Author Response: The Role of Riboflavin Concentration and Oxygen in the Efficacy and Depth of Corneal Crosslinking

We would like to thank Dr. Lin for his very interesting comments[1] on our paper.[2] We agree that our article and Lin's published mathematical modeling of riboflavin/ultraviolet (UVA) light corneal cross-linking (CXL) efficacy[3–5] highlight the importance of the type I anaerobic process in CXL, the dose-curve of stromal riboflavin concentration, and the significance of riboflavin photo-degradation during UVA exposure and its role in the efficacy of CXL.

All our experimental treatments were conducted using an accelerated (ACXL) technique (9 mW/cm^2 for 10 minutes) with a total energy dose 5.4 J/cm^2 and without oxygen supplementation or pulsing. The fact that we could repeatedly show improved CXL efficacy with increasing riboflavin concentrations supports the likely dominance of the type I photochemical (anaerobic) pathway in riboflavin/UVA CXL, and adds weight to the suggestion by Kamaev et al.[6] that the aerobic type II process serves only a transient and perhaps initiating role. Based on our and Lin's work, we believe that with a manipulation and maintenance of high stromal riboflavin concentrations during CXL, it might be possible to achieve the same efficacy in ACXL as that seen with the standard Dresden protocol (SCXL), without the need for supplemental oxygen, pulsing, or extension of the UVA dosage.

Lin sates in his letter that in his numerical calculations, the type II pathway follows the Bunsen-Roscoe reciprocal law and is proportional to the UV dose,[5] while the type I process does not. We believe that the photo-degradation of riboflavin during UVA exposure, which leads to reduced stromal riboflavin concentrations and consequently a reduction in the type I process, is likely responsible for this lack of adherence to the Bunsen-Roscoe reciprocal law; this highlights the potentially very important role for riboflavin supplementation during UVA exposure or the need for the use of greater riboflavin stromal concentrations, to maintain sufficient stromal riboflavin to drive and optimize the type I reaction.

The rate of photo-degradation of riboflavin during CXL with varying UVA intensities is yet undetermined. Kamaev et al.[6] suggested a rate of 1×10^{-2} to 2×10^{-2} min^{-1} with 3 mW/cm^2 exposure, which if correct, would result in approximately 1% of the riboflavin being degraded every 30 seconds of irradiation; this equates to 60% photolysis at 30 minutes if riboflavin is not replenished. The rate of photo-degradation is anticipated to be much higher with increased UVA fluences, with Kamaev et al.[6] postulating that a 30 mW/cm^2 fluence would result in approximately 8% of the riboflavin being degraded every 30 seconds of UVA exposure. In the SCXL during UVA irradiation of 3 mW/cm^2 for 30 minutes, riboflavin drops are typically administered every 2 to 5 minutes to replenish the stromal riboflavin. However, during ACXL procedures, riboflavin replenishment is often not undertaken or is done so at a relatively reduced frequency. It can be easily postulated that photo-degradation during high intensity UVA exposure, without supplemental riboflavin application, will significantly reduce riboflavin stromal concentrations, especially in the superficial stroma, and thereby limit the efficacy of the procedure. However, whilst we agree with Lin that the addition of supplemental riboflavin during UVA exposure should improve the efficacy of ACXL, we believe that the use of supplemental drops during short exposure procedures may be problematic due to the masking effect of the riboflavin meniscus at the stromal surface.[7] Hence, based on our own work and the modeling of Lin, we postulate that the use of higher concentrations of riboflavin (up to 0.4%) would be more effective in improving the efficacy of ACXL without any need for pulsing, supplemental oxygen, or increased UVA dosing. Indeed, we argue that it appears that the current 0.1% riboflavin formulations are suboptimal in terms of efficacy and urgent laboratory clinical studies are necessary to evaluate higher riboflavin dosages, with their subsequent implementation into clinical practice. This is particularly pertinent given recent publications showing that up to 24% of pediatric patients treated with the Dresden protocol (which is considered the gold standard treatment), show evidence of progression of ectasia over a 10-year follow-up.[8] In addition, as we discussed in our paper, the use of higher concentration riboflavin formulations has the potential not only to improve CXL efficacy but also safety. We predict that the use of higher riboflavin stromal concentrations that result in increased UVA absorption within the anterior stroma and theoretically reduce the amount of UVA radiation at the endothelium may allow for the treatment of thinner corneas. Indeed, from Lin's own modeling, it appears that with riboflavin formulations of 0.3%, corneas with thicknesses of less than 300 μm might safely undergo CXL.[9]

We note from Lin's modeling that when the riboflavin concentration increases from 0.1% to 0.2%, type I photochemical CXL efficiency is expected to increase by a factor of 1.43, while the depth of CXL is reduced by 1.48. Whilst it would be expected that increased absorption of UVA within the anterior stroma by higher riboflavin concentrations might result in an increased but more superficial CXL effect, our results that show an increase in the dry weight of undigested corneal tissue mass with increasing dosage but no difference in the overall disc diameter[2] suggest that this is not the case. We postulate that if the riboflavin is not replenished, photo-degradation would lead to a gradual reduction of stromal riboflavin concentration in the most superficial layers of the stroma and thereby allow more UVA to reach the mid stroma where there is still a high riboflavin concentration, thus allowing deeper and perhaps more optimal CXL. Further modeling and investigations are required to elucidate this in greater detail, but higher riboflavin formulations may not only be more efficacious and safer but reduce the need for riboflavin supplementation during ACXL.

Finally, Lin discusses the lack of efficacy of epithelium-on CXL. We agree, that reports of reduced efficacy compared to SCXL are related to limited riboflavin absorption through the intact corneal epithelium and subsequent low stromal riboflavin concentrations, coupled with an inability to easily replenish stromal riboflavin through the intact epithelium, and have demonstrated this using two-photon fluorescence.[10] Using iontophoresis, we have achieved higher stromal concentrations of up to 60% to 80% of that achieved with SCXL.[11] We believe that with higher concentration trans-epithelial iontophoretic riboflavin formulations of 0.6% to 0.8% we can achieve stromal concentrations of 0.3% to 0.4%, which may lead to an optimized epithelium-on ACXL approach that is more efficacious than the current SCXL protocol.

Finally, we thank Lin for the interest in our paper and for the comparison of his theoretical modeling with our laboratory measurements of residual stromal mass following enzymatic digestion. The close agreement between the theoretical and experimental findings further validates our methodology as a

iovs.arvojournals.org | ISSN: 1552-5783

Investigative Ophthalmology & Visual Science

relatively simple, reproducible, and what appears to be accurate way of assessing CXL efficacy in vitro.

David P. S. O'Brart[1]
Naomi A. L. O'Brart[2]
Nada H. Aldahlawi[3]
Sally Hayes[3]
Keith M. Meek[3]

[1]Keratoconus Research Institute, Department of Ophthalmology, St. Thomas' Hospital, London, United Kingdom; and the [2]Moorfields Eye Hospital, City Road, London, United Kingdom; [3]Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Maindy Road, Cardiff, Wales.
E-mail: davidobrart@aol.com

References

1. Lin JT. The role of riboflavin concentration and oxygen in the efficacy and depth of corneal crosslinking. *Invest Ophthalmol Vis Sci*. 2018;59:4449–4450.
2. O'Brart NAL, O'Brart DPS, Aldahlawi NH, Hayes S, Meek KM. An investigation of the effects of riboflavin concentration on the efficacy of corneal cross-linking using an enzymatic resistance model in porcine corneas. *Invest Ophthalmol Vis Sci*. 2018;59:1058–1065.
3. Lin JT. A proposed concentration-controlled new protocol for optimal corneal crosslinking efficacy in the anterior stroma. *Invest Ophthalmol Vis Sci*. 2018;59:431–432.
4. Lin JT, Cheng DC. Modeling the efficacy profiles of UV-light activated corneal collagen crosslinking. *PLoS One*. 2017;12: e0175002.
5. Lin JT. Efficacy S-formula and kinetics of oxygen-mediated (type-II) and non-oxygen-mediated (type-I) corneal cross-linking. *Ophthalmol Res*. 2018;8:1–11.
6. Kamaev P, Friedman MD, Sherr E, Muller D. Cornea photochemical kinetics of corneal cross-linking with riboflavin. *Invest Ophthalmol Vis Sci*. 2012;53:2360–2367.
7. Wollensak G, Aurich H, Wirbelauer C, Sel S. Significance of the riboflavin film in corneal collagen crosslinking. *Cataract Refract Surg*. 2010;36:114–120.
8. Mazzotta C, Traversi C, Baiocchi S, et al. Corneal collagen cross-linking with riboflavin and ultraviolet a light for pediatric keratoconus: ten-year results. *Cornea*. 2018;37:560–566.
9. Jui-Teng L. Efficacy and z* formula for minimum corneal thickness in ultraviolet light cross-linking. *Cornea*. 2017;36: 30–31.
10. Gore DM, O'Brart D, Dunsby C, French P, Allan BD. Transepithelial riboflavin absorption in an ex-vivo rabbit corneal model. *Invest Ophthamol Vis Sci*. 2015;56:5006–5011.
11. Gore DM, O'Brart D, Dunsby C, French P, Allan BD. A comparison of different corneal iontophoresis protocols for promoting transepithelial riboflavin penetration. *Invest Ophthalmol Vis Sci*. 2015;56:7908–7914.

Citation: *Invest Ophthalmol Vis Sci.* 2018;59:4451–4452.
https://doi.org/10.1167/iovs.18-24795

Investigative Ophthalmology & Visual Science