stroma, both of which are greater in the anterior compared to the posterior stroma[19]. Such changes are probably short-term alterations, due to the effects of the osmolarity of the riboflavin solutions used rather than actual effects of cross-linking and are not likely to be long-term changes[20].

Safety considerations UVA is cytotoxic. It can cause keratocyte apoptosis and most significantly endothelial cell damage and death and permanent lens and retinal injury. Cell cultures studies have found an increased cytotoxic irradiance level with UVA irradiation combined with photosensitizing riboflavin for keratocytes, which in the clinical setting would occur in human corneas to a depth of 300μm[21]. With regard to endothelial damage, studies have demonstrated a cytotoxic threshold level above 0.35mW/cm^2, UVA exposure for 30 minutes. This should not be reached in the clinical setting with corneal thickness greater than 400μm[15]. In-vivo studies have confirmed these results and have demonstrated that with UVA exposure of 3mW/cm^2 for 30 minutes, over 85–90% of UVA radiation is absorbed by the riboflavin in the anterior 400μm of the stroma, so that the irradiance at the level of the endothelium is less than 0.18mW/cm^2, i.e. 50% less than the cytotoxic level. This situation is the same for other internal structures, such as the lens and retina, where the level of UVA radiation reaching these structures is less than 3% of the cytotoxic threshold[13].

Indications for CXL The main aim of CXL is to stop the progression of corneal ectasia, consequently the best candidates for this treatment are patients suffering from primary or post-refractive surgery ectasia with documented progression of the disease. Although the criteria to classify ectasia as progressive have not been defined, changes in refraction, uncorrected visual acuity (UCVA), best-corrected visual acuity (BCVA), and topographical parameters are to be included[13-15,22]. Progression of ectasia, (table 1), was defined as an increase in Kmax of 1 diopter (D) in 1 year, or a change in either myopia and/or astigmatism ≥3D in 6 months, a mean central K-reading change ≥1.5D observed in three consecutive topographies in 6 months, or a mean central corneal thickness decrease ≥5% in three consecutive tomographies in the previous 6 months[22,25,27,30,31].

Table 1. Signs of keratoconus progression

Changes in refraction, a change in either myopia and/or astigmatism ≥3D in 6 months. Loss of one line or more of UCVA & BCVA.
Change in topographical and/ or tomographic parameters • An increase in Kmax of 1D) in 1 year • A mean central K-reading change ≥1.5D observed in three consecutive topographies in 6 months • A mean central corneal thickness decrease ≥5% in three consecutive tomographies in the previous 6 months.

Contraindications to standard CXL treatment are the presence of corneal thickness of less than 400µm, prior herpetic infection, severe corneal scarring or opacification, history of poor epithelial wound healing, severe ocular surface disease, history of immune disorders, and pregnancy/breast-feeding[25,34,39].

Standard procedure and clinical results The standard Dresden protocol, includes initial epithelial removal, the application of 0.1% riboflavin solution for 30 minutes followed by 30 minutes of UVA irradiation with a wavelength of 370nm and power of $3mW/cm^2$ ($5.4J/cm^2$). The first published clinical trial was carried out by Wollensak and co-workers[14] and showed that CXL was effective in halting the progression of keratoconus. In the last few years, several prospective and retrospective studies with a considerable follow-up period documented the effectiveness of the standard procedure in halting the progression of primary and secondary corneal ectasia, and in many cases, with an improvement of visual performance and topographical indices. Most of the reports were about clinical outcomes of standard epi-off CXL. In the follow-up after treatment, the main parameters evaluated are the maximal keratometry (Kmax) and the BCVA. The follow-up periods ranged between one and six years. All authors reported stabilization or flattening of corneal keratometry and stabilization or improvement of visual acuity after standard epi-off procedure. Long-term comparative analysis showed that functional results after CXL among pediatric and young patients (up to 26 years) were better than in patients over 27 years[22-39].

Effect of treatment on corneal curvature

The reduction in Kmax noted in most studies, indicated that in many patients CXL leads to flattening of the cornea. Suggested mechanisms: 1- The refractive outcomes were achieved by a simultaneous flattening of the cone apex and a steepening of the part of the cornea symmetrically opposite the cone[23,26]. 2- Flattening results from the contractive properties of the keratocytes as they migrate to repopulate the wound. There may also be some rearrangement of the collagen and the surrounding matrix brought about by cross-linking[20]. 3- The stiffening/shortening effect of collagen fibrils on a non-central cone, CXL would tend to pull the cone towards the corneal center, thus leading to a flattening effect. Finite element modelling does indeed suggest that the effects of CXL would depend on the position of the cone and the topographic effects of CXL may be greatest if treatment is centered on the cone[22,28].

Effect of the treatment on intraocular pressure & endothelial cell density

The majority of the studies showed no significant changes in intraocular pressure or endothelial count[14].

Effect of the treatment on cornea clarity

All trials have indicated a time-dependence of the effects of CXL, both in terms of transient haze[54] and edema in the early stages, as well as in refractive outcome, which seems to improve over the first year or more following treatment[26,30,31,51-52].

Complications of standard procedure

Treatment failure is usually defined as continued progression with an increase in maximum K readings of 1.0D over the preoperative value[22,24,38].

Keratoconus worsening was considered if patients presented an increase of more than

0.1 logMAR UCVA and BCVA and/or an increase of keratometric values by more than 0.75D during the follow-up[26,28,30,77]. Although the great majority of eyes are stabilized after CXL. Koller and co-workers[74] in 177 eyes described progression in 8 eyes (7.6%). They identified eyes with advanced keratoconus with maximum keratometry values greater than 58D of being at greatest danger of progression.

Corneal haze after standard CXL procedure, is a relatively common complication reported by 10-90% of patients. In-vivo confocal microscopy showed an increased stromal reflectivity associated to edema and keratocyte activation mainly evident 3–6 months after treatment, while in the late postoperative period, anterior and intermediate stromal layers showed a reduction of cellular density and fibrosis of extracellular matrix[52-54,77].

Infective Keratitis Several cases of infective keratitis following CXL treatment have been described including bacterial, protozoal, herpetic, and fungal keratitis[78-80].

Rare adverse events following traditional CXL included diffuse lamellar keratitis at LASIK interface, corneal melting and persistent corneal edema due to endothelial failure[81].

Technique modifications Trans - epithelial cross-linking (TE - CXL)/Epi - on technique

The diffusion process of riboflavin in the stroma is limited by corneal epithelial tight junctions[13-15], but epithelial debridement is considered the cause of the most important complications after CXL treatment such as intraoperative and postoperative pain, infective keratitis and abnormal wound healing response[77-80]. Riboflavin penetration through the epithelium can be increased by different strategies such as changing the physicochemical properties of the riboflavin molecule by adding chemical enhancers in the riboflavin formulation or performing a mechanical disruption of corneal epithelium. Several methods of TE-CXL have been proposed in which the anti-swelling agent dextran is typically omitted on the basis that its high molecular weight may inhibit the penetration of riboflavin solution across the epithelium, (table 2).

Table 2. Riboflavin solutions

- **Isotonic**
  Riboflavin 5-phosphate (0.1%) with 20% dextran T500 for normal corneas ≥ 350μm, for standard CXL approach.
- **Hypotonic**
  Riboflavin 5-phosphate (0.1%) without dextran for thin corneas below 400μm. Creates corneal swelling effect by osmosis
- **Trans-Epithelial**
  Riboflavin 0.25% plus EDTA, BAK. CXL procedures without corneal epithelial removal. Reduces trauma and risk of post-operative complications.
- **Rapid**
  Riboflavin (0.1%) with HPMC. Indicated for A-CXL (3min – 15min) with 400μm cornea thickness.
- **Plus/Extra**
  Riboflavin (0.25%). Indicated for Lasik-plus (immediate post-LASIK treatment). Intended to prevent corneal ectasia and improving corneal stability.

In these procedures, chemical agents, such as benzalkonium chloride (BAC)[58] ethylenediaminetetraacetic acid (EDTA)[60] are added to the riboflavin solution

(individually or in combination) to loosen the tight junctions of the epithelial cells and thereby facilitate passage of riboflavin into the stroma without the need for epithelial removal. Advantages of TE-CXL are: 1) It offers patients a faster and less invasive treatment than that provided by the standard technique. 2) It facilitates the treatment of pediatric, uncooperative patients and those with thinner corneas. Disadvantage of this technique is that its effectiveness remains uncertain[62,63]. Experimental comparative studies in rabbit corneas have shown that CXL of corneas with an intact epithelium using BAC 0.0005% results in an increase in biomechanical rigidity (Young's modulus) of about one-fifth of that induced by standard CXL with epithelial debridement[58]. This is presumably due to limited riboflavin absorption[59].

Iontophoresis is a non-invasive system aimed to enhance the delivery of charged molecules into tissues using a small electric current. Riboflavin is an effective molecule for iontophoretic transfer as it is small, negatively charged at physiological pH and is easily soluble in water. Riboflavin, in the formulation used for iontophoresis, is negatively charged. It has been shown that an iontophoresis imbibition lasting five minutes achieves a sufficient riboflavin concentration in the corneal stroma for CXL treatment, with the advantage of shortening the imbibition time while preserving epithelial integrity. Ex-vivo biomechanical studies on rabbit and human cadaveric corneas showed that TE-CXL with iontophoresis imbibition induced an increase of the biomechanical resistance of human cornea comparable to that obtained with the standard CXL procedure. Preliminary clinical results of iontophoresis assisted corneal CXL are promising. The technique halts keratoconus progression without significant complications, however, longer follow-up and studies with larger patient populations are needed to assess the real effectiveness of this technique[64,65]. Other methodologies currently under pre-clinical investigation to facilitate trans-epithelial riboflavin stromal absorption include the use of ultrasound[83], nano-emulsion systems[84], and other epithelial permeation enhancers such as d-alpha-tocopheryl poly (ethyleneglycol) 1000 succinate (Vitamin E-TPGS)[85].

Accelerated corneal crosslinking (A-CXL)
It was introduced in clinical practice to shorten the time required for a CXL procedure. This technique is based on the Bunsen-Roscoe law of photochemical reciprocity[101]. According to this physical theory it is theoretically possible to deliver the same energy dose ensuring a proportional biological effect by setting different UVA powers and exposure times in order to accelerate and shorten the crosslinking procedure. According to "equal-dose" principle 10mW/cm^2 for 9 minutes, 30mW/cm^2 for 3 minutes, 18mW/cm^2 for 5 minutes, 45mW/cm^2 for 2 minutes at constant energy dose of 5.4J/cm^2 are the same as the standard 3mW/cm^2 for 30 minutes, a basic concept leading to A-CXL[101]. An energy dose of 7.2J/cm^2 was demonstrated to be effective both in terms of corneal strengthening and anti-enzyme activity compared with the standard dose of 5.4J/cm^2, respectively tested by biaxial corneal extensiometry and papain digestion (Avedro's laboratory unpublished data, presented by M. D. Friedman, Ph.D at 8th International CXL Congress, Geneva 8 December 2012). Currently, commercially available ultrafast devices can achieve an irradiance intensity of 43mW/cm^2. Studies conducted on pig corneas have shown that increasing the illuminance intensity to

$10mWcm^{-2}$ and reducing the exposure time to 9 minutes produces a similar increase in corneal stiffness to that gained using the standard procedure[66]. Several recent in vivo studies using different protocols showed the procedure to be safe and effective in stopping ectasia progression[69-72].

Femto-second laser stromal pocket

Kanellopoulos AJ[57] reported efficacy of CXL in early keratoconus with riboflavin delivered in a femtosecond laser-created pocket, thus avoiding the need to remove the epithelium or use other drugs.

Treatment of very thin kerato-conic cornea

In order to overcome the contra-indication of treating corneas with a thickness bordering on 400µm, Hafezi and co-workers, and other authors[73,75] replaced the standard iso-osmolar riboflavin solution (containing dextran) with a hypo-osmolar riboflavin solution (without dextran) to swell the cornea to an acceptable thickness prior to cross-linking. Using the modified technique, Hafezi and co-workers[73] treated 20 patients with thin corneas (minimum preoperative stromal thickness of 323µm) and reported a cessation of keratoconus progression in all cases. A minimal preoperative stromal thickness of 330µm is required for successful CXL using the modified protocol. X-ray scattering studies have shown that this phenomenon of increasing corneal thickness in cross-linked corneas is caused not by an increase in the diameter of the collagen fibrils but by an increase in the spacing between individual fibrils.

Contact lens-assisted collagen cross-linking (CA-CXL)

Contact lens-assisted CXL was introduced by Jacob and co-workers 2014. A Soflens daily disposable soft contact lens (14mm diameter, 8.6mm basal curvature; Bausch & Lomb) of 90µm thickness made of hilafilcon and without UV filter was immersed in iso-osmolar riboflavin 0.1% in dextran for 30 minutes, before it was applied onto the de-epithelialized, riboflavin-saturated cornea. The pre-corneal riboflavin film with contact lens created an absorption medium in the pre-corneal space by artificially increasing the thickness of the "riboflavin-filter". At a mean follow-up time of 6.1 ± 0.3 months (range: 6–7 months), the mean postoperative depth of the stromal demarcation line was measured at 252.9µm. No significant endothelium loss or signs of postoperative endothelial damage were observed. The advantage of the CA-CXL is that it is not dependent on the swelling properties of the cornea and that the cornea is not subjected to edema, which may cause Descemet membrane folds and endothelial damage .The disadvantages are that the surface irradiance at the level of the corneal stroma is reduced by 40–50% in CA-CXL secondary to absorption by the riboflavin film and soaked contact lens. Furthermore, oxygen diffusion, which is crucial in the CXL process, might be hindered by the contact lens and the effect of CXL may be reduced[82].

New CXL Techniques

While riboflavin/UVA CXL has been shown to be effective, other methodologies which are potentially more rapid and less invasive, are currently under investigation. Rocha and co-workers[83] reported a flash-linking process with UVA and polyvinyl pyrrolidone with may have the potential to photo-chemically

cross-link the cornea in only 30 seconds. Paik and co-workers[84] investigated the topical application of short chain aliphatic beta-nitro alcohol. Cherfan and co-workers[8] demonstrated an almost four-fold increase in corneal stiffness with no reduction in keratocyte viability with rose-bengal 0.1% administration and green light application and a less than 5 minutes total treatment time.

Small incision lenticule extraction (Smile)-assisted CXL

It involves application of refractive lenticule extracted from the patients undergoing small incision femtosecond lenticule exrtraction (SMILE) procedure for myopia. The lenticule is placed over the de-epithelialized surface of the cornea so that the thickest portion of the lenticule corresponds to the thinnest portion of the cornea. Refractive lenticules of variable thickness (20 to 140 mm) can be obtained following SMILE depending on the extent of the refractive error to be corrected. Placing the central lenticule over the apex of the cone enabled the surgeon to augment the corneal thickness where required while sparing the remaining stroma to be crosslinked. An even demarcation line indicative of CXL was seen in all cases[100]. Long-term preservation of myopic lenticules would enable more widespread use of this technique.

Combined treatment

CXL has also been used in conjunction with keratorefractive procedures such as PRK and LASIK in an attempt to improve long-term stability and reduce the possible occurrence of post-surgery ectasia. The idea of performing PRK in patients with stable keratoconus has been proposed (Athens protocol). Consequently, the possibility of combining CXL and PRK was introduced in clinical practice. Several clinical reports demonstrated stability in corneas that had undergone a combination of CXL and PRK, either sequentially or combined. Patients experienced improvement in spherical equivalent (SE), defocus equivalent, UCVA and BCVA, high order aberrations and Kmax with stabilization of keratoconus progression during a follow up period of 12–24 months[89-93]. A single contralateral eye study in of CXL after hyperopic LASIK demonstrated less regression of correction over the limited follow-up period[48]. The timing of the ablation treatment and CXL as well as the interval between the two procedures has become topics of discussion. It was reported that patients who underwent both PRK and CXL procedures on the same day obtained better clinical and topographical results with a lower rate of corneal haze, compared to patients treated sequentially[94]. This may be related to the unpredictable refractive outcomes when excimer ablation is performed on cross-linked tissues. However, performing both procedures concurrently on the same day may cause an irregular healing process with the formation of persistent stromal haze, probably related to keratocyte activation, which permanently affects visual performance[95,96].

CXL and intracorneal rings

Several studies reported results confirming that combining CXL and intra-corneal rings (ICRS) implantation improved UCVA and BCVA, refraction, and keratometry during variable follow-up periods. Moreover, it was reported that after one or both ring explantations, the refractive effects may be stable or reversible while the topographic changes seem to be maintained[96-99]. Therefore, while collagen crosslinking can be performed before, in conjunction with, or

after ICRS implantation, the ideal method for combining these two treatments is still undefined.

Conclusion

CXL with riboflavin and UVA 370nm radiation appears to be capable of arresting the progression of ectatic corneal disorders, with most studies reporting significant improvements in visual, keratometric, and topographic measurements. Follow-up is limited to 5-15 years but suggests sustained stability and enhancement in corneal shape with time. Epi-on investigations suggest some efficacy but less than with epi-off treatments. Accelerated techniques with higher UVA fluencies and shorter treatments times, delivering the same UVA energy dosage, are the subject of recent investigations, with some laboratory and clinical studies suggesting good results. Combined CXL with PRK and ICRS shows promise but long-term follow-up is needed. Sight-threatening complications of CXL are rare. Clinical studies of CXL have shown great promise in stabilizing keratoconus and post-refractive surgery ectasia. While further randomized, prospective and long-term follow up studies are necessary, it is very likely that in the future corneal ectasia can be halted at an early stage and avoid the need for rigid contact lenses and keratoplasty. Future refinement in techniques will allow for safer and more rapid procedure with less patient discomfort.

FINANCIAL DISCLOSURE

The author declares no financial interests to disclose.

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