Original Article

Photophysical and photodynamic analysis of different formulations of riboflavin

Matias Osaba [1,2], Tomas Cristian Tempesti [3] and Victor Eduardo Reviglio [1,2]

[1] *Instituto de la Vision Cerro de las Rosas, Sanatorio Allende - Sede Cerro, Cordoba, Argentina*
[2] *Facultad de Ciencias de la Salud, Universidad Catolica de Cordoba, Cordoba, Argentina*
[3] *INFICQ (CONICET), Departamento de Quimica Organica, Facultad de Ciencias Quimicas, Universidad Nacional de Cordoba, Cordoba, Argentina*

ABSTRACT

Background: Riboflavin (Rb) has been used in the ophthalmological procedure known as corneal cross-linking (CXL). Pathologies requiring this treatment include keratoconus, corneal ectasia, and infectious keratitis. Rb is instilled via different molecules that are transported into the tissues. However, each vehicle imparts different properties that alter the photodynamic behavior of Rb, leading to variable concentrations of free radicals within the medium. The objective of this study was to measure the concentrations of free radicals produced by commonly used Rb formulations. To determine the free radical production level of each formulation, L-tryptophan (L-Tryp) was used as a model substrate because it can be efficiently photo-oxidized.

Methods: We investigated the photodegradation of L-Tryp and its kinetics upon light exposure. The spectra were recorded using a Shimadzu UV-1800 PC spectrophotometer and a Cary Eclipse fluorescence spectrophotometer. A high-power solid-state LED light source was used for irradiation. L-Tryp degradation was performed using a 9-W LED lamp, and steady-state photolysis was conducted in quartz cells. The observed rate constants for L-Tryp degradation were determined by analyzing the changes in absorbance and fluorescence intensity. Data analysis was performed using Origin software.

Results: We examined the characteristics of the photophysical and photodynamic action of the carriers in different commercially available Rb formulations. These included a) Rb with dextran, b) Rb without dextran, c) VibeX Rapid® (hydroxypropylmethylcellulose as a vehicle), d) Trans-Epithelial Kit (I) (sodium chloride as a vehicle), and e) Trans-Epithelial Kit (II) (benzalkonium chloride as a vehicle), using L-Tryp as a model substrate, and focusing on absorption and emission spectra. VibeX Rapid® exhibited the highest photo-degradation constant. The study affirmed the stability of Rb formulations for CXL and highlighted the efficacy of VibeX Rapid® in L-Tryp photo-oxidation and this rationalizes its current use as a CXL agent.

Conclusions: We demonstrated that formulations for transport of Rb are of crucial importance in CXL applications. Rb in the VibeX Rapid® formulation is more effective in generating photo-degradation, and this reflects its superior performance in CXL. Future experiments should be designed and conducted to quantitatively differentiate the production of free radicals. Studies involving human participants could shed light on the clinical efficacy and safety of the available Rb formulations.

KEYWORDS

drug carrier, corneal cross linking, epi off CXL, epi on CXL, vitamin B2, riboflavin, L- tryptophan, ultraviolet spectrophotometry, fluorescence spectrophotometry

Correspondence: Matias Osaba, Instituto de la Vision Cerro de las Rosas, Sanatorio Allende - Sede Cerro, Cordoba, Argentina. Email: doctorosaba@gmail.com. ORCID iD: https://orcid.org/0000-0002-2146-2402

How to cite this article: Osaba M, Tempesti TC, Reviglio VE. Photophysical and photodynamic analysis of different formulations of riboflavin. Med Hypothesis Discov Innov Optom. 2023 Winter; 4(4): 181-187. DOI: https://doi.org/10.51329/mehdioptometry189

Received: 29 August 2023; Accepted: 29 November 2023

INTRODUCTION

Riboflavin (Rb) is a photosensitive drug used to treat keratoconus, infectious keratitis, and corneal ectasias through a procedure known as corneal cross-linking (CXL) [1]. When Rb is irradiated with ultraviolet A (UVA) light, it excites and produces free radicals that catalyze the formation of additional covalent bonds between corneal collagen molecules [2].

Usually, CXL is performed using the Dresden protocol [3]. It consists of instilling one drop of Rb in the eye every 2 min for 30 min and then applying it at the same frequency for 30 min while irradiating the cornea with a UVA lamp. If both eyes must be simultaneously treated (as in cases of bilateral keratitis or keratoconus [4]), the treatment lasts for approximately 2 h. This generates fatigue in the patient and the health care team. The newest Rb formulations provide superior diffusion through the tissue in less time, and the newest UVA equipment can radiate more UVA light in less time, thereby shortening the treatment time to a few minutes [2].

In addition, owing to its physicochemical properties, Rb is a candidate for iontophoresis-assisted transepithelial administration. This is because Rb is a relatively small molecule, negatively charged at physiological pH, and soluble in water [5]. Currently, various Rb formulations are produced in the pharmaceutical industry. All have the same Rb molecule but differ in their distinct carriers. These different vehicle properties result in variable diffusion capacities for Rb in the corneal tissue [6, 7].

In this work, based on the premise that Rb formulations are excited by UV light [8] and facilitate energy transfer by generating reactive oxygen species and singlet oxygen ($^1O_2$), we aimed to measure the amount of free radicals produced by each formulation. Although there are several techniques to determine how much each formulation produces, the use of L-tryptophan (L-Tryp) is noteworthy. This substrate can be efficiently photo-oxidized by both type I and type II reaction mechanisms in the presence of a photosensitizer [9, 10].

We believe that with a better understanding of the behavior of different Rb formulations, the most appropriate one can be selected based on parameters such as lesion severity, required treatment duration, corneal depth, endothelial count, corneal thickness, and identity of microorganisms. According to the reviewed literature, there is currently no research that has analyzed and compared the photophysical and photodynamic behaviors of different Rb formulations available on the market. For this study, we analyzed the following formulations: a) Rb with dextran [11], b) Rb without dextran [11], c) VibeX Rapid® (hydroxypropylmethylcellulose [HPMC] as a vehicle) [12], d) Trans-Epithelial Kit (I) (sodium chloride as a vehicle), and e) Trans-Epithelial Kit (II) (benzalkonium chloride as a vehicle) [13] (Table 1). We analyzed the photophysical and photodynamic actions of the different carriers in commercially available Rb formulations, using L-Tryp as a model substrate.

METHODS

This study was approved by the Ethics Committee of the Instituto de la Vision del Cerro de las Rosas, Sanatorio Allende-Sede Cerro, Cordoba, Argentina.

In this study, absorption spectra were recorded on a Shimadzu UV-1800 PC spectrophotometer (Shimadzu Corporation, Tokyo, Japan) in the 200 – 800 nm wavelength range using 1-cm path length quartz cells. Fluorescence spectra were recorded on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, CA, USA) with a Peltier temperature controller set at 25.0°C [14].

The UV-visible light source used for irradiation was a high-power solid-state LED light source (COLOR X^{NES} LUSTROUS, Green Technology of Lighting, Taiwan) equipped with a 9-W LED lamp. The light intensity at the treatment site was 0.05 mW/cm^2 (SE-9087 Digital Light Meter, Extech Instruments, Nashua, NH, USA) [15].

The photo-oxidation of L-Tryp is used as a substrate model for potential target compounds of biological interest. This substrate can be efficiently photo-oxidized by both type I and type II reaction mechanisms [9, 10, 16]. All

Table 1. Riboflavin formulations

Drug name	Pharmaceutical company	Composition
Riboflavin with dextran	Farmacia Magister, Buenos Aires, Argentina	0.1% riboflavin solution
Riboflavin without dextran	Farmacia Magister, Buenos Aires, Argentina	0.1% riboflavin solution
VibeX Rapid® (hydroxypropylmethylcellulose as a vehicle)	Avedro, Inc., Waltham, MA, USA	0.1% riboflavin solution
Trans-Epithelial Kit (I) (sodium chloride as a vehicle)	Avedro, Inc., Waltham, MA, USA	0.22% riboflavin solution
Trans-Epithelial Kit (II) (benzalkonium chloride as a vehicle)	Avedro, Inc., Waltham, MA, USA	0.25% riboflavin solution

chemicals were obtained from Sigma-Aldrich (Milwaukee, WI, USA) (L-Tryp) and commercial sources (Rb: Generic and Avedro) (VibeX Rapid® Avedro, Inc., Waltham, MA, USA) (Table 1) and were used without further purification. The solvents (GR grade) were obtained from Merck (Darmstadt, Germany) and distilled. Ultrapure water was obtained using Labconco (Kansas City, MO, USA) equipment model 90901-01.

L-Tryp degradation was carried out with a 9-W (400 nm-Δ14 nm) LED lamp, high-power solid-state LED light source COLOR X^{NES} LUSTROUS (Green Technology of Lighting) in a device with a cuvette holder and adjustable distance. Measurements were performed every 60 s during irradiation.

For steady-state photolysis, solutions of L-Tryp (7.35 mM) and the same concentrations of the five Rb formulations were irradiated in 1-cm path length quartz cells (2.5 mL) with light at λ_{max} = 400 nm from the 9-W LED. The light fluence rate was determined as 0.05 mW/cm^2. The kinetics of L-Tryp oxidation were studied by following the decrease in absorbance (A) at λ_{max} = 218 nm and the fluorescence intensity (F) at λ_{max} = 362 nm.

The observed rate constants (k_{obs}), corresponding to L-Tryp degradation in terms of decay, were obtained by a linear least-squares fit of the semi-logarithmic plot of ln A0/A or ln F0/F versus time [16]. The data were processed using OriginPro 9.0 (64-bit) Version 2022 (OriginLab Corporation, Northampton, MA, USA).

RESULTS

The L-Tryp and Rb absorption spectra were measured in Milli-Q water to determine the wavelength at which the L-Tryp decay reading was obtained. Figure 1A illustrates that the band at 218 nm (L-Tryp) was appropriate for following photodegradation, and Figure 1B displays the absorption spectra of the different Rb formulations.

The λ_{max} absorption values are presented in Table 2. The Rb formulations were irradiated at 400 nm, and the UV spectrum was measured every 60 s to follow the decay of the band at 218 nm (Figure 2A). In Figure 2, a significant correlation with decay as a function of time, corresponding to L-Tryp degradation (by both type I and type II mechanisms), was observed. Subsequently, a pseudo-first order kinetic graph (Figure 2B) was

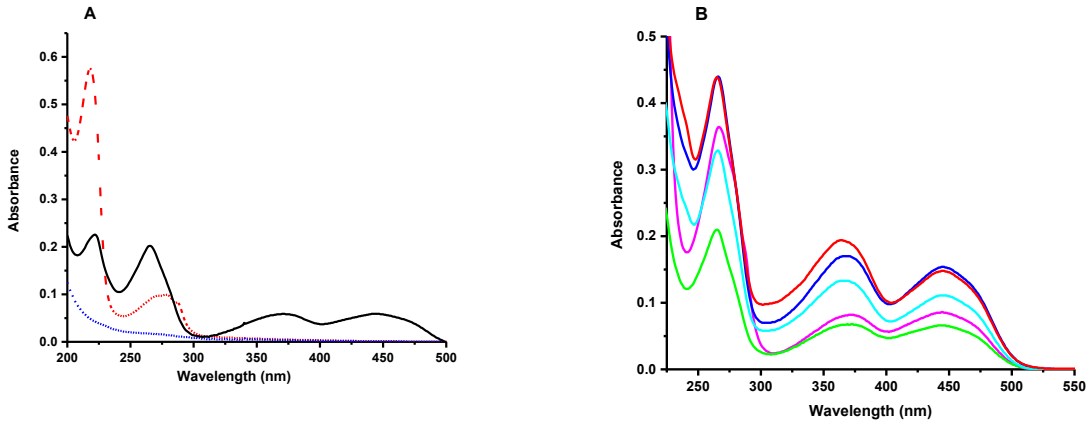

Figure 1. (A) Absorption spectra of Rb without dextran (–), L-Tryp (---), and water (•••). (B) Absorption spectra of different formulations of Rb (Table 1): – VibeX Rapid®, – Trans-Epithelial Kit (I), – Trans-Epithelial Kit (II), – Rb with dextran, and – Rb without dextran. Abbreviations: nm, nanometers; Rb, riboflavin; L-Tryp, L-tryptophan.

Table 2. Spectroscopic data for the Rb formulations tested, along with k_{obs} with L-tryp

Rb formulation	Name	Rb Concentration (%)	Absorption λ_{max} (nm)	Emission λ_{max} (nm)	Kinetic Absorption Measurement k_{obs} ($s^{-1}M^{-1}$)	Kinetic Emission Measurement k_{obs} ($s^{-1}M^{-1}$)
Rb with dextran	Generic	0.1	266	525	3.44×10^{-4}	-
Rb without dextran	Generic	0.1	266 -	525	3.35×10^{-4}	-
Rb/HPMC	VibeX Rapid® (Avedro)	0.1	266	525	8.66×10^{-4}	6.7×10^{-4}
Rb/NaCl	Trans-Epithelial Kit (I) (Avedro)	0.22	266 -	525	7.77×10^{-4}	4.06×10^{-4}
Rb/BAC	Trans-Epithelial Kit (II) (Avedro)	0.25	266	525	6.97×10^{-4}	3.27×10^{-4}

Abbreviations: Rb, riboflavin; nm, nanometers; HPMC, hydroxypropylmethylcellulose; NaCl, sodium chloride; BAC, benzalkonium chloride. Note: λ_{max}, absorption and emission wavelength; k_{obs}, observed rate constants.

constructed to obtain the k_{obs} degradation constants from the slope. The values obtained for each Rb formulation are listed in Table 2.

The degradation of L-Tryp was then evaluated by fluorescence, following the emission band of L-Tryp at 362 nm, where the effect of the different Rb values was observed (Figure 3A). The slopes observed for the different Avedro formulations are presented in Figure 3B, and the corresponding k_{obs} values are presented in the Kinetic Emission Measurement column in Table 2.

Both experiments followed by UV absorption and emission displayed the same trend, while formulations with or without dextran (generic) demonstrated no noticeable changes in their constants. The other three formulations (Avedro) were affected, and VibeX Rapid® had the highest photo-degradation constant (Table 2).

The different Rb formulations used for CXL showed no changes in absorption or emission maxima. Figure 3A presents an example of fluorescence decay (in this case, Vibex Rapid®); the other formulations displayed similar behavior. This confirms that Rb remained unchanged and in its monomeric state throughout the experiment.

The photoprocess followed first-order kinetics with respect to L-Tryp concentration, as shown in Figure 3A for [L-Tryp] = 25 μM. From the plots in Figure 3B, the values of k_{obs} L-Tryp were calculated for VibeX Rapid®, Trans-Epithelial Kit (I), and Trans-Epithelial Kit (II). The results displayed in Table 2 indicate lower k_{obs} L-Tryp values for Trans-Epithelial Kit (II) (3.27×10^{-4} s^{-1} M^{-1}) and Trans-Epithelial Kit (I) (4.06×10^{-4} s^{-1} M^{-1}) in comparison with that observed for VibeX Rapid® (6.7×10^{-4} s^{-1} M^{-1}). These results show that Rb in the VibeX Rapid® formulation is more effective in generating photo-degradation, and this rationalizes its current use as a CXL agent.

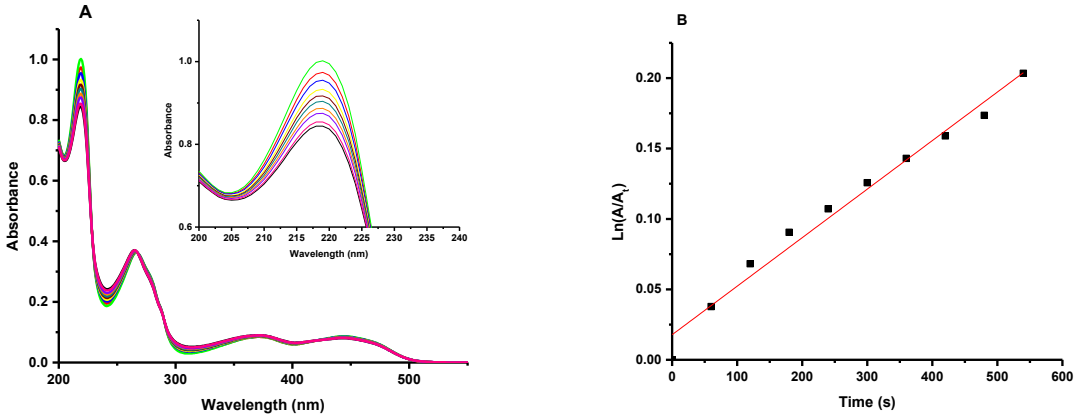

Figure 2. (A) Absorption spectra of Rb (Mg) and L-Tryp, inside decay at 218 nm. The inset image represents a magnification of the 200 – 240 nm region. (B) First-order plots for the photo-oxidation of L-Tryp photosensitized by Rb (Mg) in water. Abbreviations: nm, nanometers; s, seconds; Rb, riboflavin; L-Tryp, L-tryptophan; Mg, Magister.

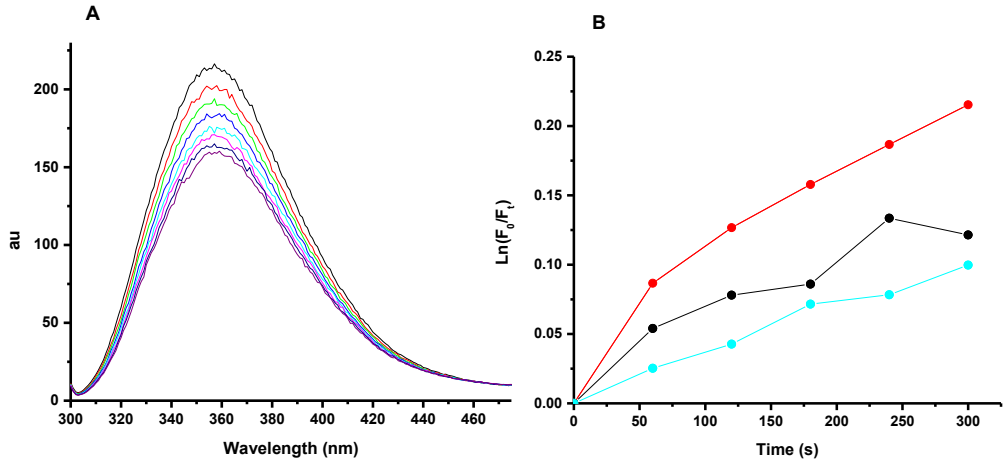

Figure 3. (A) Emission spectra of VibeX Rapid® at 362 nm. (B) First-order plots for the photo-oxidation of L-Tryp photosensitized by (–•–) VibeX Rapid®, (–•–) Trans-Epithelial Kit (I), and (–•–) Trans-Epithelial Kit (II) (Table 1) in water. Abbreviations: nm, nanometers; au, arbitrary units; Ln(F_0/F_t), natural logarithm of (F_0/F_t); s, seconds; Rb, riboflavin; L-Tryp, L-tryptophan.

DISCUSSION

Our research team has long investigated the photochemical behavior of Rb and other substances in relation to UV light from CXL [17]. We analyzed how different Rb formulations reacted to UV irradiation under the same experimental conditions. All Rb formulations reacted with L-Tryp and degraded it in different ways. However, the Rb VibeX Rapid® formulation, containing HPMC, was exceptional [18]. Moreover, Trans-Epithelial Kit (I) and Trans-Epithelial Kit (II) continued in the order of reactivity against the model substrate L-Tryp.

Some authors argue that, unlike Rb with dextran, which causes corneal thinning, Rb combined with HPMC stabilizes intraoperative corneal thickness, preventing damage to the endothelium [19-21]. Another feature of this Rb solution is its rapid diffusion compared to that of Rb with dextran [22]. This more efficient tissue diffusion accelerates the CXL process by adjusting the power of UVA radiation and the exposure time [23]. Our results indicate lower k_{obs} L-Tryp values for Trans-Epithelial Kit (II) and Trans-Epithelial Kit (I) compared to that of VibeX Rapid®, indicating that Rb in the VibeX Rapid® formulation is more effective in generating photo-degradation, and this justifies its current use as a CXL agent.

The new concept of accelerated CXL [24] includes UVA pulses, allowing the type II photochemical mechanism to be constantly restarted [25], and an additional concentration of oxygen allows a greater release of $^1O_2$ to interact with collagen molecules. Oxygen depletion has been proposed as a reason for the formation of new covalent junctions [26, 27]. Many authors argue that the medium in which Rb is delivered is essential to shorten the exposure time and improve the cost-effectiveness of CXL [28, 29]. In the current study, the performance of VibeX Rapid® was superior to that of the other formulations.

The photochemistry of Rb, which breaks down into lumiflavin and lumichrome [30], is well understood, and these products are nontoxic to humans [31]. Rb and its breakdown products interact with DNA, making this system attractive for photodisinfection and pathogen reduction [32]. The highest photodegradation constant we observed was that of VibeX Rapid®.

Previous studies have evaluated changes in corneal thickness during CXL performed using isotonic Rb solution with and without dextran; the use of Rb solution without dextran caused a steady increase in corneal thickness during the procedure [11]. Similarly, some published studies have demonstrated no significant decline in corneal thickness after CXL using Rb with HPMC compared to Rb with dextran [19]. In the experiment developed for our research, all the Rb formulations had similar behavior; however, VibeX Rapid® performed superiorly and produced a greater amount of $^1O_2$.

The pharmaceutical industry is continually developing new Rb formulations that may be marketed in the next few years. Among these, Rb combined with cyclodextrin derivatives provides even greater penetration and permeability [33]. In the future, the characteristics of new formulations of Rb may exceed those of VibeX Rapid® [34]; therefore, new studies will certainly be necessary. Importantly, there are various methods to measure the ability of a substance to produce free radicals and degrade L-Tryp, and they could provide information of great scientific value [35]. In the current study, the observed rate constants, or k_{obs}, corresponding to L-Tryp degradation observed as decay, were obtained by a linear least-squares fit of the semi-logarithmic plot of ln A0/A or ln F0/F versus time [36].

The study failed to involve human participants to investigate the clinical efficacy and safety of the available Rb formulations. One of the strengths of this work is its uniqueness; no other studies have conducted or documented a series of experiments such as those of our research group. This knowledge could make significant contributions to the fields of ophthalmology and photodynamics. Future experiments should be designed and conducted to quantitatively differentiate the levels of free radical production.

CONCLUSIONS

This study underscores the critical significance of the Rb transport formulations used in CXL. Specifically, we observed that VibeX Rapid®, Trans-Epithelial Kit (I), and Trans-Epithelial Kit (II) promote the degradation of L-Tryp to a greater extent than that of generic Rb. Rb in the VibeX Rapid® formulation is more effective in generating photo-degradation, and this reflects its superior performance in CXL. The transport of Rb is of paramount importance, as it dictates the presence of Rb at the site of action, ultimately influencing treatment outcomes that should be investigated in future clinical trials.

ETHICAL DECLARATIONS

Ethical approval: This study was approved by the Ethics Committee of the Instituto de la Vision del Cerro de las Rosas, Sanatorio Allende-Sede Cerro, Cordoba, Argentina.
Conflict of interests: None.

FUNDING

None.

ACKNOWLEDGMENTS

This work was supported by Consejo Nacional de Investigaciones Cientificas y Tecnicas (CONICET) of Argentina, Secretaria de Ciencia y Tecnologia, Universidad Nacional de Cordoba (SECYT-UNC), and Fondo para la Investigacion Cientifica y Tecnologica (*FONCYT*).

REFERENCES

1. Hafezi F, Randleman JB. PACK-CXL: defining CXL for infectious keratitis. J Refract Surg. 2014;30(7):438-9. doi: 10.3928/1081597X-20140609-01 pmid: 24983827
2. Ashwin PT, McDonnell PJ. Collagen cross-linkage: a comprehensive review and directions for future research. Br J Ophthalmol. 2010;94(8):965-70. doi: 10.1136/bjo.2009.164228 pmid: 19666925
3. Wan KH, Ip CKY, Kua WN, Chow VWS, Chong KKL, Young AL, et al. Transepithelial corneal collagen cross-linking using iontophoresis versus the Dresden protocol in progressive keratoconus: A meta-analysis. Clin Exp Ophthalmol. 2021;49(3):228-241. doi: 10.1111/ceo.13918 pmid: 33667017
4. González Castellanos JC, Osaba M, Reviglio V, Canchi MT, Arrigone MC, Reviglio VE. Early treatment of bilateral fungal keratitis with corneal cross-linking as adjuvant therapy. Oxf Med Case Reports. 2020;2020(6):omaa032. doi: 10.1093/omcr/omaa032 pmid: 32551125
5. Rubinfeld RS, Caruso C, Ostacolo C. Corneal Cross-Linking: The Science Beyond the Myths and Misconceptions. Cornea. 2019;38(6):780-790. doi: 10.1097/ICO.0000000000001912 pmid: 30882538
6. da Paz AC, Bersanetti PA, Salomão MQ, Ambrósio R Jr, Schor P. Theoretical basis, laboratory evidence, and clinical research of chemical surgery of the cornea: cross-linking. J Ophthalmol. 2014;2014:890823. doi: 10.1155/2014/890823 pmid: 25215226
7. Messmer EM. Update on corneal cross-linking for keratoconus. Oman J Ophthalmol. 2013;6(Suppl 1):S8-S11. doi: 10.4103/0974-620X.122288 pmid: 24391374
8. Idrus EA, Utti EM, Mattila JS, Krootila K. Photoactivated chromophore corneal cross-linking (PACK-CXL) for treatment of severe keratitis. Acta Ophthalmol. 2019;97(7):721-726. doi: 10.1111/aos.14001 pmid: 30593737
9. Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. Biochem J. 2016;473(4):347-64. doi: 10.1042/BJ20150942 pmid: 26862179
10. Lan M, Zhao S, Liu W, Lee CS, Zhang W, Wang P. Photosensitizers for Photodynamic Therapy. Adv Healthc Mater. 2019;8(13):e1900132. doi: 10.1002/adhm.201900132 pmid: 31067008
11. Oltulu R, Şatirtav G, Donbaloğlu M, Kerimoğlu H, Özkağnici A, Karaibrahimoğlu A. Intraoperative corneal thickness monitoring during corneal collagen cross-linking with isotonic riboflavin solution with and without dextran. Cornea. 2014;33(11):1164-7. doi: 10.1097/ICO.0000000000000249 pmid: 25211359
12. Mazzotta C, Traversi C, Caragiuli S, Rechichi M. Pulsed vs continuous light accelerated corneal collagen crosslinking: in vivo qualitative investigation by confocal microscopy and corneal OCT. Eye (Lond). 2014;28(10):1179-83. doi: 10.1038/eye.2014.163 pmid: 25060847
13. Raiskup F, Pinelli R, Spoerl E. Riboflavin osmolar modification for transepithelial corneal cross-linking. Curr Eye Res. 2012;37(3):234-8. doi: 10.3109/02713683.2011.637656 pmid: 22335811
14. Lazzeri D, Rovera M, Pascual L, Durantini EN. Photodynamic studies and photoinactivation of Escherichia coli using meso-substituted cationic porphyrin derivatives with asymmetric charge distribution. Photochem Photobiol. 2004;80(2):286-93. doi: 10.1562/2004-03-08-RA-105 pmid: 15362952
15. Velazquez FN, Miretti M, Baumgartner MT, Caputto BL, Tempesti TC, Prucca CG. Effectiveness of ZnPc and of an amine derivative to inactivate Glioblastoma cells by Photodynamic Therapy: an in vitro comparative study. Sci Rep. 2019;9(1):3010. doi: 10.1038/s41598-019-39390-0 pmid: 30816179
16. Milanesio ME, Alvarez MG, Silber JJ, Rivarola V, Durantini EN. Photodynamic activity of monocationic and non-charged methoxyphenylporphyrin derivatives in homogeneous and biological media. Photochem Photobiol Sci. 2003;2(9):926-33. doi: 10.1039/b212890j pmid: 14560810
17. Reviglio VE, Osaba M, Sambuelli G, Kuo IC. Phototoxic Effect of Topical Fluoroquinolones Administered Before Corneal Crosslinking in a Murine Model. J Ocul Pharmacol Ther. 2017;33(2):73-78. doi: 10.1089/jop.2016.0060 pmid: 28106466
18. Mesen A, Bozkurt B, Kamis U, Okudan S. Correlation of Demarcation Line Depth With Medium-Term Efficacy of Different Corneal Collagen Cross-Linking Protocols in Keratoconus. Cornea. 2018;37(12):1511-1516. doi: 10.1097/ICO.0000000000001733 pmid: 30157054
19. Jain V, Gazali Z, Bidayi R. Isotonic riboflavin and HPMC with accelerated cross-linking protocol. Cornea. 2014;33(9):910-3. doi: 10.1097/ICO.0000000000000188 pmid: 25014154
20. Zaheer N, Khan WA, Khan S, Khan MAM. Comparison of Changes in Central Corneal Thickness During Corneal Collagen Cross-

Linking, Using Isotonic Riboflavin Solutions With and Without Dextran, in the Treatment of Progressive Keratoconus. Cornea. 2018;37(3):340-346. doi: 10.1097/ICO.0000000000001496 pmid: 29283924
21. Velpandian T. Intraocular penetration of antimicrobial agents in ophthalmic infections and drug delivery strategies. Expert Opin Drug Deliv. 2009;6(3):255-70. doi: 10.1517/17425240902798119 pmid: 19327043
22. Ehmke T, Seiler TG, Fischinger I, Ripken T, Heisterkamp A, Frueh BE. Comparison of Corneal Riboflavin Gradients Using Dextran and HPMC Solutions. J Refract Surg. 2016;32(12):798-802. doi: 10.3928/1081597X-20160920-03 pmid: 27930789
23. Hagem AM, Thorsrud A, Sandvik GF, Drolsum L. Randomized Study of Collagen Cross-Linking With Conventional Versus Accelerated UVA Irradiation Using Riboflavin With Hydroxypropyl Methylcellulose: Two-Year Results. Cornea. 2019;38(2):203-209. doi: 10.1097/ICO.0000000000001791 pmid: 30365412
24. Ng AL, Chan TC, Cheng AC. Conventional versus accelerated corneal collagen cross-linking in the treatment of keratoconus. Clin Exp Ophthalmol. 2016;44(1):8-14. doi: 10.1111/ceo.12571 pmid: 26140309
25. Wernli J, Schumacher S, Spoerl E, Mrochen M. The efficacy of corneal cross-linking shows a sudden decrease with very high intensity UV light and short treatment time. Invest Ophthalmol Vis Sci. 2013;54(2):1176-80. doi: 10.1167/iovs.12-11409 pmid: 23299484
26. Richoz O, Hammer A, Tabibian D, Gatzioufas Z, Hafezi F. The Biomechanical Effect of Corneal Collagen Cross-Linking (CXL) With Riboflavin and UV-A is Oxygen Dependent. Transl Vis Sci Technol. 2013;2(7):6. doi: 10.1167/tvst.2.7.6 pmid: 24349884
27. Kamaev P, Friedman MD, Sherr E, Muller D. Photochemical kinetics of corneal cross-linking with riboflavin. Invest Ophthalmol Vis Sci. 2012;53(4):2360-7. doi: 10.1167/iovs.11-9385 pmid: 22427580
28. Fischinger I, Seiler TG, Wendelstein J, Tetz K, Fuchs B, Bolz M. Biomechanical Response After Corneal Cross-linking With Riboflavin Dissolved in Dextran Solution Versus Hydroxypropyl Methylcellulose. J Refract Surg. 2021;37(9):631-635. doi: 10.3928/1081597X-20210610-04 pmid: 34506235
29. Said DG, Elalfy MS, Gatzioufas Z, El-Zakzouk ES, Hassan MA, Saif MY, et al. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. Ophthalmology. 2014;121(7):1377-82. doi: 10.1016/j.ophtha.2014.01.011 pmid: 24576886
30. Hardwick CC, Herivel TR, Hernandez SC, Ruane PH, Goodrich RP. Separation, identification and quantification of riboflavin and its photoproducts in blood products using high-performance liquid chromatography with fluorescence detection: a method to support pathogen reduction technology. Photochem Photobiol. 2004;80(3):609-15. doi: 10.1562/2004-04-14-TSN-139 pmid: 15382964
31. Seghatchian J, de Sousa G. Pathogen-reduction systems for blood components: the current position and future trends. Transfus Apher Sci. 2006;35(3):189-96. doi: 10.1016/j.transci.2006.10.002 pmid: 17110168
32. Wainwright M, Baptista MS. The application of photosensitisers to tropical pathogens in the blood supply. Photodiagnosis Photodyn Ther. 2011;8(3):240-8. doi: 10.1016/j.pdpdt.2011.04.001 pmid: 21864797
33. Martin CB, Tsao ML, Hadad CM, Platz MS. The reaction of triplet flavin with indole. A study of the cascade of reactive intermediates using density functional theory and time resolved infrared spectroscopy. J Am Chem Soc. 2002;124(24):7226-34. doi: 10.1021/ja0123711 pmid: 12059249
34. Mazzotta C, Raiskup F, Hafezi F, Torres-Netto EA, Armia Balamoun A, Giannaccare G, et al. Long term results of accelerated 9 mW corneal crosslinking for early progressive keratoconus: the Siena Eye-Cross Study 2. Eye Vis (Lond). 2021;8(1):16. doi: 10.1186/s40662-021-00240-8 pmid: 33931101
35. Huvaere K, Cardoso DR, Homem-de-Mello P, Westermann S, Skibsted LH. Light-induced oxidation of unsaturated lipids as sensitized by flavins. J Phys Chem B. 2010;114(16):5583-93. doi: 10.1021/jp9121744 pmid: 20377218
36. Schiavi PC, Svetaz L, Petenatti E, Sortino M, Tempesti TC, Funes M. Extracts of Trichocline sinuata (Asteraceae) as natural sensitizers in the photodynamic inactivation of Candida albicans. Photochem Photobiol. 2023. doi: 10.1111/php.13871 pmid: 37877243