CARDIFF UNIVERSITY
PRIFYSGOL CAERDYDD

ORCA – Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository:https://orca.cardiff.ac.uk/id/eprint/44020/

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Hayes, Sally, O'Brart, David P., Lamdin, Letitia S., Doutch, James, Samaras, Kostas, Marshall, John and Meek, Keith Michael Andrew 2008. Effect of complete epithelial debridement before riboflavin–ultraviolet-A corneal collagen crosslinking therapy. Journal of Cataract and Refractive Surgery 34 (4) , pp. 657-661. 10.1016/j.jcrs.2008.02.002

Publishers page: http://dx.doi.org/10.1016/j.jcrs.2008.02.002

Please note:
Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See http://orca.cf.ac.uk/policies.html for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.

Effect of complete epithelial debridement before riboflavin–ultraviolet-A corneal collagen crosslinking therapy

Sally Hayes, PhD, David P. O'Brart, MD, Letitia S. Lamdin, James Doutch, BSc, Kostas Samaras, MD, John Marshall, PhD, Keith M. Meek, PhD

PURPOSE: To evaluate the importance of complete epithelial removal before riboflavin–ultraviolet-A (UVA) corneal collagen crosslinking therapy.

SETTING: School of Optometry and Vision Sciences, Cardiff University, Wales, United Kingdom.

METHODS: Riboflavin eyedrops were applied at 5-minute intervals for 35 minutes to the anterior corneal surface of 36 porcine eyes (12 with no epithelial trauma but treated with tetracaine eyedrops, 12 with superficial epithelial trauma but with an intact basal epithelium, and 12 with a fully removed epithelium). The corneal surface of 6 tetracaine-treated eyes, 6 eyes with superficial epithelial trauma, and 6 eyes with a fully removed epithelium was exposed to UVA light for 30 minutes during riboflavin administration. The light transmission spectra of the enucleated corneas were analyzed with a spectrophotometer and compared with those of 9 untreated porcine corneas.

RESULTS: Corneas with a fully removed epithelium treated with riboflavin showed an abnormal dip in the transmission spectrum between 400 nm and 510 nm ($P<.01$). This was attributed to the presence of riboflavin in the corneal stroma. The spectra of riboflavin-treated corneas with no epithelial trauma but tetracaine administration and those with superficial epithelial trauma did not differ from those of the non–riboflavin-treated controls. Exposure to UVA following riboflavin administration did not alter corneal light transmission.

CONCLUSIONS: Complete removal of the corneal epithelium is an essential component of riboflavin–UVA crosslinking therapy as superficial epithelial trauma and tetracaine administration alone are not sufficient to permit the penetration of riboflavin into the corneal stroma. Failure to achieve adequate stromal absorption of riboflavin may impair the efficacy of the crosslinking process.

J Cataract Refract Surg 2008; 34:657–661 © 2008 ASCRS and ESCRS

Keratoconus is a common noninflammatory, degenerative disorder of the cornea, characterized by stromal thinning and conical ectasia that results in irregular astigmatism and associated visual loss.[1] In most keratoconic patients, vision can be restored through the use of hard contact lenses. In cases of advanced disease, corneal transplantation may be required because of inadequate contact lens fitting, contact lens intolerance, or corneal scarring. Keratoconus remains one of the most common indications for corneal transplantation.[2–5]

Riboflavin (vitamin B_2)/ultraviolet-A (UVA) (370 nm) corneal collagen crosslinking is a new therapeutic modality that may be the first treatment available to stabilize the keratoconic process.[6] It is thought to increase the biomechanical stability of the corneal stroma[7,8] and the resistance to enzymatic digestion[9] by inducing crosslinkage between the stromal collagen molecules. The riboflavin has the dual function of acting as a photosensitizer for the production of oxygen-free radicals, which induce physical crosslinking of collagen,[10] and absorbing the UVA irradiation and preventing damage to deeper ocular structures such as the corneal endothelium, the lens, and the retina.[11,12] The technique has been shown to be safe, with no loss of corneal transparency, no endothelial damage (provided the cornea is thicker than 400 μm), and no damage to deeper ocular structures.[11,13,14]

The riboflavin–UVA corneal collagen crosslinking method described by Wollensak et al.[13] states that the epithelium should be removed before treatment to allow penetration of riboflavin into the corneal stroma. Despite this recommendation, some clinicians perform the technique with the epithelium intact to

© 2008 ASCRS and ESCRS
Published by Elsevier Inc.

0886-3350/08/$—see front matter

doi:10.1016/j.jcrs.2008.02.002

reduce the patient's postoperative discomfort.[15] They advocate the use of multiple applications of the topical anesthetic agent tetracaine 1% before the procedure to loosen the epithelial tight junctions and allow riboflavin to penetrate the corneal stroma.[15] To evaluate the importance of epithelial removal in facilitating the entry of riboflavin into the corneal stroma, we measured the light transmission spectra of porcine corneas after the administration of riboflavin eyedrops following complete epithelial debridement, superficial epithelial trauma, or no epithelial trauma with the preoperative and perioperative administration of topical tetracaine 1%. These measurements were compared with those in untreated control corneas and the absorption spectrum of the riboflavin solution itself. We also examined the effects of the crosslinking treatment on light transmission by exposing several corneas to UVA irradiation in combination with riboflavin eyedrops.

MATERIALS AND METHODS

Ninety porcine eyes were transported on ice from a local abattoir within 24 hours of death. Visual examination of each specimen for the presence of corneal scarring or opacity resulted in the exclusion of 5 eyes. The remaining 85 eyes were stored overnight in a sealed bag at 4°C. Of these, 45 eyes were selected at random for inclusion in the study and divided into the following treatment groups:

1. Controls: The corneal epithelium was removed completely from 5 eyes using a scalpel blade; the epithelium was left intact in 4 additional corneas.
2. Riboflavin only (superficial epithelial trauma, basal epithelium intact): Following scraping of the superficial epithelium for 10 to 15 seconds with a scalpel blade and visual inspection to ensure the basal layers were intact, riboflavin drops (10 mg riboflavin-5-phosphate in 10 mL dextran T-500 20%) were applied to the anterior corneal surface of 6 eyes at 5-minute intervals for 30 minutes.
3. Riboflavin plus UVA (superficial epithelial trauma, basal epithelium intact): Following superficial epithelial trauma, riboflavin drops were applied to the anterior corneal surface of 6 eyes. After 5 minutes, the corneas were exposed to a 3 mW/cm^2 dose of UVA (370 nm) 1 cm from the anterior surface of the cornea. The crosslinking treatment lasted 30 minutes, during which time riboflavin drops were applied at 5-minute intervals.
4. Tetracaine plus riboflavin (no epithelial trauma): Topical tetracaine 1% and riboflavin eyedrops were administered to the intact, nontraumatized anterior corneal surface of 6 eyes at 5-minute intervals over a 35 minute period to simulate 5-minute preoperative and 30-minute operative time periods.
5. Tetracaine and riboflavin plus UVA (no epithelial trauma): Tetracaine 1% and riboflavin eyedrops were applied to the anterior corneal surface of 6 eyes with an intact, nontraumatized epithelium. After 5 minutes, the corneas were exposed to a 3 mW/cm^2 dose of UVA (370 nm) 1 cm from the anterior surface of the cornea. The crosslinking treatment lasted 30 minutes, during which time further riboflavin and tetracaine drops were applied at 5-minute intervals.
6. Riboflavin only (epithelium completely removed): After a central 10.0 mm area of the corneal epithelium was completed debrided with a scalpel blade, riboflavin drops were applied to 6 eyes at 5-minute intervals for 30 minutes.
7. Riboflavin plus UVA (epithelium completely removed): After the central corneal epithelium was removed completely, riboflavin drops were applied to the anterior corneal surface of 6 eyes. After 5 minutes, the corneas were exposed to a 3 mW/cm^2 dose of UVA (370 nm) 1 cm from the anterior surface of the cornea. The crosslinking treatment lasted 30 minutes, during which time riboflavin drops were applied at 5-minute intervals.

Immediately after the treatment, each cornea with a 3.0 mm scleral rim was dissected from the globe and placed in a specially designed sample holder. The natural curvature of the cornea was maintained by clamping the scleral rim in the sample holder and injecting silicone oil (Dow Corning 200/5cS, BDH Laboratory Supplies) into the chamber behind it. Silicone oil was also injected into the front chamber of the holder to maintain a uniform refractive index and reduce light scatter.[16] The sample holder was then positioned into the spectrophotometer (PYE Unicam, SP8-100 UV/VIS) so light passed through the center of the cornea in the anterior–posterior direction. A transmission spectrum was measured for each cornea at 10 nm intervals in the range of 400 to 700 nm. Using the method detailed by Kostyuk et al.,[16] the transmission spectrum for each sample was normalized against a baseline transmission spectrum of the chamber filled with silicone oil. A further transmission spectrum over the same wavelength range (400 to 700 nm) was obtained for the riboflavin solution alone.

RESULTS

Removing the epithelium had no significant affect on the transmission spectra of the control corneas. In each case, a gradual increase in light transmission occurred between 400 and 700 nm. Based on this finding, the spectra of all control corneas (with or without

Accepted for publication November 28, 2007.

From the School of Optometry and Vision Sciences (Hayes, Lamdin, Doutch, Meek), Cardiff University, Cardiff; and the Department of Ophthalmology (O'Brart, Samaras, Marshall), and Kings College London, The Rayne Institute (O'Brart, Marshall), St. Thomas' Hospital, London, United Kingdom.

No author has a financial or proprietary interest in any material or method mentioned.

Supported by a grant from the Medical Research Council, grant number G0600755.

Madhavan S. Rajan, MD, FRCOphth, FRCS, made significant contributions to the concept and design of this investigation.

Corresponding author: Keith Meek, PhD, School of Optometry and Vision Sciences, Cardiff University, Maindy Road, Cathays, Cardiff CF24 4LU, United Kingdom. E-mail: meekkm@cf.ac.uk.

epithelia) were averaged for comparison with riboflavin-only and riboflavin-plus-UVA–treated corneas. Figure 1 shows the mean transmission spectra of control corneas, corneas treated with riboflavin only, and corneas treated with riboflavin plus UVA. The standard error bars associated with each spectrum are the result of variations in the hydration of corneas within the treatment groups.

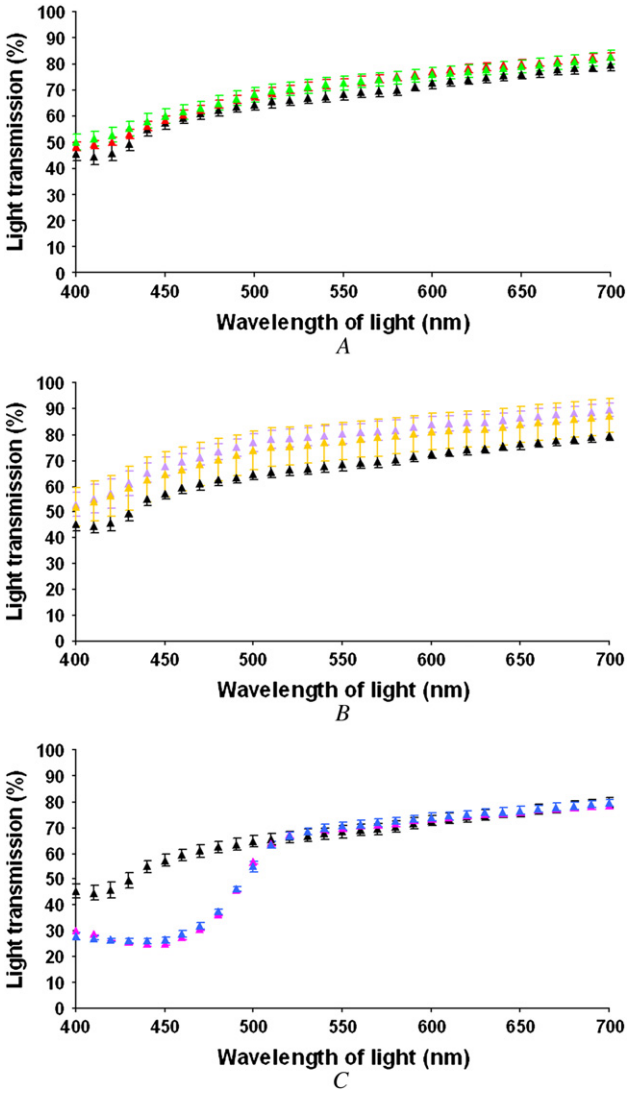

Figure 1. *A*: Mean light transmission spectra of 9 untreated porcine corneas (black triangle), 6 riboflavin-only–treated corneas with superficial epithelial trauma (red triangle), and 6 riboflavin-plus-UVA–treated corneas with superficial epithelial trauma (green triangle); S.E. bars are shown. *B*: Mean light transmission spectra of 9 untreated porcine corneas (black triangle), 6 riboflavin-and-tetracaine–treated corneas with an intact epithelium (purple triangle), and 6 riboflavin-and-tetracaine-plus-UVA–treated corneas with an intact epithelium (yellow triangle); S.E. bars are shown. *C*: Mean light transmission spectra of 9 untreated porcine corneas (black triangle), 6 riboflavin-only–treated corneas with epithelium completely removed (pink triangle) and 6 riboflavin-plus-UVA–treated corneas with epithelium completely removed (blue triangle); S.E. bars are shown.

The transmission spectra of corneas with superficial epithelial trauma (but with the basal epithelium intact) treated with riboflavin only or with riboflavin and UVA did not differ from each other or from those of the control corneas (Figure 1, *A*). Similarly, the transmission spectra of corneas with no epithelial trauma treated with riboflavin and tetracaine alone or with riboflavin, tetracaine, and UVA did not differ from each other or from that of the control corneas (Figure 1, *B*). Complete removal of the epithelium before riboflavin-only or riboflavin-plus-UVA treatment resulted in a dramatic reduction in light transmission between 400 nm and 510 nm ($P < .01$) (Figure 1, *C*). At 450 nm, light transmission was a mean of 32% lower in riboflavin-treated corneas that had had complete epithelial removal than in the untreated control corneas. This dip in light transmission can be attributed to the presence of riboflavin within the tissue, which absorbs light between 400 nm and 510 nm (Figure 2). The light transmission spectra of corneas with a fully removed epithelium treated with riboflavin plus UVA did not differ from the spectra of corneas treated with riboflavin alone (Figure 1, *C*).

DISCUSSION

Riboflavin–UVA corneal collagen crosslinking is the first therapeutic modality that may halt the progression of the ectatic process in keratoconus and post-keratorefractive surgery ectasia.[6] Riboflavin is a key component of the photochemical crosslinking treatment as it increases corneal absorption of UVA to approximately 95%[8] and thereby protects the deeper ocular structures, especially the endothelium, from UVA damage.[11,12]

Thus far, clinical and laboratory studies of corneal collagen crosslinking have advocated the complete removal of the epithelium to allow penetration of riboflavin into the corneal stroma.[9,12–14,16–18] However, to reduce the early postoperative discomfort experienced

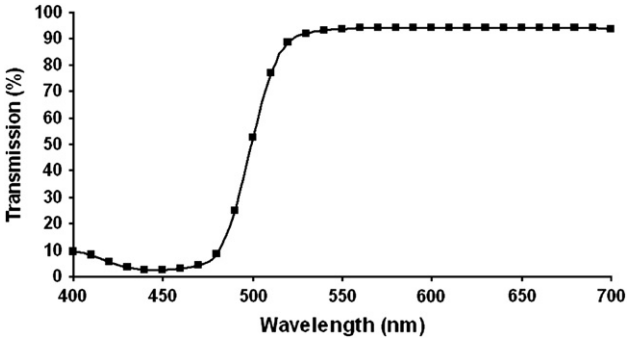

Figure 2. Light transmission spectrum of riboflavin solution.

by the patient (as a result of epithelial removal), some clinicians have performed the procedure with the epithelium intact.[15] They postulate that topical anesthetic drops can loosen epithelial tight junctions, allowing penetration of riboflavin into the corneal stroma. In this study, we used spectrophotometry to investigate the importance of epithelial removal by assessing the ability of riboflavin to penetrate the stroma of corneas treated with topical anesthetic eyedrops (tetracaine 1%), of those with superficial epithelial trauma (in which the basal epithelium remains intact), and of those with a fully removed epithelium.

We have shown that in the immediate postoperative period, the light transmission spectra of fully deepithelialized porcine corneas treated with riboflavin eyedrops are altered by the presence of riboflavin within the stroma and subsequent exposure of the cornea to UVA light does not produce further changes in the transmission spectra. As riboflavin is an essential vitamin and naturally occurring compound that decomposes in the presence of light at wavelengths below 500 nm, the acute changes in light transmission due to riboflavin absorption will be short-lived; in the clinical setting, the yellow discoloration of the cornea due to riboflavin, which is clearly visible following the treatment, is clear 24 hours later (D. O'Brart, personal observation).[13]

The normality of the transmission spectra of corneas that had superficial epithelial trauma but an intact basal epithelium clearly suggests the need to remove all epithelial cell layers before treatment to permit stromal penetration of riboflavin. In our study, the concomitant use of tetracaine 1%, even if given preoperatively and intraoperatively, did not appear to allow stromal penetration of riboflavin through an intact corneal epithelium. The presence of an intact basal epithelial layer appears to act as an effective barrier to riboflavin absorption by the corneal stroma, which is not sufficiently altered by superficial epithelial trauma or tetracaine eyedrops. Such considerations are very important as failure to achieve adequate stromal absorption of riboflavin is likely to severely limit the crosslinking process.[11,14] It is of note, however, that although inadequate stromal absorption of riboflavin will undoubtedly result in increased UV irradiance of the endothelium, lens, and retina, at energy levels of 3 mW/cm^2 toxic levels are unlikely to be reached.[11,14,15]

Our results do not agree with the clinical results of Chan et al.[15] and Pinelli (R. Pinelli, MD, "Corneal Collagen Cross-Linking with Riboflavin (C3-R) Treatment Opens New Frontiers for Keratoconus and Corneal Ectasia," EyeWorld, May 2007; pages 34–36. Available at: http://www.eyeworld.org/article.php?sid=3797. Accessed January 12, 2008), who performed the crosslinking procedure with the epithelium intact and advocated the use of tetracaine 1% to loosen the epithelial tight junctions. Chan et al. reported a possible additive effect in reduction in refractive cylinder, steep and mean keratometry values, and lower–upper keratometry ratios, with corneal collagen crosslinking following inferior Intacs insertion compared with inferior Intacs insertion alone in keratoconic eyes. However, their results must be interpreted relative to the nonrandomized, retrospective nature of their study and that keratoconus is a very heterogeneous condition in which accurate and repeatable refractive, keratometric, and topographic measurements may be difficult to obtain. In addition, the crosslinking treatment of their patients was performed immediately after insertion of the intrastromal ring segment and it may be that epithelial trauma associated with the procedure (both mechanical and due to exposure) together with the deep stromal incision required for ring segment insertion may have facilitated corneal stromal riboflavin absorption. Pinelli documented similar outcomes following riboflavin–UVA crosslinking treatment with and without epithelial removal. Similar to Chan et al., he reported fluorescence in the anterior chamber, which was attributed to riboflavin penetration, although direct measurements were not made. While such findings are of interest, we do not believe they give direct evidence that riboflavin can be significantly absorbed into the corneal stroma without removal of all layers of the corneal epithelium before topical administration and they are not supported by the spectrophotometric measurements in this study.

Analysis of the light transmission spectra of porcine corneas following riboflavin–UVA corneal crosslinking treatment and control corneas suggests the epithelium must be completely removed to allow adequate penetration of riboflavin into the stroma.

REFERENCES

1. Krachmer JH, Feder RS, Belin MW. Keratoconus and related noninflammatory corneal thinning disorders. Surv Ophthalmol 1984; 28:293–322
2. Javadi MA, Motlagh BF, Jafarinasab MR, et al. Outcomes of penetrating keratoplasty in keratoconus. Cornea 2005; 24:941–946
3. Reeves SW, Stinnett S, Adelman RA, Afshari NA. Risk factors for progression to penetrating keratoplasty in patients with keratoconus. Am J Ophthalmol 2005; 140:607–611
4. Mamalis N, Anderson CW, Kreisler KR, et al. Changing trends in the indications for penetrating keratoplasty. Arch Ophthalmol 1992; 110:1409–1411
5. Al-Yousuf N, Mavrikakis I, Mavrikakis E, Daya SM. Penetrating keratoplasty: indications over a 10 year period. Br J Ophthalmol 2004; 88:998–1001
6. Wollensak G. Crosslinking treatment of progressive keratoconus: new hope. Curr Opin Ophthalmol 2006; 17:356–360

7. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine cornea after riboflavin/ultraviolet-A-induced crosslinking. J Cataract Refract Surg 2003; 29:1780–1785
8. Spörl E, Schreiber J, Hellmund K, et al. Untersuchungen zur Verfestigung der Hornhaut am Kaninchen. [Cross-linking effects in the cornea of rabbits]. Ophthalmologe 2000; 97:203–206
9. Spoerl E, Wollensak G, Seiler T. Increased resistance of cross-linked cornea against enzymatic digestion. Curr Eye Res 2004; 29:35–40
10. Andley U. Photooxidative stress. In: Albert DM, Jakobiec FA, eds, Principles and Practice of Ophthalmology. Philadelphia, PA, Saunders, 1994; 575–590
11. Wollensak G, Spoerl E, Wilsch M, Seiler T. Endothelial cell damage after riboflavin-ultraviolet-A treatment in the rabbit. J Cataract Refract Surg 2003; 29:1786–1790
12. Spoerl E, Wollensak G, Dittert D-D, Seiler T. Thermomechanical behaviour of collagen cross-linked porcine cornea. Ophthalmologica 2004; 218:136–140
13. Wollensak G, Spörl E, Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003; 135:620–627
14. Spoerl E, Mrochen M, Sliney D, et al. Safety of UVA-riboflavin cross-linking of the cornea. Cornea 2007; 26:385–389
15. Chan CCK, Sharma M, Boxer Wachler BS. Effect of inferior-segment Intacs with and without C3-R on keratoconus. J Cataract Refract Surg 2007; 33:75–80
16. Kostyuk O, Navolina O, Mubard TM, et al. Transparency of the bovine corneal stroma at physiological hydration and its dependence on concentration of ambient ion. J Physiol 2002; 543:633–642. Available at: http://jp.physoc.org/cgi/reprint/543/2/633. Accessed January 12, 2008
17. Wollensak G, Wilsch M, Spoerl E, Seiler T. Collagen fiber diameter in the rabbit cornea after collagen-crosslinking by riboflavin/UVA. Cornea 2004; 23:503–507
18. Kohlhass M, Spoerl E, Schilde T. Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. J Cataract Refract Surg 2006; 32:279–283

First author:
Sally Hayes, PhD

School of Optometry and Vision Sciences, Cardiff University, Cardiff, United Kingdom