

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

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**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.

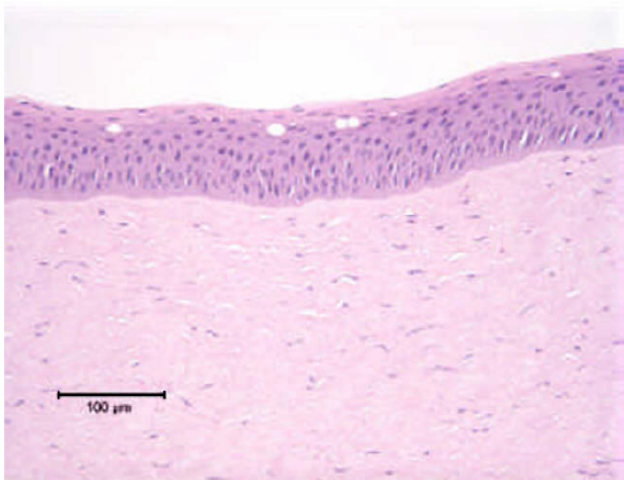
**CONCLUSIONS:** Complete removal of the corneal epithelium is an essential component of riboflavin-UVA crosslinking therapy as superficial epithelial trauma and tetracaine administration alone are not sufficient to permit the penetration of riboflavin into the corneal stroma.

This study was determined to be flawed. Please read the letter to the editor disputing its conclusions on the next two pages to follow:

## Effect of epithelial debridement in corneal collagen crosslinking therapy in porcine and human eyes

In their recent article,<sup>1</sup> Hayes et al. concluded that their results in cadaveric porcine eyes differed from the results in live human corneas<sup>2</sup> and generalized that “[c]omplete removal of the corneal epithelium is an essential component of riboflavin–ultraviolet-A (UVA) crosslinking.” We believe that this conclusion is unfounded for several reasons:

1. The epithelial thickness of porcine and human corneas is different. The epithelial thickness of a porcine cornea is approximately 100  $\mu\text{m}$  (Figure 1), which is twice as thick as that of humans.<sup>3,4</sup> The thicker porcine epithelium represents a greater barrier for riboflavin penetration than the human epithelium. The results of Hayes et al. cannot be generalized to human corneas.
2. The riboflavin protocol in the study was different from the procedure in humans. Riboflavin was applied at 5-minute intervals for 35 minutes (7 drops); however, the protocol recommended by Wollensak et al.<sup>5</sup> and in subsequent human studies, including our protocol,<sup>2</sup> riboflavin was applied every 2 to 3 minutes for 30 minutes (10 to 15 drops). The study by Hayes et al. equates to 30% to 50% less riboflavin applied over the time period.
3. The study evaluated the wrong wavelengths. The study evaluated light transmission spectra of riboflavin in corneas between 400 nm and 700 nm. Human collagen crosslinking has been shown to work at 370 nm UVA. In Figure 1, C, of the article, it appears that extrapolating these 2 lines (to the left of



**Figure 1.** Freshly excised porcine cornea showing epithelium with scale bar<sup>3</sup> measuring approximately 100  $\mu\text{m}$ , which is twice the thickness of the human epithelium. Reprinted with permission of Taylor & Francis Ltd (<http://www.tandf.co.uk/journals>).<sup>3</sup>

the graph) for the controls and epi-off porcine corneas would likely show them converging at 370 nm. This would suggest there is no difference between controls and epi-off at 370 nm.

4. The study measured riboflavin outcomes indirectly while disregarding direct clinical observation of riboflavin penetration into the anterior chambers of human corneas. Light transmission spectrum was used as an indirect marker for the presence of stromal absorption of riboflavin. (A dip in the transmission is assumed to have resulted from the presence of riboflavin absorption in the stroma. This premise has not been proven in human corneas as there is no human correlate cited by the authors.) Hayes et al. commented on Chan et al.'s<sup>2</sup> paper that “we do not believe they give direct evidence that riboflavin can be significantly absorbed into the corneal stroma,” even though Chan et al. described direct visualization of riboflavin fluorescence in the anterior chamber after corneal application. This is direct evidence that riboflavin penetrated the cornea when the epithelium was pretreated with tetracaine, which was also applied every 10 minutes during the C3-R procedure.

Another study corroborated Chan et al.'s results that riboflavin penetrates the epithelium in humans with the use of tetracaine. In a prospective study by Pinelli, fluorescence of riboflavin was found in the anterior chamber with epi-on, which is evidence of penetration (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 August 1, 2008). Pinelli showed that at 6 to 9 months postoperatively, the clinical benefits in human corneas are the same for uncorrected visual acuity, best spectacle-corrected visual acuity, mean keratometry, mean spherical equivalent, root-mean-square error, pachymetry, and endothelial cell count with epi-on and epi-off methods. Patient discomfort and satisfaction questionnaires were better with the epi-on technique.

5. The study did not describe any statistical tests used. There was no statistical methodology in Hayes et al.'s paper. The authors simply reported a *P* value in “Results,” which is insufficient and brings into question the results of the study.
6. Was there observer bias? O’Brart personally observed that a “yellow discoloration of the cornea due to riboflavin” was visible after the procedure. However, he made an epi-off observation in only 1 cornea (“the cornea”) as described in the article; no observations were made in epi-on corneas. It seems misleading to the reader, with an inherent observer bias.

In conclusion, the study is methodically flawed and misleading by extrapolating porcine results to human corneas. The authors conclude that epi-on crosslinking does not occur in porcine eyes and therefore will not work in humans. This is like placing deceased pigs in a tanning booth and concluding that since they did not tan, tanning booths will not work for humans. Studies of proven human efficacy of epithelium-on collagen crosslinking provide clinical evidence of crosslinking.

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