Editorial

Keratoconus & cross linking

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Keratoconus is a progressive, asymmetric, noninflammatory disease of the cornea characterized by steepening, distortion, and apical thinning. Protrusion and asymmetric distortion of the cornea induce myopia and astigmatism in regular and irregular forms, leading to marked visual impairment. In most cases, the cornea assumes a conical shape owing to the degeneration of the stromal tissue.

In the early to mid-1990s, scientists worked on the identification of biological glues and their activation by heat or light to affect an increase the resistance of stromal collagen. This work perhaps marked the beginning of the search for therapeutic targeting of the underlying pathogenic mechanisms of keratoconus. The gluing effect was found to be mediated by oxidative mechanisms associated with hydroxyl radical release. A similar mechanism was also seen to be involved in the active glycosylation of age-dependent tropocollagen, which was demonstrated in aging corneas. Alongside Riboflavin and UVA light exposure has been shown to produce similar effects in further studies. This knowledge followed by the developed the collagen crosslinking treatment (CXL) which demonstrated a clinical success. Corneal rigidity increased by approximately 70% following CXL treatment with riboflavin and UVA compared with those that did not receive treatment.

Dresden protocol of CXL treatment includes: Instillation of proparacaine hydrochloride 0.5% drops for topical anesthesia and subsequent deepithelialization of the central 9mm of the cornea. After deepithelialization, a mixture of 0.1% riboflavin in 20% dextran solution was instilled to the cornea for 30 minutes (2 drops every 2 minutes) before irradiation, until the stroma was completely penetrated and aqueous was stained yellow. After that, an 8.0-mm diameter of central cornea was irradiated for 30 minutes by UV-A light with a wavelength of 370 nm and an irradiance of 3mW/cm^2. Instillation of riboflavin drops (1 drop every 2 minutes) was continued during irradiation, as well, to sustain the necessary concentration of the riboflavin. Moreover, balanced salt solution was applied every 6 minutes to moisten the cornea. Recently, in an attempt to reduce patient treatment time, accelerated CXL protocols using higher fluencies and shorter exposure times have been postulated. The envisaged safe and effective use of accelerated CXL is based on the Bunsen-Roscoe law of reciprocity which predicts that the same subthreshold total cytotoxic corneal endothelial UVA dosage can be administered by increasing UVA fluency while simultaneously reducing exposure time. But in all alternatives protocols the demarcation lines of cross linked tissue was shallower that the Dresden protocol. Only accelerated protocols that increase the total energy about 40% presented similar treatment depth as the standard protocol. Moreover, these protocols lack data from animal experiments regarding its toxicity and biomechanical efficiency. Beyond these, the future of the CXL seems to be the personalized treatment like customized pachymetric-guided epithelial removal or to add riboflavin within a corneal pocket created by a femtosecond laser or in intracorneal ring channels. In order to expand the clinical effectiveness of CXL treatment and to make the step from standard to the alternative future is essential to understand the science behind the process. In the next paragraphs there is an attempt to explain the fundamental theory of CXL treatment.

The role of Riboflavin (Vitamin B2) is the precursor of the coenzymes, Flavin adenine dinucleotide (FAD) and Flavin mononucleotide (FMN) and as such required for a variety of flavoprotein enzyme reactions including activation of other vitamins. Is a yellowish compound *that absorbs light and acts as* efficient photosensitizers. Because the UV light causes an effect only where it is absorbed, it is desirable that the treatment of CXL to be designed so that as much as possible of the irradiation is absorbed in the corneal stroma tissue. Riboflavin has peaks in its absorption spectrum at 270, 366, and 445nm. The effects on tissues of UV light at these frequencies vary: at 270nm there high absorption in DNA, and tissue damage photo-conjunctivitis, and photo-keratitis. At 445nm there is potential for photo-chemical damage to the retina–blue light hazard. At 366nm there is absorption by pigmented tissues, but relative high transmission of DNA, so this frequency is the optimal one for cross-linking the cornea. Riboflavin-catalyzed photosensitization, photo-oxidation, and photo polymerization classically involve the production of singlet oxygen, which then reacts with available groups nearby in the corneal stroma. These reactions may involve tyrosine residues, histidine residues, advanced glycation end products or changes in secondary or tertiary structure. Beyond its crosslinker role, Riboflavin also protects substance by shielding the deeper ocular structures (eg, endothelium, lens, and retina) from UVA.

The effect of 30min corneal presoaked Applied riboflavin must diffuse into the cornea stroma, and this process requires a certain time. The aqueous humor without riboflavin does not have any relevant absorption at 370 nm, but clinically it starts to stain after 5 minutes of surface exposure to riboflavin. Thirty minutes after riboflavin application onto the deepithelialized cornea, the concentration of riboflavin exceeds 0.04% at any level up to 400 µm deep. Moreover an absorption coefficient of 0.7 cm has been measured, corresponding to a concentration of 0.002% riboflavin in the aqueous humor. Lambert-Beer law yields a reduction of the irradiance by a factor of 5.5, considering an anterior chamber depth of 3 mm and the measured absorption coefficient of 0.7 cm.

The role of dextran

The deturgescent agent dextran intends to give an iso-osmolar solution according to the cornea. The osmolality of corneal stroma is 380-420 mosmol/l, and the standard riboflavin/dextran solution is 400 mosmol/l, so application to the de-epithelialised surface theoretically does not lead to swelling of the cornea. The role of intensity and time of UV-A light. The damage mechanism from the UV light depends on its wavelength, it's intensity, and the irradiation time. Photokeratitis has been shown to occur in the cornea at wavelengths of 270 to 315 nm (UVB). Cataract development is related to various dose values for the wavelengths between 290 and 365nm.The retina is damaged by thermal or blue light in the wavelength range of 400-1400 nm. Under these circumstances, a balance between the cell damage, the biologic protective mechanisms, and the biologic repair mechanisms is achieved. In rabbits, the threshold radiant exposure for damage has been shown to be 70 J/cm^2 for the lens and 42 J/cm^2 for the cornea and the threshold irradiance for retinal damage was 4.3 mW/cm^2. Using the standard irradiance of 3 mW/cm^2 and a minimal stromal thickness of 400µm, the lens, the endothelium and the retina is completely safe because their cytotoxicity threshold is not reached.

The limitation of 400µm corneal pachymetry. In rabbit corneas 24 hours after standard CXL treatment, keratocyte apoptosis was found in 300µm deep. Smaller irradiances led to shallower cell depth following Lambert-Beer law. In cell cultures established from porcine keratocytes, the damage threshold of the irradiance of UVA in combination with 0.025% riboflavin solution was determined to be 0.45 mW/cm^2 which is 10 times lower than UVA alone. According to Lambert-Beer law this irradiance is achieved after passing through ~300 µm of stroma saturated with riboflavin. In agreement with these experimental data, confocal microscopy of CXL corneas revealed cell death of keratocytes 270-350 µm deep in the stroma but 6 months after treatment, a repopulation of the whole stroma with a normal keratocyte density had taken place. Epithelium Debate. There is a debate concerning epithelium removal prior the CxL treatment. The removal of the epithelium is essential for the homogeneous diffusion of riboflavin into the corneal stroma. Without epithelial removal the biomechanical effect is less than 50% of the standard crosslinking procedure.

Riboflavin or Rose Bengal; UV-A, Green light or near-infrared femtosecond laser; UV absorption or two photon absorption; half an hour, ten seconds or even two; with or without epithelium; Science of CXL traditional treatment is the base of a vary armamentarium against ectatic corneas.

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Conflicts of interest

The author declares there are no conflicts of interest.