Editorial

Reflections on the Controversies on Collagen Corneal Cross-linking for Arresting Keratoconus

Since the first publication on collagen corneal cross-linking (CXL) for arresting the progression of keratoconus (KC) by the Dresden group,[1] several hundreds of publications have been published, most of them reporting positive results of CXL in arresting the progression of the disease, improvement of uncorrected visual acuity (UCVA) and best spectacle corrected visual acuity, reduction of astigmatism and of keratometry (K)max. In April 2016, the treatment was approved by the Food and Drug Administration after a controlled randomized study. The beneficial effect of the treatment was reported even after 10 years.[2]

The Conventional Protocol

As reported by the Dresden group, the conventional protocol (C-CXL) implies the removal of the central 8 to 9 mm of the epithelium (epi-off), instillation of riboflavin 0.1% with dextran 20% every 5 minutes for half an hour until a strong yellow flare is seen by the slit lamp in the anterior chamber. Then the cornea is irradiated by ultraviolet A (UVA) of 370 nm, 3 mW/cm^2 for half an hour at a distance of 5 cm from the cornea; during the treatment balanced salt solution is instilled every 2 minutes and riboflavin every 5 minutes.[1] After the completion of the treatment, a therapeutic contact lens (CL) is inserted and removed after full reepithelialization, which takes between 3 and 4 days; antibiotic drops are instilled till full epithelialization and lens removal, then steroid eye drops are instilled for 1 month. The effects of CXL are limited to the anterior 300 µm of corneal stroma, the riboflavin acts as a photosensitizer and photoprotector, it is highly reactive with oxygen species, triggering formation of cross-links that consist of intra- and interfibrillary covalent bonds.[3] A minimal corneal thickness of 400 µm is recommended in order to avoid endothelial cell damage.

Since the first steps C-CXL, it has been challenged by alternative treatment protocols:

Epithelium on Protocol

The epithelium is not removed, the aim is to avoid the pain and eye irritation after C-CXL and the complications related to epithelial removal, such as keratitis, delayed epithelialization, corneal infiltrates, perforation, anterior uveitis, and transient corneal scarring.[4-6] The results reported in the literature regarding the effectiveness of epithelium on protocol (epi-on) CXL are controversial, and even when effectiveness is reported, the depth of CXL and the refractive changes as expressed by the reduction of astigmatism and Kmax are inferior to what is achieved by epi-off CXL.[7-12] The decreased effect is due to the absorption of UVA by the epithelium, the inability of the riboflavin to penetrate the tight junctions of the epithelium although various forms of riboflavin and absorption enhancers were used. The effect in terms of stress and strain measurements after the treatment in porcine eyes was fifth of what is achieved with epi-off, 64% increase in corneal rigidity *vs* a 320% in CXL with epi-off.[13] Nonhomogeneous uptake of riboflavin in the stroma was observed in partial grid-pattern epithelial removal.[14] Epi-on CXL is useful in cases in which epithelial removal is not desired, such as pediatric cases, uncooperative patients, and patients with thin corneas.[15]

Iontophoresis Corneal Cross-linking

Iontophoresis CXL (I-CXL) is used to enhance the absorption of riboflavin; the technique is efficient in halting the progression of KC, reduces the treatment time, and improves the riboflavin diffusion.[16,17] Similar stiffness of corneas treated by I-CXL or C-CXL in rabbits was reported.[18]

Accelerated Corneal Cross-linking

Several new CXL devices offer high UVA irradiation intensity with different time settings that exist in the market; the aim is to reduce the treatment time by reducing the exposure of the cornea to 10 minutes instead of 30 minutes and even to 5 and 3 minutes to maintain a total dose of 5.4 J/cm^2. According to Bunsen-Roscoe's law of reciprocity, the effect of photochemical or photobiological reaction is directly proportional to the total irradiation dose, irrespective of the time span over which the dose is administered. Does CXL work in this way on the cornea? This is partially true because the *ex vivo* results in porcine corneas show that the Bunsen-Roscoe reciprocity law is only valid for

illumination intensities up to 40 to 50 mW/cm^2 and illumination times of more than 2 minutes.[19] No significant difference between the rapid 9 minutes at 10 mW/cm^2 = 5.4 J/cm^2 and standard 30 minutes at 3 mW/cm^2 = 5.4 J/cm^2 groups was observed in the median of Young's modulus.[20] Decreased stiffening effect with increasing UVA intensity was reported[21] clinically similar results to C-CXL were reported,[22] accelerated CXL (A-CXL) is effective in stopping the progression of KC.[23-25] Effectiveness was reported in A-CXL even with epi-on.[26] On the contrary, a decreased depth of the demarcation line (DL) after using the A-CXL protocol with 10 minutes of irradiation time and 9 mW/cm^2 suggests a reduced effectiveness.[27] The DL is an optical line that separates the treated from the untreated stroma and is supposed to reflect the depth of treatment.[28] Even with higher UV dose (6.6 J/cm) A-CXL showed a smaller topographic flattening effect than did the C-CXL.[29]

Accelerated Pulsed Corneal Cross-linking

Oxygen is depleted early in the A-CXL, and oxygen is necessary for the process of cross-linking;[30] pulsed protocols with A-CXL delivering UV light with an on–off pattern could allow a better diffusion of the oxygen into the corneal stroma and subsequent deeper effect.[31] The DL was significantly deeper using pulsed rather than continuous light exposure,[32] and deeper apoptotic effect was noted with pulsed A-CXL.[33] Advanced oxidation protein product levels indicative of oxygen concentration and reactive oxygen species were higher in accelerated pulsed CXL compared with C-CXL and A-CXL in rabbits' eyes.[34] Lower levels of nitric oxide, indicative of oxidative stress, were in aqueous humor of rabbits' eyes by pulsed accelerated CXL compared with A-CXL and C-CXL.[35] Do these last two researches in rabbits' eye indicate more efficacy and safety of accelerated pulsed CXL? Can we apply this to human KC? Clinically improved postoperative functional outcomes compared with continuous light treatment were reported and with deeper stromal penetration.[33] Deeper DL was reported in accelerated pulsed CXL *vs* A-CXL using energy of 30 mW/cm^2 and energy dose of 7.2 J/cm^2.[32]

Cretan Protocol

The Cretan Protocol is an epi-off CXL; the epithelium is removed by excimer laser, 50 µm of "epithelium" is removed by the laser. The refractive results were reported to be superior to the Dresden protocol. The explanation of this beneficial effect of the laser ablation of the epithelium is that the epithelium is thinner at the KC apex and by this few microns of the stroma will be removed, more astigmatism reduction is obtained, and there is more improvement in visual acuity.[36] Another research reports no statistically significant difference at 12 and 24 months postoperatively in the Createan protocol group compared with C-CXL, the Kmax at 6 months was flatter in the first group, but this effect did not last.[37]

Customized CXL with up to 10 J/cm^2 centered on the maximum of the posterior float was reported and compared with C-CXL. Epithelial healing time, ΔKmax, and regularization index were significantly better in the customized CXL group; the other examined parameters were similar in the two groups. Energies up to 15 J/cm^2 were used according to the topographic corneal curvature, with three levels of energy, no difference between the customized CXL, and the C-CXL was noted, although the DL was deeper in the areas of the cornea radiated by higher energies.[38]

Why do we have Different Results in different Clinical and Basic Science Researches?

- KC is not the same in different patients and even in the same patient it is asymmetric, in shape, anterior and posterior elevation of the cone, thickness of the cornea, the distribution of the thickness in the cornea, the degree of corneal irregularity, and the rate of progression.
- Eye rubbing may cause or aggravate KC;[39,40] certainly, this will affect progression pre- and postoperatively.
- Different UVA energies and different beam profiles are used. Deeper CXL centrally than peripherally has been observed; this is probably due to the top-hat beam profile of UVA optical system that does not compensate for natural corneal curvature, so the UVA beam enters the cornea at a nonorthogonal angle in the corneal periphery.[41] There are no published papers that demonstrate the superiority of enhanced peripheral beam profile over the C-CXL, it is worth to mention that the change in the beam profile was accompanied with increased beam energy to 9 mW/cm^2 (IROC, Zurich, Switzerland).
- Different riboflavin solutions are used, with and without dextran, hypertonic, isotonic, and hypotonic, with different riboflavin concentrations of 0.1 to 0.25%, different ways of riboflavin instillation, with or without lid speculum, the patient in sitting or in supine position, different intervals and durations of the instillation of

riboflavin, is the corneal surface washed from riboflavin before starting the UVA irradiation? Riboflavin instilled during treatment and when and how much? In case of epi-on CXL what are the enhancers used? Benzalkonium chloride? Gentamicin? Tetracaine? Etc,, is alcohol added before in order to loosen the tight junctions of the epithelium? Is some mechanical disruption of the epithelium added by sponge or by the Daya disruptor? (A metallic device for performing multiple holes in the epithelium).

- How much CXL does each KC patient need? We still do not know. In some cases a less effective treatment may be sufficient to halt the progression, and this may explain the effectiveness of epi-on CXL.
- The photochemically induced effect of CXL in the cornea cannot be evidenced directly by staining methods or microscopic techniques. However, CXL induces several changes to collagen-containing tissue, from which indirect signs of the cross-linking effect can be deduced.[42]
- Most of the published studies are not randomized controlled studies (RCS), only four RCS were reported. In the published researches differences exist in measuring and reporting the outcomes.[43]
- In *ex vivo* experiments, we have most of the earlier mentioned variables, added to them are the age of the cornea, the hydration of the cornea, the time from death to the experiment, is whole eye used or only cornea? How the cornea is cut for the stress–strain measurements before and after the treatment? What device is used to measure the stress and strain, what formulas are used to compensate for the nonlinear distension of the cornea during stress–strain measurements?
- In the *in vivo* studies what are the animals used for the experiments? Rats, rabbits, or pigs?
  Can we apply the conclusions of these animal studies to KC eyes in humans?
- There is no consensus on the definition of KC progression; this may affect the indications for CXL and the evaluation of the posttreatment results. According to Global Consensus on Keratoconus and Ectatic Diseases,[44] progression is a consistent change in at least two of the following parameters: Steepening of the anterior corneal surface, steepening of the posterior corneal surface, and thinning and/or changes in the pachymetric rate of changes. Here are some of the criteria considered as a sign of progression: Increase in Kmax of 1 to 1.5 D or more and deterioration in UCVA of more than 0.2 log MAR,[43] patient's self-report of deteriorating visual acuity, need for new CL fitting more than once in 2 years, increase of ≥1.00 D in manifest refraction's astigmatism or of ≥0.50 D in manifest refraction spherical equivalent, worsening of uncorrected distance visual acuity/corrected distance visual acuity >0.50 Snellen lines, increase of topographic symmetry index surface asymmetry index/surface irregularity >1.00 D, reduction of the thinnest point at corneal optical coherence tomography pachymetry ≥10 µm, or ≥5% decrease in the thinnest pachymetry in the preceding 6 months and reduction in corneal thickness (thinnest point) >10 µm lasting more than 6 months.[46]

CONCLUSION

The above-mentioned tens of variables exist in every single CXL treatment and certainly affects the results of the treatment. The different parameters used to define progression and the different devices used to measure them and noninterchangeability of these devices make the indication and the evaluation of CXL results extremely variable. The best strategy of treatment, i.e., able to obtain the best clinical efficacy together with the maximum safety profile is still to be defined. There is a need for reliable and accurate techniques for measuring corneal biomechanical properties before and after treatment. Designed prospective randomized controlled trials comparing traditional CXL and all the alternative procedures are required.

P.S

The *International Journal of Keratoconus & Ectatic Corneal Diseases* as a stage for KC researchers and clinicians has dedicated in this issue five articles on CXL: "Assessing Progression of Keratoconus and Cross-linking Efficacy: The Belin ABCD Progression Display" to determine the quantitative values and to assess their suitability as progression determinants by Micheal Belin et al; "WST-11 as an Alternative to Riboflavin for Corneal Collagen Crosslinking for the Treatment of Keratoconus" by Sajjad Abbas et al; "Technology of the Local Cross-linking (Part 1): Keratotensotopography and Vacuum-compression, Topographic Test—New Diagnostic Possibilities for Studying the Local Biomechanical Properties of Cornea" by Sergei Anisimov et al; "Two Years' Experience in Keratoconus Treatment using Collagen Cross-linking" by Dimitrii Dementiev et al"; "Reevaluating the Effectiveness of Corneal Collagen Crosslinking and Its True Biomechanical Effect in Human Eyes" by Damien Gatinel. The last article doubts the efficacy and the utility of CXL for arresting KC.

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