Cross-linking for microbial keratitis

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The success of collagen cross-linking as a clinical modality to modify the clinical course in keratoconus seems to have fueled the search for alternative applications for this treatment. Current clinical data on its efficacy is limited and laboratory data seems to indicate that it performs poorly against resistant strains of bacteria and against slow growing organisms. However, the biological plausibility of crosslinking and the lack of effective strategies in managing infections with these organisms continue to focus attention on this potential treatment. Well-conducted experimental and clinical studies with controls are required to answer the questions of its efficacy in future.

Key words: Collagen cross-linking, microbial keratitis, riboflavin, fungus, acanth

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The combination of riboflavin and ultraviolet A light (UVA) exposure has been extensively used in collagen cross-linking (CXL) for the treatment of ectatic disorders of the cornea.[1] The antimicrobial effect of a similar photochemical reaction using riboflavin and UVA has been successfully exploited in the field of transfusion medicine for inactivation of various microorganisms in blood products.[2,3] This has inspired various groups to investigate the potential beneficial effects of CXL in the management of microbial keratitis. Herein, we attempt to put together the available evidence exploring the use of this modality in corneal infections.

Evidence from Laboratory Studies

Martins *et al*., first reported on the antimicrobial effect of riboflavin and UVA *in vitro*. An inhibition of growth was seen in both drug sensitive as well as drug resistant bacteria, but no effect was observed on the growth of *Candida albicans*.[4] Subsequently, Schrier *et al*., reported the effectiveness of a combination of riboflavin and UVA exposure for 30 minutes against *Staphylococcus aureus* as well as *Pseudomonas aeruginosa*.[5] Makdoumi *et al*., tested the effects of riboflavin and UVA on bacterial suspensions in a fluid solution. They demonstrated that exposure for 60 minutes achieved a high degree of eradication of bacteria *in vitro*, whereas an exposure for 30 minutes achieved only a limited eradication.[6] *In vitro* and animal-model studies from multiple centers failed to show a beneficial effect of this treatment against *Acanthamoeba*.[7,8] Using a rabbit model, Galperin *et al*., demonstrated a reduction in severity and intensity of *Fusarium solani* keratitis using CXL.[9]

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In contrast, no effect of a similar treatment was observed on *Fusarium solani* and *C. albicans* isolates in an *in vitro* experiment performed by Kashiwabuchi *et al*.[10]

Clinical Reports

Studies on CXL in microbial keratitis are summarized in the Table 1. Most are small case series or single case reports. The modality has been studied in bacterial, fungal, viral, and *Acanthamoeba* keratitis. Except one, all are retrospective studies. Case selection appears arbitrary. A lack of homogeneity in terms of predisposing factors, clinical presentation and responsible organisms makes interpretation of results difficult. Some reported cases are presumed infections with negative microbiology. Others are diagnosed based on confocal microscopy. Treatment protocols range from a single 5-minute exposure of UVA to multiple sessions lasting up to 45 minutes. The concentration of riboflavin used has also been variable. Surgical procedures performed during active infection include keratoplasty, amniotic membrane transplantation, phototherapeutic keratectomy, flap amputation, intracorneal voriconazole injection, and enucleation. Outcome assessment is largely subjective, as a clear definition of what constitutes resolution or healing is missing in a majority of reports. Almost all results are confounded by the concurrent use of standard of care medical therapy. Though these reports seem to indicate that CXL may be an option in the treatment of infection, one cannot conclusively say it is effective as no control groups are available to compare against.

Our Experience

We have carried out *in vitro* experiments to test the effects of a combination of riboflavin and UVA exposure on drug sensitive as well as multi-drug resistant bacteria, fungi, and *Acanthamoeba*. We used the standard Dresden protocol including 30 minutes of soaking time with 0.1% riboflavin in dextran and a 30-minute exposure of 3 mW/cm^2 to 370 nm UV light. We conducted our experiments at LV Prasad Eye Institute in four arms, control, riboflavin only, UV only, and combined

Table 1: Cross-linking for microbial keratitis

Study (year)	No. of eyes	Organisms identified	Outcomes	Adjunctive medication	Additional surgery during active infection	Comments
Iseli *et al.*[11] (2008)	5	Non-tuberculous mycobacterium-three eyes *Fusarium*, *Acremonium*-one eye each	Progression of keratitis stopped	Antibiotics, antifungals, corticosteroids	Keratoplasty in one eye	Post LASIK interface infection in four eyes
Micelli Ferrari *et al.*[12] (2009)	1	*Escherichia coli*	Healed	Antibiotics	None	Single 5-minute treatment session
Ehlers *et al.*[13] (2009)	8	Not mentioned clearly "*Varicella zoster* virus keratitis" in one eye "*Acanthamoeba* keratitis/recurrence" in three eyes "Bacterial keratitis" in two eyes "*Fusarium* recurrence/fungal keratitis"-one eye each	No clear effect in four eyes "Some healing" in two eyes Healing in two eyes	Not mentioned	Amniotic membrane transplant in three eyes Keratoplasty in one eye Enucleation in one eye Keratoplasty followed by enucleation in one eye	Characteristics of ulcers, treatment details, causative organisms and outcomes not described clearly
Morén *et al.*[14] (2010)	1	None	Healed	Antibiotics, antifungal, and anti-*Acanthamoeba* drugs	None	Presumed infectious keratitis, microbiology negative
Kozobolis *et al.*[15] (2010)	2	*Streptococcus viridans* in one eye, None in one eye	Healed	Antibiotics	None	Ulceration in bullous keratopathy, microbiology negative in one case
Makdoumi *et al.*[16] (2010)	7	*Staphylococcus aureus*-2 eyes, *Moraxella lacunata, Hemophilus influenzae, Staphylococcus epidermidis* None-in three eyes	Healed	Antibiotics	Amniotic membrane transplant in two eyes	Small ulcers, antibiotics given for very short time or not given prior to CXL
Khan *et al.*[17] (2011)	3	*Acanthamoeba*-two eyes None in one eye	Healed	Antibiotics, povidone iodine, trifluridine, anti-*Acanthamoeba* drugs	None	Two treatment sessions for each eye
Garduño-Vieyra *et al.*[18] (2011)	1	*Acanthamoeba*	Healed	Antibiotics	None	Resolution without use of anti-*Acanthamoeba* drugs
Anwar *et al.*[19] (2011)	2	*Staphylococcus aureus* in one eye *Aspergillus* in one eye	Healed	Antibiotics, antifungals	None	Multiple deep vessels with intra-corneal bleed in one eye
Kymionis *et al.*[20] (2012)	1	Atypical *Mycobacterium*	Healed	Antibiotics, corticosteroid	Phototherapeutic keratectomy, flap amputation	
Panda *et al.*[21] (2012)	7	None in six cases One case-hyphae on KOH mount, bacterial growth on blood agar, and *Acanthamoeba* positive in polymerase chain reaction	Healed	Antibiotics, antifungals, antiviral, and anti-*Acanthamoeba* drugs	None	Microbiology negative in six cases, confusing results in one case

Contd...

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Study (year)	No. of eyes	Organisms identified	Outcomes	Adjunctive medication	Additional surgery during active infection	Comments
Price *et al*.[22] (2012)	40	Bacteria-24 eyes Fungus-seven eyes Acanthamoeba-two eyes (Confocal microscopy) Herpes simplex-one eye None in six eyes	Resolution-25 eyes Lost to follow up-two eyes Outcomes not reported in six eyes	Antibiotics, antifungal, anti-viral, and anti-acanthamoeba drugs	Keratoplasty in six eyes Intracorneal voriconazole injections-two eyes Superficial keratectomy in one eye	Multiple different treatment protocols used Heterogeneous group of infections included
Li *et al*.[23] (2013)	8	*Fusarium*-six eyes *Aspergillus*-two eyes	Healed	Antibiotic and antifungal drugs	None	Small ulcer sizes
Rosetta *et al*.[24] (2013)	4	*Pseudomonas*-two eyes Acanthamoeba-one eye *Streptococcus pneumonia*-one eye	Healed	Antibiotics, anti-*Acanthamoeba* drugs	None	Modified protocol-no epithelial debridement, use of hypo-osmolar riboflavin
Ferrari *et al*.[25] (2013)	1	Herpes virus	Corneal melt, thinning, impending perforation	Antibiotics, antiviral drugs	Keratoplasty	

riboflavin + UV. We found the group with combined riboflavin and UV exposure had the greatest efficacy in reducing growth of the exposed microbes. In our experience, the treatment was most effective against bacterial isolates, with drug resistant strains requiring multiple exposures. We have not been able to demonstrate arrest the growth of fungi or *Acanthamoeba in vitro* with this treatment. Looking at the mixed published results as well as our own experimental data, we have been hesitant thus far to use CXL as a therapeutic option in cases of microbial keratitis. We have, however, managed cases treated elsewhere, which seemed to show equivocal results. We haven't yet seen a patient with either fungal or *Acanthamoeba* keratitis where CXL helped in resolution of the infectious process following failure of specific therapy. We believe the next steps should be aimed at evaluating the response of the cornea with active keratitis to CXL and changes that occur over time rather than a cross-sectional observation. The ideal model for this kind of data would be an animal model of keratitis treated with CXL to observe changes in histology at various stages following the exposure.

Discussion

The promise of a simple, effective, and safe alternative to anti-microbial medication or keratoplasty is somewhat of a holy grail in the management of microbial keratitis. Harnessing the antimicrobial properties of UVA-activated riboflavin sounds biologically plausible. Putative mechanisms include the genome damage resulting from intercalation of activated flavins with nucleic acids and direct free radical insult to microbial deoxyribose nucleic acid (DNA), in addition to the presumably increased resistance of the cornea to melting due to CXL-induced stiffening. Proof of principle exists, as demonstrated by the successful use of this technology in transfusion medicine.

In vitro studies show mixed results. The modality seems to be able to inhibit growth of bacteria, with drug-resistant strains requiring greater exposure time. Studies on fungi have conflicting results, and activity against *Acanthamoeba* seems even less convincing. Clinical reports are marred by poor study designs. Most cases reported include ulcers that would probably heal well with adequate duration of standard of care therapy. Ethical constraints preclude the use of CXL in isolation, without first using anti-microbial drugs. Potential concerns include endothelial damage in corneas that are already thin, as well as corneal melts and reactivation of viral keratitis. Most investigators have directly extrapolated the protocols using in CXL for keratoconus. Gray areas include optimum concentration of riboflavin and UVA exposure duration needed to combat microbes.

Based on available evidence, it is difficult to say whether CXL is effective in cases of microbial keratitis where we need it the most-drug resistant organisms, advanced keratitis and keratitis caused by organisms such as fungi and *Acanthamoeba*, which are refractory to conventional medical therapy. An ideal study would be prospective, with an adequate sample size, well-defined inclusion and exclusion criteria, appropriate outcome measures, and unbiased assessment as well as a robust interpretation of data. The preliminary results reported by Price *et al*., are part of an ongoing prospective, multi-center study.[22] We hope the eventual results would plug some of gaps in existent knowledge.

Conclusion

Parallels in transfusion medicine and data from laboratory experiments indicate the photochemical reaction used in CXL holds promise as a future therapeutic option for microbial keratitis. Clinical reports are inconsistent and difficult to interpret. Well-designed studies investigating the safety and efficacy of this modality in appropriately chosen cases of microbial keratitis are sorely needed.

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