Collagen corneal cross linking with riboflavin (C3R)
Technology and Treatment

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Abstract

Corneal collagen cross-linking (CXL) is a new modality of treatment, based on collagen cross linking in the corneal stroma with the help of UVA and the photosensitizer riboflavin to increase the formation of intra and interfibrillar covalent bonds by photosensitized oxidation. In vitro studies have shown that the cornea absorbs approximately 30% of UVA light with an additional 50% of UVA absorption occurring in the lens. CXL is proven to halt the progression of corneal ectasias such as keratoconus, pellucid marginal degeneration and iatrogenic post Lasik ectasia by strengthening the corneal stroma. CXL may also be effective in the treatment of infectious keratitis due to elimination of infectious agent by UVA irradiation. Combination of CXL with refractive vision improving interventions, such as intracorneal ring segment implantation, limited topography-guided photoablation and conductive keratoplasty have shown success.

Keywords: Corneal collagen cross-linking, riboflavin, corneal ectasia, bullous keratopathy.

Introduction

The term crosslinking (CXL) is used to express the formation of chemical bridges. It can be formed by chemical reactions initiated by heat, pressure, or radiation, resulting in change of the physical properties of the cross-linked material. Corneal cross linking is also known as C3R or CXL or CCL.C3R. It is a new approach to increase the mechanical and chemical stability of corneal tissue. It utilizes riboflavin as a photosensitizer and UVA to increase the formation of intra and interfibrillar covalent bonds by photosensitized oxidation. In vitro studies have shown that the cornea absorbs approximately 30% of UVA light with an additional 50% of UVA absorption occurring in the lens.1 UVA absorption can be considerably increased using Riboflavin. With 0.1% riboflavin and an irradiance of 3 mW/cm² of UVA, almost 95% of UVA light will be absorbed within the cornea reducing irradiance to 0.15 mW/cm² (at the endothelial level), which is well below 0.36 mW/cm², the threshold considered cytotoxic for the endothelium.2-4 The irradiation levels may still exceed the threshold leading to keratocyte apoptosis in the anterior stromal layer and a demarcation line between the treated and untreated cornea.2-4 Caporossi et al performed confocal microscopy analysis in humans after X-linking in vivo. They detected the effective depth of treatment by identifying distinct vertical and lateral transition areas at a depth of 270 to 330 μm. The anterior stroma showed oedema with only a few keratocyte nuclei and poor reflectivity and the posterior stroma showed regular keratocyte population with normal reflectivity. CXL changes the intrinsic biomechanical properties of cornea, increasing its strength by almost 300%. CXL is a non-surgical procedure and it is only known procedure that is proven to halt the progression of the keratoconus.

Biochemistry of C3R

The UVA 365nm irradiates the riboflavin molecules causing them to lose their internal chemical balance and produce oxygen free radicals. The riboflavin molecule is unstable, it can be stabilised by linking it to two collagen fibrils leading to formation of crossed bridge between the collagen fibrils, producing a general strengthening of the cornea.

Technique

Photo polymerization using UV-A is activated by a photosensitizer (riboflavin) and a wavelength (U V-A) which is deeply absorbed enough to protect deeper layers of the eye. Riboflavin has two important functions in this technique; absorption of UV-A radiation and acts as a photo-sensitizer, leading to generation of reactive oxygen species.

Riboflavin and UV-A Light
Riboflavin, a naturally occurring photosensitizer is also known as Vitamin B2, a precursor of Flavin mononucleotide (FMN). UV-A has deleterious effect on the ocular structures but with the use of riboflavin as photosensitising agent, transmission rate of UV-A is only 7% across the cornea, limiting the UV-A irradiance of the lens and retina.

**Physiology of collagen corneal cross linking**

In this procedure, riboflavin eye drops are applied to cornea which is then activated by UV light. Riboflavin is exited into its triplet state generating reactive oxygen species (ROS) by using UVA at 365nm. Reactive oxygen species is mainly singlet oxygen and to a much less degree superoxide anion radicals; which further react with various molecule including chemical covalent bonds bridging amino group collagen fibrils. Because of the absorption maximum of riboflavin at 365 nm, this wavelength was specially chosen for the treatment with UV light. This achieves 90% absorption of the UV light in a 400 µm thick de-epithelialized cornea without endangering the lens or the cornea. It is caused by an increase in collagen fibre diameter due to interfibrillar and intrafibrillar covalent bonds by photosensitised oxidation cross-linking. The cornea becomes more compact due to cross linking and are more resistant to biochemical deformation or ectasia.

**Exclusion criteria**
- Corneal thickness less than 400 µm at the thinnest position.
- Active ocular disease.
- Herpes keratitis.
- Diabetes.
- Pregnancy.
- Previous ocular surgery other than laser refractive surgery.
- Immunocompromised patients.
- Patients with known sensitivity.

**Clinical indications for CL-UVR treatment**

1. Progressive keratoconus

Fig.1 : Picture showing conical protrusion

Keratoconus is a corneal non-inflammatory degeneration. It is characterized by bilateral conical protrusion and thinning (Figure1). It commonly occurs in the second decade of life. Differences between normal and keratoconic corneas is seen in biochemical and immunohistochemical studies of the proteoglycans in the matrix. With CXL, covalent binding occurs between collagen molecules leads to stabilization of the collagen scaffolds and changing of several tissue properties.  

2. Iatrogenic post refractive keractasia (post Lasik ectasia)

Fig.2 : Picture showing corneal ectasia

Keractasia characterized by a progressive corneal steepening, can occur centrally or inferiorly and can be associated with severe refractive changes, loss of BCVA, with stromal thinning of the cornea (Figure2). CXL should be considered for patients with post LASIK progressing ectasia to strengthen artificially weakened corneal stroma and stabilize the cornea.

3. Pellucid marginal degeneration

Fig.3 : Picture showing corneal thinning in pellucid marginal degeneration

It is a rare ectatic disorder that typically affects the inferior peripheral cornea in a crescentic fashion, mostly in males, between the second and fifth decades of life (Figure3). It has been observed that patients with PMD have large amounts of against-the-rule astigmatism, making it difficult to fit in contact lenses than in patients with keratoconus.
4. Infectious keratitis and the melting process

Fig. 4: Picture showing corneal melting

CXL treats corneal melts (Figure 4) or infectious keratitis by strengthening of collagenolytic cornea because of cross linking and elimination of the infectious agent by UVA irradiation.\(^1\)

5. Bullous keratopathy

Fig. 5: Picture showing bullous keratopathy

Wollensak et al. has shown the anti oedematous effect of CXL on the cornea (Figure 5). The bullous changes markedly improve and the patients do not report of any pain or discomfort.\(^2\)

Pre-operative work up for CXL-UVR treatment

A detailed ophthalmological examination is required which includes the following:

- Visual acuity assessment (UCVA, BCVA, Contrast sensitivity)
- Intraocular pressure recording
- Slit lamp examination specially for Vogt's striae, Fleischer's ring and corneal scarring
- Slit lamp photographs
- Pentacam
- Evaluation of central corneal thickness and thinnest pachymetry
- Corneal topography
- OCT examination

Pre-requisites

- Clear cornea.
- Contact lens tolerance or good spectacle corrected visual acuity.
- Disorders should be progressive in nature.
- Thinnest corneal pachymetry higher than 400 µm.
- No central corneal scarring.
- Maximum corneal curvature should not exceed 60 D.

Machine-Technical details

Fig. 6: Parts of machine

- UV-A light is harmful to normal eye. It is necessary to use specified goggle (shown in below Figure 7), while treating the patient.

Fig. 7: Goggle

Rear panel description

Fig. 8: Rear panel

- The input power supply (110/230V AC) is given through the power chord.
- The power switch is meant to turn ON and turn OFF the power supply to the device.
- Three Pin Footswitch connector used to connect the footswitch.
- Aiming Beam Power control Knob.
Head housing (optical) assembly

- Important section of machine, from where UV-A light is projected to the eye.
- Adjust two aiming beams (Red LED) to become one point (focus point) from the Head housing assembly using footswitch and it should be placed 65mm distance from the patient’s eye (cornea).

Solar meter

- Used to measure UV-A light component. The output is expressed in terms of Mw/cm².

Footswitch

- Allows the user to move pillar up/downs.

Surgical procedure

CXL with removal of epithelium (epithelium off)
The treatment is performed in an operation theatre under sterile conditions. Removal of the epithelium is required for efficient penetration of riboflavin. Procedure is done under topical anesthesia after cleaning and draping of the eye. Scraping of the corneal epithelium out to 7 mm is required for better diffusion of riboflavin. Ultrasound pachymetry should be performed at the thinnest point of the deepithelialized cornea, to ensure a minimal corneal thickness of 400 m. Riboflavin solution, 0.1% in 20% dextran is instilled every 3 min for 30 min to saturate the cornea with riboflavin and for corneal hydration.

Prior to treatment, calibration of the intended irradiance of 3 mW/cm² surface irradiance (5.4 J/cm² surface dose) is done using a UVA meter at a working distance of 6 cm. Irradiance is performed.

After the treatment, bandage contact lens is placed after a drop of topical antibiotics is instilled. Instillation of topical antibiotics four times daily until contact lens removal is advised to the patient. The contact lens is removed on the third day and the patients are instructed to instil topical steroids four times daily.
times daily followed by a tapering schedule over 2 months.

CXL without removal of epithelium (Epithelium on or transepithelial cross linking)
The corneal epithelium is left intact, which requires a longer riboflavin loading time. It is likely less painful and would be ideal if keractasia is efficiently stabilised. Several substances have been used to increase the permeability of riboflavin by loosening the tight junctions of the epithelial layer. Various clinical studies have been conducted to evaluate the clinical effects of transepithelial CXL on keratoconic eyes pre-treated with substances enhancing epithelial permeability. Enhanced riboflavin permeability with trometamol, BAC, EDTA, and gentamicin have been tried. The results of clinical observations varied from “less effective than standard CXL” to “moderately effective” to “appearing to halt keratoconus progression, with a statistically significant improvement in visual and topographic parameters.” Alcohol 20% has also been applied to devitalize corneal epithelium. Samaras et al. in their study concluded that complete removal of the corneal epithelium appears to be necessary to allow sufficient riboflavin absorption into the stroma to alter the normal light transmission properties of the porcine cornea.

Complications after CXL

1. Infectious disease
Kymionis et al. published a case report which showed that CXL can induce herpetic keratitis with iritis even in patients with no history of herpetic disease. Australian authors reported a case of polymicrobial keratitis caused by Streptococcus salivarius, Streptococcus oralis and coagulase-negative Staphylococcus sp. in a patient.

2. Corneal haze
Greenstein et al. conducted a study to determine the natural history of CXL associated corneal haze measured by Scheimpflug imagery (densitometry) and slit-lamp bio microscopy in patients with keratoconus or iatrogenic keractasia. Changes in haze did not correlate with postoperative clinical outcomes.
Herrmann et al described a temporary subepithelial haze in a patient who underwent CXL for keratoconus and treated with topical steroids. The haze disappeared several months after the procedure.

3. Corneal scarring
On retrospective evaluation of haze development after riboflavin-ultraviolet (UV) A-induced corneal collagen cross-linking (CXL), 8.6% of the eyes developed significant stromal scarring at 1 year after CXL. Based on these results, advanced keratoconus should be considered at higher risk of corneal scarring after CXL due to low corneal thickness and high corneal curvature.

4. Cell density loss
Kymionis et al reported the outcomes after CXL in patients with thin corneas(<400 m). In 1 year of follow up time, they detected a significant decrease in endothelial cell density from 2733 ± 180 cells/mm² to 2441 ± 400 cells/mm².

5. Corneal melt
Gokhale and Vemuganti reported a case of acute corneal melt with perforation in a patient with keratoconus after uncontrolled use of topical diclofenac and proparacaine eyedrops who had corneal melting following CXL.

Combination of CXL with refractive surgeries
Collagen CXL stops, slow, or, in many cases, partially reverses the ectatic process in eyes with keratoconus and ectasia after LASIK, as measured by visual acuity and corneal curvature. Corneal curvature irregularity must be significantly reduced and regularized. Some of the means to regularize the ectatic cornea in combination with CXL are described below.

1. CXL combined with intracorneal rings
A prospective comparative randomized consecutive study was conducted in Dunya Eye Hospital, Istanbul, Turkey. It compared 2 sequences of combined intrastromal corneal ring segment (ICRS) implantation and ultraviolet/riboflavin-mediated corneal collagen crosslinking (CXL) in progressive keratoconus. They concluded that Implantation of ICRS followed by CXL resulted in greater improvement of keratoconus. Miranda et al. reported a reduction of spherical equivalent and max K of more than 2.50 D and 6.00 D, respectively, using Ferrara rings.

2. CXL combined with topoguided photorefractive keratectomy.
Kymionis et al. conducted a prospective study to present the results after simultaneous photorefractive keratectomy (PRK) followed by corneal collagen cross-linking (CXL) for progressive keratoconus and showed favorable results. PRK followed by CXL seems to be a promising treatment capable of offering functional vision in patients with keratoconus.

4. CXL combined with conductive keratoplasty
Kymionis et al. evaluated the combined effect of conductive keratoplasty (CK) followed by corneal collagen cross-linking (CXL) in 2 patients with keratoconus and showed that corneal remodeling with CK in patients with keratoconus seems to have a temporary effect despite the subsequent application of CXL.

Conclusion
Corneal CXL halts the progression of corneal ectasia, with very low incidence of complications or side effects. It is an efficacious procedure in the treatment of keratoconus and iatrogenic ectasia. CXL has shown to reduce the corneal curvature, spherical equivalent refraction and refractive cylinder in eyes with corneal instability and progressive irregular astigmatism seen in patients with keratoconus and ectasia. This has been used as an adjuvant treatment to conventional therapeutic modalities in infectious keratitis, corneal melting processes and bullous keratopathy. A sequential or simultaneous combination of limited topography-guided PRK and CXL seems to be a promising treatment capable of offering functional vision in patients with keratoconus.

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