The rationale behind photodynamic collagen crosslinking therapy (CXL) as a means of strengthening keratoconic corneas is based on the observations of Andreassan et al,1 who demonstrated that the biomechanical strength of the cornea is abnormally low in keratoconus patients. Andreassan suggested that the lack of biomechanical strength may play a role in the progression of the disease. Furthermore, it has been shown that keratoconus rarely occurs in instances where corneal stiffening is increased by enhanced collagen crosslinking, such as in the elderly2 and patients with diabetes.3-5

The biomechanical basis of increased corneal strength is said to be the formation of covalent crosslinks that occur when the photosensitizer, riboflavin, is applied to the de-epithelialized surface of the cornea and subsequently exposed to UV-A light. Free radicals created by the excitation of riboflavin at its absorption peak of 370 nm are thought to interact with amino acids in neighboring collagen molecules to form strong chemical bonds. Although the precise nature of these crosslinks is not yet known, it has been suggested that they take the form of covalent dityrosine bonds.6

The observed increase in fibril diameter in rabbit corneas following riboflavin/UV-A treatment7 has been put forward as evidence that CXL induces crosslinking; it is supposed to be the result of collagen molecules being pushed farther apart by the newly formed crosslinks,7 a phenomenon that also occurs in glycation-induced collagen crosslinking8 and age-related crosslinking.9 Yet, to the best of our knowledge, there is still no direct evidence that covalent, UV-A-induced crosslinks are the basis of enhanced biomechanical strength in the cornea after CXL treatment; further work is therefore necessary.

ARE CROSSLINKS CONFINED TO COLLAGEN?

The question of whether crosslinks are confined to collagen or occur nonspecifically in tissue has also not yet been addressed. In theory, collagen fibril surfaces are too widely separated to allow direct interfibrillar linkages; however, corneal collagen closely associates with other bridging collagens and proteoglycan molecules, and there is possible scope for indirect interfibrillar crosslinking via these molecules. Such crosslinking may be expected to influence the swelling behavior of the cornea.

Although it has been shown that crosslinked corneas swell less than normal,10 this work was carried out on the anterior cornea, which does not swell much anyway.11 Furthermore, the solution of riboflavin and dextran—which we have shown to reduce sheep corneal thickness by up to 17% during 35-minute exposure—was applied only to the UV-A—treated corneas and not to their controls. As a consequence, it is unclear from this study whether the observed resistance to stromal swelling in CXL-treated corneas is due to presence of dextran within the tissue, which persists for up to 24 hours,12 or indirect interfibrillar crosslinking.

Clinical studies have shown that CXL effectively halts the progression of keratoconus and, in some cases, results in slight regression.13-14 To date, only one in vivo human study of corneal rigidity following CXL has been reported.15 In this study, corneal elasticity in 12 eyes was determined using a contact ultrasonic device, revealing a decrease in corneal elasticity after riboflavin/UV-A crosslinking.

Corneal hysteresis is an alternative biomechanical measurement. It is purported that corneal hysteresis, as measured by the Ocular Response Analyzer (Reichert Ophthalmic Instruments, Inc., Depew, New York) is an indicator of corneal viscoelasticity.16 This machine allows simple in vivo measurement of corneal biomechanics in the clinic. To date, there have been few and contradictory studies concerning the question of whether CXL increases corneal hysteresis.17,18

TAKE-HOME MESSAGE

- The biomechanical strength of the cornea is abnormally low in keratoconus patients, which may play a role in the progression of the disease.
- Crosslinking may halt the progression of keratoconus, however, the lack of demonstration that CXL stiffens the cornea is a cause for concern.
- Long-term follow-up is needed to determine the durability of the strengthening effect of CXL and the potential for late-stage complications.
Preliminary studies conducted at our laboratory showed an increase in ovine corneal hysteresis immediately after CXL (Samuel Heinz, Felix Barker, and Rachel North, personal communication, February 2009), but interpretation of these results in terms of corneal stiffening must be made with caution until the precise relationship between corneal hysteresis and elasticity is resolved. Moreover, it must be acknowledged that biochemical measurements may be confounded by changes in parameters, such as corneal thickness and hydration, which occur during crosslinking. The lack of a clear in vivo demonstration that the cornea is stiffened by the CXL procedure remains a cause for concern.

Despite the lack of in vivo studies, quantitative biomechanical stress-strain studies have been performed to examine the effect of CXL on the strength and stability of the cornea. Spoerl et al. obtained stress-strain measurements from 160 riboflavin/UV-A crosslinked pig corneas using a microcontrolled material tester. This study provided evidence of significant tissue stiffening following treatment.

The same technique was used to show that CXL increased corneal rigidity by approximately 320% in humans, 85% in rabbits, and 70% in pigs. It also increased Young’s modulus of elasticity by factors of 4.5, 1.6, and 1.8 in humans, rabbits, and pigs, respectively. It has been suggested that differences in corneal rigidity in pigs because crosslinking is the most likely explanation for the lower posttreatment corneal rigidity in pigs20 as compared with that of the human cornea (850 vs 550 mm Hg).

The greater thickness of the porcine cornea compared with that of the human cornea (850 vs 550 mm) is the most likely explanation for the lower posttreatment increase in corneal rigidity in pigs20 because crosslinking is limited to the anterior 200 to 300 mm of the cornea23 and therefore involves only 35% of the total thickness of the pig cornea as opposed to 54% in the human cornea.20

The depth-dependent strengthening effect of riboflavin/UV-A crosslinking, which is greatest in the anterior 200 mm of the stroma, correlates closely with the intensity of the UV-A light source24 and with riboflavin corneal diffusivity,21 which in turn is dependent upon the presence or absence of the epithelium.22 The optimum technique parameters for maximal crosslinking and minimal tissue damage have been identified by Spoerl et al.22

Clinical studies with 3 to 5 years’ follow-up after riboflavin/UV-A therapy have demonstrated the usefulness of this treatment in halting keratoconus progression.24 In vivo studies of rabbit corneas have provided evidence that the stiffening effect is maintained over 8 months;22 however, the exact rate of connective tissue turnover in the keratoconic corneal stroma is not yet known. Repeat treatments may be necessary to prevent recurrence of disease progression. Time will tell of the long-term durability of the strengthening effect and the potential for late-stage complications incurred as a result of increasing corneal rigidity.

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