RIBOFLAVIN IN PHOTODYNAMIC INACTIVATION OF PATHOGENS AND PHOTODYNAMIC THERAPY

Pogorelaya M, Martynov A, Romanova E.

Mechnikov institute of microbiology and immunology

Riboflavin or vitamin B2 is the main biochemical source of a flavin moiety in the cell, readily forming flavin mononucleotide (FMN) and flavinadenine dinucleotide (FAD), which play a vital role in the cellular metabolism. Riboflavin is involved in such cell metabolic process like cellular respiration – the process in which biochemical energy from nutrients is converted into adenosine triphosphate (ATP).

Riboflavin was discovered in 1920, isolated in 1933 and first synthesized in 1935. Riboflavin is on the World Health Organization's List of Essential Medicines.

Besides its key role in providing cellular metabolism in humans and animals via dietary supplementation, riboflavin as a photosensitizer is used in disinfection systems, which provides antimicrobial effects. This disinfection technique, in which photosensitizer is applied, called antimicrobial photodynamic therapy (aPDT).

Antimicrobial photodynamic therapy (aPDT) is a promising approach for the photoinactivation of pathogens in blood and blood derivatives. It has been reported, that the advantage of antimicrobial photodynamic therapy is that there are no resistant strains to it. For now, there are three photosensitizers have been approved: methylene blue (MB) for plasma and riboflavin and amotosalen for plasma and platelets [1].

Photochemical mechanisms applied in photodynamic therapy

In its ground state a photosensitizer molecule (PS) is a singlet because it has two electrons with opposite spins. Absorption of a photon of light (*hv*) with the appropriate quantum energy (with appropriate wavelength) leads to the excitation of one electron into a higher-energy orbital (Figure 1). This singlet excited-state PS is unstable and loses its excess energy either as emission of light (fluorescence) or production heat (internal conversion). The excited singlet PS may undergo a process known as ‘intersystem crossing’(IC) to form a more stable excited triplet state with parallel spins. The triplet-state PS molecule can decay back to the ground state (by emitting a phosphorescent photon) but this is a ‘forbidden process’ by the quantum selection rules, so the triplet state is much more stable than the singlet state having a lifetime of microseconds compared with only nanoseconds for the excited singlet. This long lifetime of the triplet state allows it sufficient time to transfer its energy by colliding with molecular oxygen (O_2), which is unique in being a molecular triplet in its ground state. This energy-transfer step leads to the formation of singlet oxygen ($^1O2$) (and ground-state PS), and the reaction is referred to as a Type II photochemical process. A Type I photochemical process can also occur whereby the excited-state PS undergoes electron transfer reactions that eventually forms reactive oxygen species (ROS). This mechanism may involve either acquisition or donation of an electron to form the radical cation or radical anion. The radical anion can react with oxygen to produce the superoxide radical anion ($O2•−$). Dismutation or one-electron reduction of $O2•−$ gives hydrogen peroxide (H_2O_2), which in turn can undergo another one-electron reduction to form the powerful oxidant – hydroxyl radicals (HO•). ROS generation via Type II mechanism is much simpler than via Type I, and most PSs used, for instance, in anti-cancer PDT are believed to operate via the Type II rather than the Type I mechanism [2].

Riboflavin and antimicrobial photodynamic inactivation

Riboflavin is naturally occurring (not synthetical) compound of antimicrobial photodynamic therapy as an antimicrobial photosensitizer with two peaks in the UVA (360 nm) and blue (440 nm) regions. [3]. Owing to antimicrobial properties have been identified for riboflavin it finds its application in blood product sterilization [4] and also acts well as a photoactivated cross-linker for corneal stiffening in patients with keratoconus and other ectatic corneal disorders [5;6].

The cationic version of riboflavin, which basically act as antimicrobial photosensitizer was designed and synthesized by Maisch et al [7].

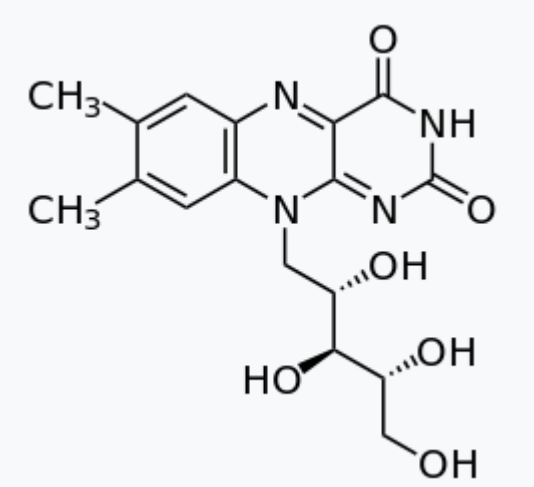

Figure 1. Riboflavin structure

DOI: 10.5281/zenodo.3726595

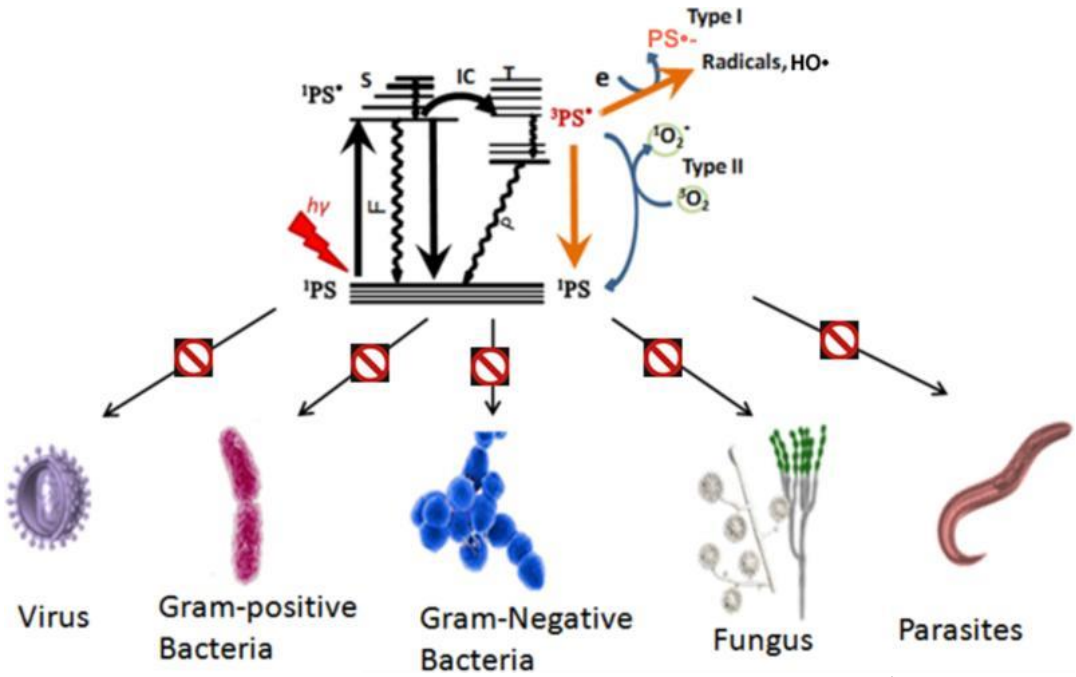

Figure 2- Jablonski diagram showing photochemical pathways in aPDI. The *ground state ^{1}PS (photosensitizer) absorbs a photon to form excited singlet state ^{1}PS* that can undergo intersystem crossing (IC) to form the triplet state ^{3}PS*. This long-lived species can undergo energy transfer (Type II) to form singlet oxygen ^{1}O2* or electron transfer (Type I) to form hydroxyl radicals HO•. Both these ROS are capable of killing a broad spectrum of pathogens (picture 1) [8].*

Flavins are photoreducible, i.e. capable of charge transfer per photon absorption, which mediates the cell signaling or gene expression in endogenous protein complexes, such as light-oxygen-voltage-sensing domains in bacteria and plants [9]. At the same time, biologically-unregulated photon-induced excitation of flavins in the ultraviolet-blue (UV-blue) spectral band can lead to formation of either singlet oxygen ($^1O_2$) via energy transfer to environmental oxygen (Type II), or hydrogen peroxide and derivatives via radicalisation (Type I) – altogether termed reactive oxygen species (ROS) and used hereafter [10].

The ROS production property of riboflavin is known for a long time and used for antiviral and antibacterial disinfection; for strengthening the corneal tissue in photorefractive surgery by the ROS-induced collagen cross-linking [11;12].

Riboflavin on account of having different meso-atoms possess excellent photophysical and photochemical properties [13]. Several studies show that photoactivated riboflavin does produce ROS [14;15]. Riboflavin absorbs photons from the illuminated light and undergoes intersystem conversion i.e. from singlet state to triplet state. This triplet state reacts with molecular oxygen, thereby producing ROS. This generated ROS attacks cellular macromolecules and disintegrates membrane integrity, thereby, killing the organism.

The study of Khan S. et al. demonstrated the antimicrobial photodynamic potential of riboflavin irradiated with white light for 2 h by using a study model with *Escherichia coli*. The treated bacterial cells exhibited abundant intracellular ROS generation and marked increment in the level of oxidative stress markers - lipid peroxidation (MDA level) and protein carbonylation - as well as significant reduction in lactate dehydrogenase (LDH) activity. Marked reduction in colony forming units of *E.coli* was also observed, that was further confirmed by optical microscopic and SEM images via registration of the bacterial death. Thus, this study shows that photoilluminated riboflavin renders the redox status of bacterial cells into a compromised state leading to significant membrane damage ultimately causing bacterial death. The activity of superoxide dismutase (SOD) and catalase, significantly reduced in samples which are exposed to photoilluminated riboflavin as compared to samples which are exposed to riboflavin without light and exposed to light alone. The decrease in the level of cellular antioxidant metabolite - glutathione (GSH) was significantly reduced in samples exposed to photoilluminated riboflavin. The specific activity of glutathione S-transferase (GST) - the GSH utilizing enzyme that is involved in detoxification process - was increased significantly in cells exposed to photoilluminated riboflavin. This study adds another dimension to the use of photoactivated riboflavin, demonstrating that clinically important multi-drug resistant

DOI: 10.5281/zenodo.3726595

bacteria are susceptible to ROS generated by photoactivated riboflavin [16].

The study of Maisch T. et al shows that synthesized flavin derivatives are able to be used as photosensitizers for topical application to decolonize bacteria from skin and mucosa. The synthesized flavin derivatives showed a high quantum yield of singlet oxygen of approximately 75 %. Multidrug resistant bacteria like MRSA (methicillin resistant *Staphylococcus aureus*), EHEC (enterohemorrhagic *Escherichia coli*), *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* were incubated with flavin derivatives *in vitro* and were subsequently irradiated with visible light for seconds only. After irradiation, the number of viable bacteria decreased up to 6 $\log_{10}$ steps depending on the concentration of the flavin derivatives and the light dosimetry. The bactericidal effect of PIB was independent of the bacterial type and the corresponding antibiotic resistance pattern. In contrast, the photosensitizer concentration and light parameters used for bacteria killing did not affect cell viability of human keratinocytes (therapeutic window). Thus, the study suggests that multiresistant bacteria can be safely and effectively killed by a combination of modified vitamin B_2 molecules, oxygen and visible light, whereas normal skin cells survive [17].

Thakuri et al. show by their study, where riboflavin was excited by blue light, killing of *S. aureus* and *P. aeruginosa* [18]. In the O'Rourke's study was shown the killing of *Bacillus subtilis* [19,20]. In the study of Makdoumi K. et all riboflavin enhanced the antibacterial effect on the exposed MRSA strain for two different wavelengths of blue light - 412 and 450 nm. Using the higher dose, CFU reduction was 99% and 98%, respectively, for 412 and 450 nm light. The bactericidal efficacy was high also in the deeper fluid layer (93%, higher dose). These findings suggest that blue light could be considered for possible implementation in deep corneal infections [21].

It was shown, that riboflavin/UV-A and pretreatment with amphotericin B allowed effectiveness against such fungal pathogens as *C. albicans*, *Fusarium sp*, and *A. fumigatus*as which may cause infection keratomycosis [22].

But, in the study of Tuncann G. et al where biofilm formation and antibiofilm activity were assessed using different photosensitizers and types of irradiation, was observed that riboflavin + UV treatment showed only minimal effect on the *Staphylococcus aureus* ATCC 35556, *Staphylococcus epidermidis* ATCC 35984, *Candida albicans* ATCC 90028, and *Candida parapsilosis* ATCC 96142 comparing with green LED and rose bengal. The lowest biofilm inhibition (biofilm inhibition indexes) was measured in group with riboflavin + UV treatment compared with LED and rose bengal and red LED with methylene blue. The groups to which riboflavin + UV was applied showed no effect on biofilm formation in *C. parapsilosis* and only minimally reduced the formation of biofilm in *S. aureus* and *S. epidermidis*, and by 24.5% in *C. albicans* [23].

Riboflavin as a photosensitizer in viruses' inactivation

Vitamin B2, illuminated by UVA or visible light, has also been developed as a nucleic acid-binding agent to be used for photoinactivation of such nucleic acid-containing pathogens in plasma, platelets, and RBCs as viruses. Several studies have revealed the effectiveness of reduction in some viruses' infectivity, including human immunodeficiency virus 1 (HIV-1), bovine viral diarrhea virus (BVDV), hepatitis B virus (HCV), pseudorabies virus [24,25].

It was shown that riboflavin with ultraviolet light irradiation has a substantial impact on reduction of dengue viruses' infectivity, that are emerging across the world pose a risk to transfusion safety. After treatment (1·81 log reduction) for DENV-4 was observed followed by (1·71 log reduction) for DENV-3, (1·45 log reduction) for DENV-2 and then (1·28 log reduction) for DENV-1. This study concluded that riboflavin and UV light may be an alternative approach for managing the risk of DENV transfusion transmission via the ability to inactivate DENV 1-4 in platelet concentrates [26].

On the subject of the risk of transfusion transmission, the study of Keil SD et all showed that riboflavin and UV light effectively reduced the titer of Middle East respiratory syndrome coronavirus (MERS-CoV) in human plasma products to below the limit of detection, suggesting that the treatment process may reduce the risk of transfusion transmission of MERS-CoV [27].

The one more study is on the virus reduction technology used for blood and plasma. In this study was obtained plasma from recovered patients after Ebolavirus (EBOV) infection, which is called "convalescent plasma, and is an effective treatment for active disease available in endemic areas. Whereas this type of plasma carries the risk of introducing other pathogens, including other strains of EBOV it must undergo the pathogen reduction process, but that which can save protective antivirus antibody titers. The «riboflavin+UV» application reduced ebolavirus (EBOV) titers to nondetectable levels in both nonhuman primate serum (≥2.8- to 3.2-log reduction) and human whole blood (≥3.0-log reduction) without decreasing protective antibody titers in human plasma. Thus, *in vivo* testing to determine whether UV+RB can improve convalescent blood product safety is indicated [28,29].

Riboflavin as a photosensitizer became a part of pathogen reduction technology

Thus, the «riboflavin+UVB» system has found their application in pathogen reduction technology – «Mirasol»® Pathogen Reduction Technology (PRT) system («Terumo BCT Inc.», Lakewood, USA), which is used for blood and plasma before transfusion. [30].

DOI: 10.5281/zenodo.3726595

After obtaining a large amount of evidences of antimicrobial activity of «riboflavin+UVB», the focus has shifted to safety of its application and some possible alterations, which may following occur in platelets and plasma.

The study of Abonnenc M. et all revealed that Mirasol®-treated platelets exhibit enhanced storage lesions compared to controls (increase of activation markers and glycolysis rate, lower hypotonic shock and double-agonist activation responses, and decrease of total antioxidant capacity). Authors also confirmed that the UV radiation alone is causing platelet lesions. Riboflavin tends to have an intracellular protective role while it decreases the extracellular antioxidant defenses. In this study were found some benefits of platelet additive solutions containing potassium and magnesium, which were confirmed to reduce the extent of storage lesions [31].

In the new study of Hermida-Nogueira et all were identified a high number of proteins related to platelet activation and platelet storage lesion that could have a role in possible transfusion adverse reactions. In this study 151 proteins were found up-regulated at day 7 of storage after «Mirasol»® treatment. This group of proteins includes C-C motif chemokine ligand 5 (CCL5) and platelet factor 4, chemokines with ability to attract neutrophils and monocytes, which could generate transfusion adverse reactions. In addition, other glycoproteins and platelet activation markers were also found elevated at day 7. Proteins related to glycolysis and lactate production were found altered with high fold changes, showing a deregulation of platelet metabolism at day 7. The obtained results provide novel information about possible effects of platelet-derived EVs on transfusion adverse reactions.

Riboflavin as an antitumor agent in photodynamic therapy

Riboflavin has been thought to be a promising antitumoral agent in photodynamic therapy, though the further application of the method was limited by the unclear molecular mechanism. Some article reveal that riboflavin is able to recognize G–T mismatch specifically and induce single-strand breaks in duplex DNA targets efficiently under irradiation. In the presence of riboflavin, the photo-irradiation could induce the death of tumor cells that are defective in mismatch repair system selectively, highlighting the G–T mismatch as potential drug target for malignant cells. Moreover, riboflavin is a promising leading compound for further drug design due to its inherent specific recognition of the G–T mismatch [32].

Several studies reveal that of PDT-mediated cytotoxicity occur in three ways: apoptotic, necrotic and autophagy-associated cell death. Some findings, show that certain PDT techniques acting via inducing of apoptotic cell death that is highly immunogenic and can stimulate antitumor immunity [33].

In the study of Akasov et al. a water-soluble form of riboflavin - flavin mononucleotide (FMN) – was shown as a promising agent for photodynamic therapy of melanoma. Blue light irradiation with dose 5 J/cm^2 of melanoma cells pre-incubated with FMN led to cell death through apoptosis. Thus, the IC50 values of human melanoma A375, Mel IL, and Mel Z cells were in a range of FMN concentration 10 – 30 μM that can be achieved in tumor tissue under systemic administration. Melanoma xenograft regression in mice was observed as a result of intravenous injection of FMN followed by blue-light irradiation of tumor site. The inhibition of tumor growth was 85 – 90 % within 50 days after PDT treatment [34].

Corneal collagen cross-linking with riboflavin as photodynamic therapy in the management of infectious keratitis

Infectious keratitis is a potentially blinding ocular disease of the cornea. Besides antimicrobial drugs, the use of corneal collagen cross-linking (CXL) as photodynamic therapy (PDT) is also considered as potential alternative in the management of infectious keratitis [35,36]. Thus, CXL has also been described as riboflavin-UVA-photodynamic-inactivation (riboflavin-UVA-PDI) [37].

Skaat A. et al presented the data when the adjunctive use of riboflavin/UVA photochemical therapy has a positive effect on refractory infectious keratitis. A retrospective analysis was performed of an interventional case series in which 6 eyes of 6 patients with severe infectious keratitis, all of whom were refractive to multidrug conventional therapy, were treated with riboflavin/UVA. In the result five of the 6 patients showed rapid reduction in symptoms and decreased infiltrate size after riboflavin/UVA photochemical therapy. Signs of infection and inflammation mostly resolved within 1 to 2 weeks after the treatment. Despite this therapy, one patient continued to deteriorate, and penetrating keratoplasty was performed. The treatment was claimed to be safe and effective and should be considered as part of the first-line therapy in severe cases of infectious keratitis [38].

The study of Song X et al revealed the impact of cross-linking (CXL) on viability, apoptosis, proliferation, activation, and cytokine secretion (FGFb, HGF, TGFβ1, VEGF, KGF, IL-1β, IL-6, and IL-8) of human keratoconus (KC) keratocytes, *in vitro*. Keratocytes were underwent UVA illumination (370 nm, 2 J/cm2) during exposure to 0.1% riboflavin and 20% Dextran. Results reveal that 24 hours after CXLcross-linking decreases viability, triggers apoptosis, and inhibits proliferation, without an impact on multipotent hematopoietic stem cell transformation and myofibroblastic transformation of KC keratocytes. CXL triggers FGFb secretion of KC keratocytes transiently (5 hours), normalizing after 24 hours [39].

DOI: 10.5281/zenodo.3726595

Riboflavin in photodynamic inactivation of pathogens and photodynamic therapy

Pogorelaya M, Martynov A, Romanova E.,
Mechnikov institute of microbiology and immunology

Riboflavin, besides its key role in providing cellular metabolism in humans and animals, is used as a compound of antimicrobial photodynamic therapy (aPDT) owing to its photosensitizing capability. PDT is a promising approach for the photoinactivation of pathogens in blood and blood derivatives. It has been reported, that the advantage of antimicrobial photodynamic therapy is that there are no resistant strains to it. Flavins are photoreducible and photon-induced excitation of them in the ultraviolet-blue (UV-blue) spectral band can lead to formation of either singlet oxygen via energy transfer to environmental oxygen, or hydrogen peroxide and derivatives via radicalisation – altogether termed reactive oxygen species (ROS) and used hereafter. Exactly the ROS production property of riboflavin is used for antiviral and antibacterial disinfection; for strengthening the corneal tissue in photorefractive surgery by the ROS-induced collagen cross-linking. Several studies reveal the antimicrobial photodynamic potential of riboflavin irradiated with the ultraviolet-blue and with visible light against methicillin resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, enterohemorrhagic *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Bacillus subtilis in vitro*. It was shown, that riboflavin/UV-A and allowed effectiveness against such fungal pathogens as *Candida albicans, Candida parapsilosis, Fusarium spp*, and *A. fumigatus*as which may cause infection keratomycosis. The photoilluminated riboflavin significantly reduced the activity of superoxide dismutase (SOD) and reduced the level of cellular antioxidant metabolite - glutathione (GSH). Along with that the specific activity of glutathione S-transferase (GST) - which is involved in detoxification process - was increased significantly in cells exposed to photoilluminated riboflavin. Riboflavin, illuminated by UVA or visible light, has also been developed as a nucleic acid-binding agent to be used for photoinactivation of such nucleic acid-containing pathogens in plasma, platelets, and RBCs as viruses. Several studies have revealed the effectiveness of reduction in some viruses' infectivity, including human immunodeficiency virus 1 (HIV-1), bovine viral diarrhea virus (BVDV), hepatitis B virus (HCV), pseudorabies virus. Thus, the «riboflavin+UVB» system has found their application in pathogen reduction technology – «Mirasol»® Pathogen Reduction Technology (PRT) system. Riboflavin has been thought to be a promising antitumoral agent in photodynamic therapy, though the further application of the method was limited by the unclear molecular mechanism. Several studies reveal that of PDT-mediated cytotoxicity occur in three ways: apoptotic, necrotic and autophagy-associated cell death. Some findings, show that certain PDT techniques acting via inducing of apoptotic cell death that is highly immunogenic and can stimulate antitumor immunity. Thus, we can conclude that, the «riboflavin+UVB» system is suitable for photoinactivation of bacteria, fungi and viruses and has a potential in antitumor treatment strategies. Further studies will reveal more and more aspects of riboflavin capabilities.

Keywords: riboflavin, photodynamic, antimicrobial, antiviral, UVA, UVB

References

1 Sousa V, Gomes ATPC, Freitas A, Faustino MAF, Neves MGPMS, Almeida A. Photodynamic Inactivation of Candida albicans in Blood Plasma and Whole Blood. Antibiotics (Basel). 2019;8(4):221. Published 2019 Nov 13. doi:10.3390/antibiotics8040221

2 Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. Biochem J. 2016;473(4):347–364. doi:10.1042/BJ20150942

3 Makdoumi K, Backman A, Mortensen J, Crafoord S. Evaluation of antibacterial efficacy of photo-activated riboflavin using ultraviolet light (UVA) Graefes Arch Clin Exp Ophthalmol. 2010;248:207–212.

4 Ettinger A, Miklauz MM, Bihm DJ, Maldonado-Codina G, Goodrich RP. Preparation of cryoprecipitate from riboflavin and UV light-treated plasma. Transfus Apher Sci. 2012;46:153–158.

5 Chan TC, Lau TW, Lee JW, Wong IY, Jhanji V, Wong RL. Corneal collagen cross-linking for infectious keratitis: an update of clinical studies. Acta Ophthalmol 2015

6 Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. Biochem J. 2016;473(4):347–364. doi:10.1042/BJ20150942

7 Maisch T, Eichner A, Spath A, Gollmer A, Konig B, Regensburger J, Baumler W. Fast and effective photodynamic inactivation of multiresistant bacteria by cationic riboflavin derivatives. PLoS One. 2014;9:e111792.

8 Hamblin MR. Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes. Curr Opin Microbiol. 2016;33:67–73. doi:10.1016/j.mib.2016.06.008

9 Conrad K. S., Bilwes A. M. & Crane B. R. Light-Induced Subunit Dissociation by a Light-Oxygen-Voltage Domain Photoreceptor from Rhodobacter

10 Agostinis P. et al.. Photodynamic Therapy of Cancer: An Update. Ca-a Cancer Journal for Clinicians 61, 250–281, doi: 10.3322/caac.20114 (2011).

11 Ruane P. H. et al.. Photochemical inactivation of selected viruses and bacteria in platelet concentrates using riboflavin and light. Transfusion 44, 877–885, doi: 10.1111/j.1537-2995.2004.03355.x (2004).

12 Wollensak G., Spoerl E. & Seiler T. Riboflavin/ultraviolet-A-induced collagen crosslinking for the treatment of keratoconus. American Journal of Ophthalmology 135, 620–627, doi: 10.1016/s0002-9394(02)02220-1 (2003).

DOI: 10.5281/zenodo.3726595

13 Sheraz M.A., Kazi S.H., Ahmed S., Anwar Z., Ahmad I. Photo, thermal and chemical degradation of riboflavin. Beilstein J. Org. Chem. 2014;10:1999–2012.
14 Khan S., Naseem I. Photocatalytic interaction of aminophylline-riboflavin leads to ROS-mediated DNA damage and cell death: a novel phototherapeutic mechanism for cancer. IUBMB Life. 2017;69:611–622.
15 Bouillaguet S., Wataha J.C., Zapata O., Campo M., Lange N., Schrenzel J. Production of reactive oxygen species from photosensitizers activated with visible light sources available in dental offices. Photomed. Laser Surg. 2010;28:519–525.
16 Khan S, P MR, Rizvi A, [et al.]. ROS mediated antibacterial activity of photoilluminated riboflavin: A photodynamic mechanism against nosocomial infections // Toxicol. 2019. Vol. 6. P. 136-142.
17 Maisch T., Eichner A., Späth A. [et al.] Fast and effective photodynamic inactivation of multiresistant bacteria by cationic riboflavin derivatives // PLoS One. 2014. Vol. 9(12):e111792.
18 Thakuri PS, Joshi R, Basnet S, [et al.] Antibacterial photodynamic therapy on Staphylococcus aureus and Pseudomonas aeruginosa in vitro // Nepal Med. Coll. J. 2011. Vol. 13(4). P. 281-284.
19 O'Rourke JF, Dowds BC. Dye-mediated photodynamic inactivation of Bacillus subtilis // Biochem. Soc. Trans. 1992. Vol. 20(1). P. 76S.
20 Yin R, Hamblin MR. Antimicrobial Photosensitizers: Drug Discovery Under the Spotlight // Curr Med Chem. 2015. Vol. 22(18). P. 2159-2185.
21 Makdoumi K, Goodrich R, Bäckman A. Photochemical eradication of methicillin-resistant Staphylococcus aureus by blue light activation of riboflavin // Acta Ophthalmol. 2017. Vol. 95(5). P. 498-502.
22 Sauer A, Letscher-Bru V, Speeg-Schatz C, [et al.]. In vitro efficacy of antifungal treatment using riboflavin/UV-A (365 nm) combination and amphotericin B // Invest Ophthalmol Vis Sci. 2010. Vol. 51(8). P. 3950-3953.
23 Tunccan ÖG, Kalkanci A, Unal EA, [et al.] The in vitro effect of antimicrobial photodynamic therapy on Candida and Staphylococcus biofilms // Turk. J. Med. Sci. 2018.Vol. 48(4). P. 873–879.
24 Schuyler R. Use of riboflavin for photoinactivation of pathogens in blood components // Transfus. Apher. Sci. 2001. Vol. 25. P. 189–190.
25 Zhu L, Tong H, Wang S, [et al.] Effectiveness of a flow-based device using riboflavin photochemistry in damaging blood-borne viral nucleic acids // J. Photochem. Photobiol. B. 2018. Vol. 183. P. 391-396.
26 Faddy HM, Fryk JJ, Watterson D, [et al.] Riboflavin and ultraviolet light: impact on dengue virus infectivity // Vox Sang. 2016. Vol. 111(3). P. 235-241.
27 Keil SD, Bowen R, Marschner S. Inactivation of Middle East respiratory syndrome coronavirus (MERS-CoV) in plasma products using a riboflavin-based and ultraviolet light-based photochemical treatment // Transfusion. 2016. Vol. 56(12). P. 2948-2952.
28 Cap AP, Pidcoke HF, Keil SD, [et al.] Treatment of blood with a pathogen reduction technology using ultraviolet light and riboflavin inactivates Ebola virus in vitro // Transfusion. 2016. Vol. 56. Suppl 1. S6-15.
29 Hermida-Nogueira L, Barrachina MN, Izquierdo I, [et al.] Proteomic analysis of extracellular vesicles derived from platelet concentrates treated with Mirasol® identifies biomarkers of platelet storage lesion // J. Proteomics. 2020. Vol. 210. P. 103529.
30 Perez-Pujol S., Tonda R., Lozano M., [et al.] Effects of a new pathogen-reduction technology (Mirasol PRT) on functional aspects of platelet concentrates // Transfusion. Vol. 45, Issue 6. P. 911-919.
31 Abonnenc M., Crettaz D., Sonego G. [et al]. Towards the understanding of the UV light, riboflavin and additive solution contributions to the in vitro lesions observed in Mirasol®-treated platelets // Transfus. Clin. Biol. 2019. Vol. 26. Issue 4. P. 209-216.
32 Yuan Y, Zhao Y, Chen L, [et al.] Selective tumor cell death induced by irradiated riboflavin through recognizing DNA G-T mismatch // Nucleic Acids Res. 2017. Vol. 45. N. 15. P. 8676–8683.
33 Garg AD, Nowis D, Golab J, [et al.] Immunogenic cell death, DAMPs and anticancer therapeutics: an emerging amalgamation // Biochim. Biophys. Acta. 2010. Vol. 1805. P. 53–71.
34 Akasov RA, Sholina NV, Khochenkov DA, [et al]. Photodynamic therapy of melanoma by blue-light photoactivation of flavin mononucleotide // Sci Rep. 2019. Vol. 9. Issue 1. P. 9679.
35 Lim L, Lim EWL. A Review of Corneal Collagen Cross-linking - Current Trends in Practice Applications // Open Ophthalmol J. 2018. Vol. 12. P. 181–213.
36 Mohammadpour M, Masoumi A, Mirghorbani M, [et al.] Updates on corneal collagen cross-linking: Indications, techniques and clinical outcomes // J. Curr. Ophthalmol. 2017. Vol. 29(4). P. 235–247.
37 Zhu Y., Reinach PS., Zhu H., [et al.] High-intensity corneal collagen crosslinking with riboflavin and UVA in rat cornea // PLoS One. 2017. Vol. 12(6):e0179580.
38 Skaat A, Zadok D, Goldich Y, [et al.] Riboflavin/UVA photochemical therapy for severe infectious keratitis // Eur. J. Ophthalmol. 2014. Vol. 24. Issue 1. P. 21-28.
39 Song X, Stachon T, Wang J, [et al.]. Viability, apoptosis, proliferation, activation, and cytokine secretion of human keratoconus keratocytes after cross-linking // Biomed. Res. Int. 2015. Vol. 2015. P. 254237.

DOI: 10.5281/zenodo.3726595