Therapeutic effect of corneal crosslinking on infectious keratitis

Efeito terapêutico do crosslinking *corneal na ceratite infecciosa*

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ABSTRACT

Purpose: *To evaluate the effect of corneal crosslinking (CXL) in the treatment of infectious keratitis resistant to medical treatment, and investigate the relation with the CXL outcome to the etiologic agent.* ***Methods:*** *The study included 11 patients who were diagnosed with bacterial (seven eyes) or fungal keratitis (four eyes) at Altino Ventura Foundation from october 2011 to may 2012. All patients were using antibiotic eye drops for at least 7 days and have had no infection improvement. Patients were evaluated prior to CXL and the postoperative period until healing of the keratitis. For CXL, eyes were first instilled with a solution containing 0.1% riboflavin and 20% dextran for 30 min at a 5-minutes interval. Riboflavin-soaked eyes were then irradiated with UVA light (370ηm ± 5ηm) at 3mW/cm² for 30 minutes.* ***Results:*** *Eyes with bacterial infection exhibited improvement of infectious symptoms after CXL whereas eyes with fungal keratitis showed no improvement. Thus, there was a statistically significant correlation (p = 0.003) between the etiologic agent and the effectiveness of healing.* ***Conclusion:*** *CXL was effective in the treatment of bacterial keratitis resistant to clinical treatment, eliminating the need for surgery. However, CXL was not effective in managing fungal keratitis.*

Keywords: *Cornea; Crosslinking reagents/therapeutic use; Infectious keratitis/drug therapy; Bacterial keratitis/drug therapy; Fungal keratitis/drug therapy*

RESUMO

Objetivo: Avaliar o efeito do *crosslinking* (CXL) no tratamento de ceratite infecciosa, resistente ao tratamento clínico, e investigar a relação com o agente etiológico. **Métodos:** Foram incluídos 11 pacientes com diagnóstico de ceratite infecciosa de etiologia bacteriana (sete olhos) e fúngica (quatro olhos) na Fundação Altino Ventura (FAV) no período de outubro de 2011 a maio de 2012. Os pacientes incluídos estavam em uso de colírios há pelo menos sete dias e não apresentavam melhora da infecção. Estes foram avaliados antes da realização do CXL e no período pós-operatório até cicatrização da úlcera. Para realização do CXL foram instiladas gotas de riboflavina a 0,1% e dextrano a 20%, a cada cinco minutos em um período de 30 minutos antes do procedimento, e durante a aplicação da luz ultravioleta A (UVA). A córnea foi exposta à UVA com comprimento de onda de 370ηm ± 5ηm e uma irradiância de 3mW/cm². **Resultados:** Os pacientes com infecção bacteriana obtiveram cura do processo infeccioso após o CXL e nenhum paciente com ceratite fúngica apresentou cicatrização. Observou-se associação significante (p = 0,003) entre o agente etiológico e a cicatrização. **Conclusão:** O CXL mostrou-se eficaz no tratamento da ceratite bacteriana resistente ao tratamento clínico, evitando a realização de transplante tectônico. Em relação à ceratite fúngica, este procedimento não influenciou na melhora do processo infeccioso.

Descritores: Córnea; Reagentes para ligações cruzadas/uso terapêutico; Ceratite infecciosa/quimioterapia; Ceratite bacteriana/quimioterapia; Ceratite fúngica/quimioterapia

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The authors declare no conflict of interest

Received for publication: 26/11/2012 - Accepted for publication: 31/7/2013

Introdução

Infectious keratitis is a common and preventable eye condition[1-3]. It is a leading cause of monocular blindness in developing countries[1-4]. Most cases are bacterial, but the condition can also be caused by fungi, protozoa, and viruses[5-9]. The current standard therapy for bacterial and fungal keratitis is based on antibiotics and antifungals[10,11]. Although this is effective in most cases, some classes of microorganisms have been shown to be resistant to available antimicrobial agents[12,13].

Thus, even with timely treatment, the inflammatory process may progress with worsening ulceration, corneal melting, and ocular perforation[4,8]. When medical treatment fails, surgical procedures such as a conjunctival flap or corneal transplantation can be used[14-16]. Studies show that therapeutic transplants represent 2.6% to 17.9% of total penetrating transplants[17].

There are reports of corneal ulcers treated successfully with corneal collagen crosslinking (CXL)[18-25]. An experimental study found increased resistance to enzymatic digestion by collagenase, pepsin, and trypsin in corneas subjected to CXL[26]. Ultraviolet A light (UVA) associated with riboflavin acts as photomediator, causing riboflavin molecules to lose their internal balance and creating oxygen free radicals that induce new links between collagen fibrils. This restructuring of collagen fibres increases the biomechanical strength of the cornea. Oxygen free radicals have an antimicrobial effect as they interfere with the integrity of the bacterial cell membrane. Thus, the combination of UVA and oxygen free radicals produced by CXL can act synergistically to treat corneal ulcers both through their antimicrobial action and the protection of collagen against degradation by proteolytic enzymes[27-31].

Currently, CXL is usually indicated for cases of keratoconus[30], pellucid marginal degeneration[31], painful bullous keratopathy[32], and iatrogenic ectasia after refractive surgery with laser in situ keratomileusis (LASIK) or photorefractive keratectomy (PRK)[30]. The therapeutic effects of CXL in infectious keratitis are still unclear[18-26].

This study aimed to evaluate the effect of CXL on infectious keratitis resistant to medical treatment.

Methods

We conducted a nonrandomized clinical intervention study. Patients were selected from the cornea and external diseases outpatient unit and the emergence service of Altino Ventura Foundation (FAV) from October 2011 to May 2012.

Eleven patients aged 18-70 years and diagnosed with bacterial or fungal corneal ulcer were included. All selected patients had been using eye drops for at least seven days without improvement of the infectious process, defined as decreased pain, decreased density of the infiltrate and stromal oedema, re-epithelisation, more distinct margins, decreased anterior chamber reaction, or decreased hypopyon. Patients with corneal thinning or perforation, endophthalmitis, changes in the position or function of the eyelids, and those who did not agree to participate in the study were excluded.

Material for cytology, bacterioscopy, and culture was collected at the onset of the condition, when the patient came to our clinic. Material was collected from the ulcer area using a Kimura spatula. The material was seeded in blood agar, chocolate agar, Sabouraud agar, and liquid medium enriched with Brain Heart Infusion (BHI); direct examination was also performed using Gram and Giemsa staining. The material was analysed by the Laboratory of Medical Mycology of the Federal University of Pernambuco (UFPE) and the Laboratory of the University Hospital of UFPE.

Patients with bacterial keratitis had been using antibiotic eye drops according to the unit's protocol, which consists of commercially-available fluoroquinolones (moxifloxacin and gatifloxacin) for superficial ulcers with less than 3 mm in any dimension and located in the peripheral cornea. In such cases, the initial dosage was one eye drop every minute for five minutes (five drops) followed by one drop every five minutes for 15 minutes (three drops), and then one drop every hour.

For severe ulcers larger than 3 mm in any dimension or affecting the visual axis, initial treatment was topical application of a combination of two stronger agents (preferably one against gram-positive and the other against gram-negative organisms). For gram-positive organisms, cefazolin (50 mg/dL) or vancomycin (25 mg/dl) were used; for gram-negative organisms, tobramycin (14 mg/dL), gentamicin (14 mg/dL), or amikacin (25 mg/dl) were used. Patients with fungal ulcers were using 5% natamycin or 0.15% amphotericin B (1.5 mg/dL) eye drops every hour associated with oral ketoconazole, 400mg per day.

Patients were assessed before and after CXL and in the postoperative period until the infectious process was cured. Patients underwent a complete ophthalmic examination, including: far visual acuity (VA) testing; biomicroscopy with examination of the corneal surface with and without fluorescein; measurement of the corneal ulcer in its greater horizontal and vertical dimensions; and graphical representation of the condition. Ultrasound imaging was performed to rule out endophthalmitis.

Thirty minutes before CXL and during the application of radiation, 0.1% riboflavin and 20% dextran eye drops were instilled every five minutes, interspersed with anaesthetic eye drops (proparacaine). If there was some epithelium in the area to be treated, it was removed with a scalpel blade number 15. The cornea was exposed to UVA light with a wavelength of 370 ± 5 nm and an irradiance of 3 mW/cm^2 for a total of 30 minutes, corresponding to a total dose of 3.4 J or a radiation exposure of 5,4 J/cm^2. This was done using the X-Link Corneal Crosslinking System (Opto™) device. Medical treatment with antibiotics and antifungals was maintained after the procedure (Figure 1).

We recorded each patient's age in years, profession, LogMar VA before and after CXL, estimated ulcer area by multiplying its vertical and horizontal size in millimetres (mm), whether the infectious process was cured, healing time, and the aetiological agent.

Cure was defined as an absence of stromal infiltrate with or without re-epithelisation.

The results of qualitative variables were presented in tables as absolute and relative frequencies. For quantitative variables, the mean, median, standard deviation, and minimum and maximum values were used to indicate the variability of data.

The Spearman correlation test was used to check whether there was a correlation between ulcer area and healing time. The nonparametric Wilcoxon test was used to check the difference in visual acuity before and after CXL. Results whose descriptive levels (p-values) were less than 0.05 were considered statistically significant. Statistical analysis was done using SPSS (Statistical Package for the Social Sciences) software for Windows version 18.0.

The study was approved by the Research Ethics Committee of FAV. All subjects were explained the aim and methods of the

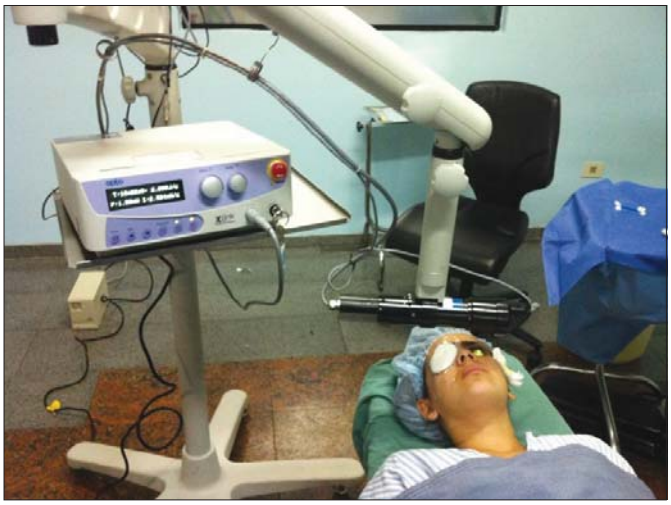

Figure 1. Patient undergoing corneal collagen crosslinking for infectious keratitis

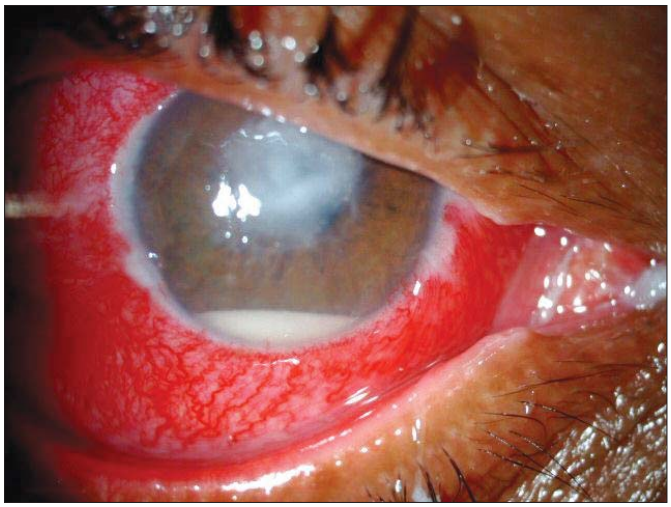

Figure 2. Corneal ulcer of fungal aetiology (*Fusarium sp.*). Patient number 10

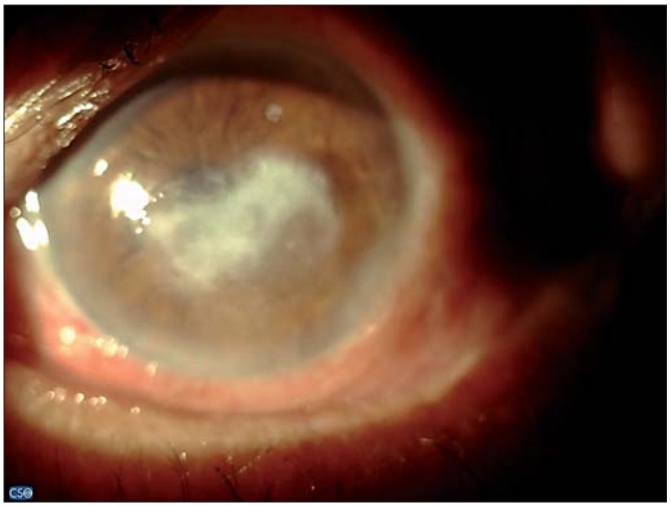

Figure 3. Bacterial ulcer by *Staphylococcus aureus*. Patient number 1

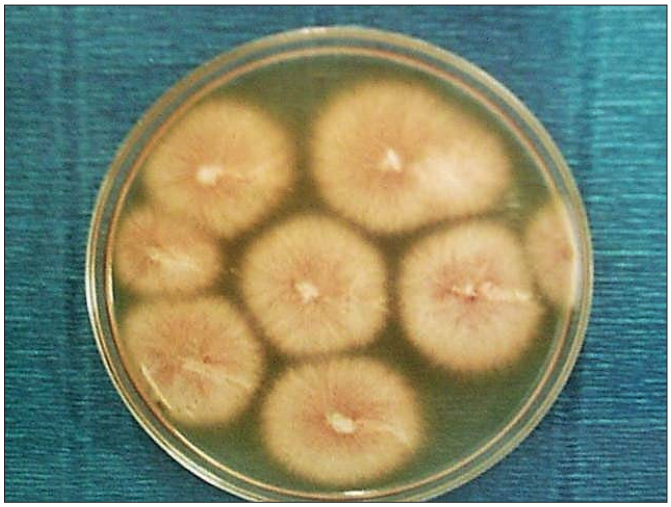

Figure 4. Culture of *Fusarium sp*. Patient number 7

study and gave their Free and Informed Consent.

The procedure was considered safe and without significant long-term problems with regard to loss of corneal and lens transparency, postoperative endothelial cell count, and retinal damage[27-29].

Results

Figure 1 shows the sample distribution according to demographic variables. Of the 11 patients, 7 (63.6%) were positive for bacteria and 4 (36.4%) for fungi. Bacterial organisms were: *Staphylococcus aureus* (two patients), *Staphylococcus epidermidis* (one patient), *Streptococcus sp.* (one patient), *Pseudomonas aeruginosa* (one patient). Two patients had negative cultures, but their cytology showed gram-positive cocci. Fungal organisms were: *Fusarium sp.* (three patients) and *Aspergillus sp.* (one patient) (Figures 2, 3 and 4).

Sample distribution according to clinical parameters of corneal ulcer is shown in Table 2. Sample distribution according to culture, ulcer characteristics, and healing time is shown in Table 3.

Analysis of the correlation between ulcer area and healing time in the seven patients with bacterial ulcers showed a positive trend, with larger ulcers taking more days to heal ($r = 0.775$ $p = 0.041$, Spearman correlation test) (Figure 5).

Regarding the best final corrected VA compared with initial VA, six (54.5%) patients improved, two (18.2%) remained unchanged and three (27.3%) worsened (Table 4). There was no statistically-significant difference between final and initial VA ($p = 0.4393$).

All patients whose VA worsened had a diagnosis of fungal ulcer.

Table 5 shows that there was a statistically-significant association ($p = 0.003$) between aetiological agent and healing.

Two patients (18.2%) with bacterial ulcer, despite having their infection cured after CXL, required surgery (conjunctival flap) to cure the epithelial defect (Table 6).

The four patients with fungal ulcers (36.4%) required additional surgery to improve the infectious process. Three patients underwent tectonic corneal transplantation and one patient required a conjunctival flap associated with anterior chamber wash with amphotericin B (Table 6).

Table 1

Sample distribution according to demographic variables

Variable	N (%)
Male patients	7 (63,6%)
Age (years)	
Mean (SD)	43,9 (14,5)
Minimum – Maximum	18 – 69
Diabetes mellitus	1 (9,1%)
Occupation	
Farmer	2 (18,2%)
Hairdresser	1 (9,1%)
House maid	2 (18,2%)
Student	2 (18,2%)
Driver	1 (9,1%)
Construction worker	1 (9,1%)
Locksmith	1 (9,1%)
General services	1 (9,1%)

Sample: 11 patients. SD, standard deviation

Table 2

Sample distribution according to clinical variables

Variable	N (%)
Ulcer size	
Mean (SD)	23,1 (10,6)
Minimum – Maximum	8,7 - 48,2
Aetiology	
Bacterial	7 (63,6)
Fungal	4 (36,4)
Cure of infection	
No	4 (36,4)
Yes	7 (63,6)
Healing time (days)*	
Mean (SD)	14,4 (2,4)
Minimum – Maximum	11 - 18
Additional surgery	6 (54,5)

Sample: 11 patients. SD, standard deviation. *Only for the 7 patients whose infection was healed

Table 3

Sample distribution according to ulcer size, area, healing time, and culture

Patient	Ulcer size (horizontal x vertical) (mm)	Estimated ulcer area (mm^2)	Cure of infection	Healing time	Culture
1	5,0x4,0	20,00	Yes	13 days	*S. aureus*
2	5,8x5,2	30,16	Yes	15 days	Negative*
3	3,2x7,0	22,40	Yes	18 days	*S. epidermidis*
4	6,6x7,3	48,18	Yes	17 days	*S. aureus*
5	4,0x6,5	26,00	Yes	14 days	Negative*
6	3,7x3,9	14,43	Yes	13 days	*P. aeruginosa*
7	5,0x4,5	22,50	No	Did not heal	*Fusarium sp.*
8	5,7x2,4	13,68	Yes	11 days	*Streptococossp*
9	3,1x2,8	8,68	No	Did not heal	*Fusarium sp.*
10	5,0x5,8	29,00	No	Did not heal	*Fusarium*
11	4,5x4,2	18,90	No	Did not heal	*Aspergilus*

*Cytology showing Gram-positive cocci. mm, millimetres

Discussion

Different strategies have been proposed to manage infections by organisms resistant to multiple drugs. There are reports of eye infections by bacteria resistant to newer antibiotics, raising concerns that the antibiotic arsenal currently available may not be sufficient in the future[12,13,33,34].

In our study, CXL associated with topical treatment resulted in resolution of the infectious process in all patients with bacterial ulcers, and none of them required tectonic transplantation. However, due to the small sample size, it cannot be said that all patients with bacterial keratitis resistant to medical treatment will improve with CXL.

Makdoumi K et al. used riboflavin and UVA as a primary treatment, without topical antibiotics, to treat bacterial ulcers caused by *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*; in 14 out of 16 patients included in

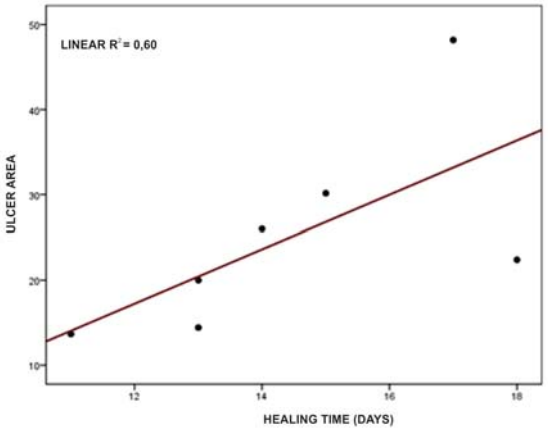

Figure 5. Relationship between ulcer area and the healing time

Table 4

Visual acuity before and after CXL

Patient	VA Initial (LogMar)	Final (LogMar)
1	0.92	0.60
2	2.00	1.80
3	3.00	3.00
4	1.92	1.70
5	2.00	1.80
6	2.00	1.60
7	3.00	3.00
8	2.00	0.92
9	0.60	2.00
10	1.80	2.00
11	1.70	1.92
Mean ± SD	1.90±0.72	1.85±0.72

p-value = 0.4393 (Wilcoxon test). VA, far visual acuity; SD, standard deviation

Table 5

Outcomes for each aetiological agent

	Cure of infection				Total	
	No		Yes			
Culture type	N	%	N	%	N	%
Bacterial	0	0	7	100,00	7	63,6
Fungal	4	100,00	0	0	4	36,4
Total	4	100,00	7	100,00	11	100,00

p-value = 0,003 (Fisher test)

Table 6

Type of culture, complications, and additional surgical procedures

Patient	Culture	Healing time (days)	Complications	Additional surgical procedures
1	*Staphylococos aureus*	13		
2	Negative*	15		
3	*Staphylococos epidermidis*	18	Epithelial defect without infection	Conjunctival flap
4	*Staphylococos aureus*	17		
5	Negative*	14		
6	*Pseudomonas aeruginosas*	13		
7	*Fusarium sp.*	Did not heal	Persistent infection	Tectonic transplantation
8	*Streptococos sp.*	11	Epithelial defect without infection	Conjunctival flap
9	*Fusarium sp.*	Did not heal	Persistent infection	Anterior chamber wash + conjunctival flap
10	*Fusarium sp.*	Did not heal	Persistent infection and corneal perforation	Tectonic transplantation
11	*Aspergilus sp.*	Did not heal	Persistent infection and corneal thinning	Tectonic transplantation

*Cytology showing Gram-positive cocci

this study the lesions healed, with consequent improvement in visual acuity without the need for antibiotic therapy[23].

Martins SA et al. studied the in vitro response of *Staphylococcus epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* to CXL. The combination of UVA and riboflavin was more effective in reducing bacterial counts than UVA alone. This effect was greater for gram-positive bacteria compared with *Pseudomonas aeruginosa*. In this study, riboflavin alone had no effect as an antibacterial agent[26].

In another in vitro study, a dose of 10.8J/cm^2, corresponding to 60 minutes of UVA irradiation, led to higher rates of eradication of every organism included in the study. Increased UVA irradiation time was more effective in fighting bacterial growth, probably due to increased oxidative stress[35]. In our study, irradiation time was 30 minutes, with a dose of 5.4 J/cm^2. This dose and time were based on treatment protocols for keratoconus and are below the threshold known to damage the endothelium, lens, and retina. Different irradiation times or concentrations of riboflavin may be needed to treat infectious keratitis, and may even depend on the microbial species being treated. Each species may respond differently to CXL, with varying sensitivity between different organisms depending on the duration of the cell cycle and cell wall structure.

The procedure can also be repeated for refractory cases; however, studies should be conducted to ensure the safety of repeated procedures in a short period of time.

In patients who develop corneal melting there is a destruction of collagen layers of the cornea due to the presence of enzymes such as proteases, collagenases, and elastase. Such patients are at increased risk of ocular perforation. Such enzymes are released at the injury site by bacteria and inflammatory cells[2]. In addition to its antibacterial effect, CXL makes the corneal stroma more resistant to the effects of enzymes such as pepsin, trypsin, and collagenase[26]. Studies show that CXL significantly changes corneal elasticity, produces a 328% increase in rigidity, and increases resistance to proteolytic enzymes. Thus, it increases resistance against microbial degradation and corneal melting[26,35-36].

Even though some clinical trials and experimental studies have shown improvement of fungal ulcers with CXL[21,23], this was not observed in our study. All of our patients with fungal ulcers underwent adjuvant surgical treatment aimed at curing the infectious process.

Fungi are more invasive of corneal tissue and may reach the anterior chamber even with an intact Descemet's membrane. This is due to enzymes that destroy tissues and antimicrobial proteins and the organisms' capacity to survive at high temperatures[37].

CXL acts on the anterior corneal stroma up until a depth of approximately 300 ìm[38]. Thus, deeper infectious corneal infiltrates may be less influenced by CXL. Fungi can penetrate deep into the corneal stroma, which might explain the lower efficacy of CXL for this type of infection.

Most therapeutic transplantations in Brazil are due bacterial and fungal keratitis[39]. In our study, penetrating keratoplasty was avoided in 100% of patients with bacterial keratitis resistant to medical treatment. Complications after therapeutic transplantation are more frequent than after optical transplantation. Therefore, CXL has the potential to prevent emergency transplants. Transplants in these situations have the disadvantage that penetrating keratoplasty tends to be used rather than lamellar grafting. Moreover, even with penetrating keratoplasty the infection can recur in up to 15% of cases. The rate of transplant rejection is also high, ranging from 14.6 to 52.0%[40-43].

In our study, two patients with bacterial keratitis required additional surgery, however the infectious process was cured and conjunctival flap surgery was conducted only to close a persistent epithelial defect. A case series described treatment with CXL in 16 eyes, of which only one required surgery to treat an epithelial defect using amniotic membrane grafting[24].

Conclusion

Our results showed that therapeutic use of CXL through corneal application of ultraviolet A light (UVA) and riboflavin associated with medical treatment was effective in treating bacterial ulcers resistant to medical treatment, thus avoiding tectonic corneal transplantation. However, cure of keratitis was not associated with improved visual acuity. Also, CXL had no effect on fungal keratitis resistant to medical therapy.

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