

Therapeutic and inducing effect of corneal crosslinking on infectious keratitis

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Abstract

• **The corneal crosslinking (CXL) with riboflavin and ultraviolet –A (UVA) is a new therapy method to successfully treat infectious keratitis in clinical practice. However, there are rare reports on the complications of CXL such as the secondary keratitis. The diverse clinical outcomes on keratitis have highlighted the necessity to further evaluate the efficacy and complications of CXL. We reviewed the positive and negative reports on UVA/riboflavin related with keratitis and provided our opinion on the therapeutic and side effect of UVA/riboflavin crosslinking on keratitis.**

• **KEYWORDS:** corneal crosslinking; keratitis; therapeutic and inducing effect

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INTRODUCTION

Infectious keratitis is a commonly encountered disease in clinical practice. Routine anti-microbial treatments are often less effective in some severe cases especially in keratitis with fungi or non-responsive bacterial. Corneal crosslinking (CXL) was firstly introduced into the treatment of keratoconus in 2003 by Wollensak *et al*^[1]. The basic principle is that the exposure of cornea saturated with riboflavin to UVA with a wavelength of 370 nm and energy density of 3 mW/cm², it induces additional covalent bindings between amino/groups of collagen fibers and aims to enhance

the corneal intrinsic biomechanical property and the stiffness of cornea to resist ectasia of cornea^[1]. Besides its original application for the keratoconus and keratectasia^[2], CXL has been utilized onto the treatment of infectious keratitis^[3], nowadays. Although the secondary infectious keratitis after CXL is rare, there are some reports on secondary keratitis infected by bacteria, fungi, herpes simplex virus and Acanthamoeba. This rare complication of CXL can cause serious ocular morbidity and have a subsequent damaging effect on the patient's vision. The surgical technique of CXL involves the removal of epithelium intraoperatively and the application of contact lens postoperatively. These factors have been associated with the occurrence of infectious keratitis after CXL. In present study, we summarized the therapeutic effect of CXL on infectious keratitis and the keratitis secondary to cornea CXL reported by previous studies, and provided our opinion on the therapeutic and inducing effect of CXL on keratitis.

TREATMENT OF KERATITIS WITH CORNEAL CROSSLINKING

In Vitro Studies of Corneal Crosslinking on Keratitis

Laboratory studies have revealed that UVA irradiation combined with riboflavin solution administration eradicates or inhibits the growth of bacteria^[4], *Staphylococcus aureus*, *Pseudomonas aeruginosa*^[5] and *Fusarium solani*^[6], however, a negative anti-microbial effect of CXL was reported by other studies on *Candida albicans*^[4,7], *Acanthamoeba*^[8-9], and *Fusarium solani*^[7]. Before crosslinking treatment, patients often receive antibiotic and steroid treatment, and it is difficult to isolate the action of the crosslinking with other treatments. It is possible that the combination of treatments and the significantly smaller number of cysts and trophozoites in the cornea during the infection allowed the eradication of the amoeba. The mostly used irradiation pattern in previous studies is 30min exposure to UVA with energy density 3 mW/cm². The anti-microbial effect of UVA seems in an exposure-duration dependent pattern, as Makdoui *et al*^[10] revealed that the 60min exposure of bacterial suspension to UVA eradicates the microorganism in solution, while 30min exposure has limited effect of eradication.

Clinical Studies of Corneal Crosslinking on Keratitis

Clinically, CXL has been described effective in treating keratitis cases with *Escherichia coli*^[11], *Acanthamoeba*^[12-14],

Pseudomonas aeruginosa^[15], *Staphylococcus aureus*, *Aspergillus*^[16-17], *Fusarium*^[17], other types of bacteria^[18], and herpes virus^[18]. However, some reports also revealed that CXL can effectively treat infectious keratitis but excluding viral keratitis^[19]. Chan *et al*^[20] also reported the role of CXL in infectious keratitis remained unclear despite the reported success in some clinical cases, Kymionis *et al*^[21] reported corneal melting resolved after CXL treating. Uddaraju *et al*^[22] reported CXL used as adjuvant therapy for recalcitrant deep stromal fungal keratitis, however, of the 13 cases enrolled in the study, five eyes in the CXL group (6 eyes) and 3 eyes in the non-CXL group (7 eyes) experienced treatment failure. In a secondary analysis, the CXL group experienced more perforations than the non-CXL group (4 vs 0)^[22-23]. CXL treatment was simultaneously combined with some surgical procedures including keratoplasty^[24], amniotic membrane transplantation^[25], conjunctival flap covering^[21], *etc*. The exposure duration of UVA during CXL treatment ranged from 5 to 45min, as well as a variation of riboflavin concentration. While the total energy is maintained, according to the Bunsen-Roscoe photochemical law of reciprocity, the effects of photochemical reaction are similar even if the time and intensity change^[3]. The assessed clinical outcomes include the healing of corneal ulcer, the stopping of the progression of corneal melting, and the disappearing of corneal edema, *etc*. To date, most clinical studies were performed in a retrospective and small scale pattern.

Basic Mechanisms of Corneal Crosslinking on Keratitis

At least three mechanisms may contribute to the therapeutic effect of CXL on infectious keratitis. Firstly, the cornea received CXL is more resistant to the digestion of protease or collagenase. Spoerl *et al*^[26] reported that the dissolution of cornea was observed on day 5, 13 and 14 in CXL treated porcine corneas immersed in trypsin, pepsin and collagenase solution, while the corresponding time was day 2, 6 and 6 in untreated corneas. Secondly, the exposure to UVA or free radicals leads to the damage of DNA of microorganism^[27], by which eliminates or suppresses the proliferation of pathogens. Last but not least, the corneal stiffness induced by CXL is a putative reason for the resistance to melting^[28].

THE SECONDARY KERATITIS DUE TO CORNEAL CROSSLINKING

Diverse Secondary Keratitis Although the CXL treatment has been utilized in infectious keratitis, CXL itself also leads to varieties of complications including secondary keratitis^[29]. These phenomena have been reported by numerous studies performed CXL on patients with non-infectious cornea disorders. 1) Bacterial keratitis has been reported 3d following CXL, and corneal scraping revealed an *Escherichia coli* infection. After treatment, the keratitis resulted in an avascularized corneal scar and permanent reduction of the visual acuity^[30]. Another cases which showed different

polymicrobial keratitis caused by *S epidermidis*^[31], *Staphylococcus aureus*. and Methi-cillin Resistant *Staphylococcus aureus* (MRSA)^[32], *Streptococcus salivarius* and *S. oralis*^[33] had been reported. Secondary keratitis after cornea scrapings has also been reported recently^[34]. Fortunately, following treatment, the ulcer decreased in size, only leaving a corneal scar. 2) *Fusarium* keratitis 3wk after healed CXL has been reported in 2010^[35]. Corneal scrapings revealed fungal hyphae, which were later identified as *Fusarium solani*. The patients were treated with topical amphotericin B and voriconazole for 2mo. The outcomes revealed *Fusarium* species could cause infectious crystalline keratopathy after CXL treatment. 3) Dendritic and geographical herpes simplex keratitis with or without iritis after CXL has been reported^[36-37]. The patients had no history of herpetic keratitis. The diagnosis of herpes simplex virus (HSV) was confirmed with polymerase chain reaction (PCR) tear analysis. It was reported emotional stress, trauma, fever, and laser surgery could trigger HSV^[38]. The UV-A induced the reactivation of a latent HSV infection in the process of CXL^[19]. The mechanism could be the trauma of corneal epithelial and stromal or the damage of corneal nerves. 4) *Acanthamoeba* keratitis associated with corneal melting 5d after CXL has been reported. The patient washed eye with tap water several times because of ignoring the bandage contact lens being inserted^[39]. The rapid progression of corneal melting led to the corneal perforation, so the therapeutic penetrating keratoplasty (PKP) was performed. During the procedure of CXL, deepithelialization and contact lens placement increase the risk of infection.

Risk Factors There are some risk factors that may predispose a patient to a corneal infection: the corneal epithelium debridement, the application of a contact lens, the postoperative overuse of a steroidal drop, ocular surface diseases (dry eye, blepharitis, keratoconjunctivitis, *etc*) and patient's poor hygiene. Epithelial removal destroyed the important defense barrier of an intact corneal epithelium. Most of the ocular pathogens cannot pass through an intact epithelium. When the epithelium is removed, the defense mechanism is loss and bacteria are free to invade into cornea^[40]. On the other hand, the application of soft contact lens increases the risk of infection because bacteria may adhere to the ocular surface and form biofilm on the lens. Besides, contact lens changes the ocular surface biochemistry. Those risk factors may reduce the resistance of the cornea to infection^[41]. The postoperative overuse of a steroidal drop may create the risk for corneal infection^[42]. Topical overusage NSAIDs could impair corneal epithelial healing, decrease corneal sensitivity, and inhibit the proliferation of keratocytes^[43]. Aside from the above-mentioned risk factors, ocular surface diseases (dry eye, blepharitis, keratoconjunctivitis, *etc*) and patient's poor hygiene may play an important role in corneal infection^[33,39].

DISCUSSION

Although the clinical practice has demonstrated the plausible effect of CXL in some cases, the result is controversial between the laboratory data and clinical outcomes, even in the clinical outcomes themselves. The effectiveness of CXL on *Acanthamoeba* have been revealed by some reports^[14-16], however, laboratory result showed a negative result on such pathogen^[10-11]. A possible reason for the controversies is that there was not a standard protocol for CXL in previous laboratory and clinical studies, in which the exposure duration, energy density, the concentration of riboflavin solution should be homogenized. However, much more data are needed before establishing a standard for CXL to be used in clinical practice. The questions needed to be elaborated include the relation of UVA energy and the anti-microbial efficacy on different types of pathogen, the effective depth of UVA in infectious cornea with different severities of ulcer, the response of endothelium to CXL in pathologic cornea, a long-term follow-up to reveal the biomechanism changes of pathological cornea after CXL, *etc.* Another reason for the varieties of clinical outcomes is the complicated condition of patients before CXL treatment and the diverse complementary treatments, including the topical or systemic administration of anti-microbial drugs, the complementary surgeries, and the wearing of contact lens after CXL. Though lack of blank control, the efficacy of CXL has been verified as the evident by the comparison between the clinical presentation before and after CXL treatment. More well designed studies with a standard CXL procedure and long-term follow-up in a multi-center and large scale pattern are necessary to further substantiate the efficacy of CXL on infectious keratitis. To avoid performing CXL on cornea with herpetic keratitis is highly suggested based on present documents. The effect of riboflavin on cornea metabolism should also be studied, for no related documents was found to date. Nowadays there are several CXL protocols, including accelerated CXL, routine CXL, and trans-epithelium CXL. The effect of different CXL modalities on keratitis secondary to CXL and the efficacy of CXL with different protocols on treating keratitis should be elaborated in the future studies.

The removal of corneal epithelium theoretically assists the penetration of the pathogen into the deep stroma of cornea and should be regarded as an important cause for the secondary keratitis after CXL. Considering the effective depth of routine CXL procedure with a UVA density of 3 mW/cm² is 300 μm in corneal stroma, some pathogen in deep stroma should not be completely eliminated with CXL alone. Furthermore, the penetration ability of UVA in pathological cornea was weaken since corneal transparency decreased. The activation of residual virus inside stroma by UVA^[32] may partly explain the viral derived keratitis after CXL. Most of the previous studies used contact lens after

CXL to promote epithelial healing and decrease pain, however, the contact lens, especially soft lens will inevitably impede the oxygenation of the cornea and interfere with the metabolism of cornea tissue, which in turn reduces the resistance ability of cornea to microbial.

To date, the mechanism of some non-infectious keratitis after CXL including cornea haze and melting is still needed to be explored. More standardized clinical studies combined laboratory works are needed to explore the mechanism underlying these phenomena.

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