Induction of corneal collagen cross-linking in experimental corneal alkali burns in rabbits

Indução de ligações covalentes de colágeno em queimaduras corneanas experimentais por álcali em coelhos

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ABSTRACT

Objective: To evaluate the effect of riboflavin-ultraviolet-A-induced cross-linking (CXL) following corneal alkali burns in rabbits.

Methods: The right corneas and limbi of ten rabbits were burned using a 1N solution of NaOH and the animals were then divided into two groups: a control group submitted to clinical treatment alone and an experimental group that was treated 1 h after injury with CXL, followed by the same clinical treatment as administered to the controls. Clinical parameters were evaluated post-injury at 1, 7, 15, and 30 days by two independent observers. Following this evaluation, the corneas were excised and examined histologically.

Results: There were no statistically significant differences in clinical parameters, such as hyperemia, corneal edema, ciliary injection, limbal ischemia, secretion, corneal neovascularization, symblepharon, or blepharospasm, at any of the time-points evaluated. However, the size of the epithelial defect was significantly smaller in the CXL group (p<0.05) (day 15: p=0.008) and the extent of the corneal injury (opacity lesion) was also smaller (day 30: p=0.021). Histopathology showed the presence of collagen bridges linking the collagen fibers in only the CXL group.

Conclusions: These results suggest that the use of CXL may improve the prognosis of acute corneal alkali burns.

Keywords: Cross-linking reagents; Riboflavin; Ultraviolet therapy/methods; Cornea/drug effects; Rabbits; Animal

INTRODUCTION

Eye burns are common and may be caused by various chemical and physical agents including acids, alkalis, high temperatures, and fire¹. They are most generally a consequence of chemical handling accidents and may result in permanent damage to the ocular surface and visual function².

Corneal alkali burns are considered an ophthalmologic emergency. Therefore, timely recognition and implementation of the appropriate treatment represent important steps in controlling the progression of early and late complications³. The literature describes various forms of treatment for corneal alkali burns. These include artificial tears, collagenase inhibitors, therapeutic contact lenses, topical fibronectin, topical vitamin C, topical citrate⁴, conjunctival transplantation⁵, amniotic membrane patching⁶, limbal transplantation⁷, and autologous serum eye drops⁸ as well as treatment of the severe inflammatory processes with topical or systemic corticosteroids.

Chemical burns may lead to devastating complications, including corneal perforation due to the rapid degradation of collagen fibers⁹. Recently, riboflavin-ultraviolet-A-induced cross-linking (CXL) was developed as a technique for enhancing collagen cross-linking in the treatment of corneal wounds. CXL has been reported to be a safe and effective method for controlling the progression of corneal ectasia⁸,⁹. The procedure stops the melting process of the cornea¹⁰ and has been shown to increase the resistance of porcine corneas by inhibiting enzymatic degradation¹¹. CXL was previously used in five cases of corneal necrosis following a bacterial infection refractory to clinical treatment¹², and it was demonstrated that this technique constitutes a useful alternative to emergency keratoplasty by increasing corneal resistance against the action of collagenolytic enzymes. Currently, CXL is used to stabilize degenerative corneal disorders such as corneal ectasia (in keratoconus patients and following refractive surgery), where it acts to increase the degree of rigidity of the stromal collagen fibers¹¹,¹².

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Corneas were anesthetized using a drop of 1% proparacaine hydrochloride (4 mg/kg of weight). Appropriate ventilatory support was provided. Ketamine hydrochloride (25 mg/kg of weight) associated with 2% xylazine hydrochloride was induced by an intramuscular injection of ketamine hydrochloride in the rabbits.

An irradiance of 3 mW/cm² and a surface dose of 5.4 J/cm²; X-link, then followed by irradiation with ultraviolet light (UVA 370 nm, with thirty minutes to the animals in the experimental group. This step was conducted under the supervision of a veterinarian, general anesthesia was induced by an intramuscular injection of ketamine hydrochloride (25 mg/kg of weight) associated with 2% xylazine hydrochloride (4 mg/kg of weight). Appropriate ventilatory support was provided.

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Figure 1. Induction of ocular burns with alkali-immersed filter disks: 14 mm diameter filter paper discs (Whatman filter paper, # 40) soaked in 1N NaOH.
Table 1. Grading of clinical parameters evaluated

<table>
<thead>
<tr>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretion&lt;sup&gt;(18)&lt;/sup&gt;</td>
<td>Absent</td>
<td>Traces of secretion in the cul-de-sac or eyelid margins</td>
<td>Small amount of secretion visible in the conjunctiva and on the eyelid margins</td>
<td>Large amount of secretion, eyeball is still visible</td>
</tr>
<tr>
<td>Hyperemia and chemosis&lt;sup&gt;(19,20)&lt;/sup&gt;</td>
<td>Absent</td>
<td>Slight conjunctival injection</td>
<td>Moderate conjunctival injection with mild chemosis</td>
<td>Moderate to intense injection of vessels, mild chemosis</td>
</tr>
<tr>
<td>Neovascularization</td>
<td>Absent</td>
<td>One vessel extending 0.5 mm from the limbus</td>
<td>One or more vessels, with fewer than five branches, extending 0.5 mm from the limbus</td>
<td>Three or more vessels, or two vessels, with more than five branches, extending 0.5 mm from the limbus</td>
</tr>
<tr>
<td>Ciliary injection&lt;sup&gt;(19,20)&lt;/sup&gt;</td>
<td>Absent</td>
<td>&lt;1 mm</td>
<td>1-2 mm</td>
<td>&gt;2 mm</td>
</tr>
<tr>
<td>Corneal edema&lt;sup&gt;(16)&lt;/sup&gt;</td>
<td>Absent</td>
<td>Present, details of the iris are visible</td>
<td>No details of the iris are visible</td>
<td>Neither the iris nor the pupil is visible</td>
</tr>
<tr>
<td>Limbal ischemia&lt;sup&gt;(16)&lt;/sup&gt;</td>
<td>Absent</td>
<td>Less than one-third of the limbus circumference</td>
<td>Between one-third and half of the limbus circumference</td>
<td>More than half the limbus circumference</td>
</tr>
<tr>
<td>Blepharospasm</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Symbblepharon</td>
<td>Absent</td>
<td>Present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelial defect</td>
<td>Absent</td>
<td>Present</td>
<td>Present</td>
<td></td>
</tr>
<tr>
<td>Corneal injury</td>
<td>Mean between the largest horizontal and the largest vertical measurements of the fluorescein-stained epithelial defect, as measured in millimeters using a surgical caliper (Figure 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

In the present study, we used 14 mm alkali-immersed filter discs (1 N NaOH) to create severe ocular burns (Roper Hall criteria, grade IV). This methodology was chosen because it has been well described and involves clinically comparable parameters that can be easily measured.

We hypothesized that CXL would allow covalent bonds to be formed between the collagen fibrils, thus promoting thickening of the collagen fibrils through the deposit of structural molecules such as proteoglycans<sup>(13,14)</sup>, and possibly making the cornea more resistant to the effect of collagenolytic enzymes. In the CXL group, histopathological examinations revealed collagen bridges linking the collagen fibers in the corneal stroma. This may indicate that the collagen fibers in the CXL group were more resistant to collagenolytic enzymes than those in the control group. Furthermore, the arrangement pattern of the stromal collagen fibers was more organized in the CXL group compared to the control group. This may also indicate a greater resistance of the collagen fibers to collagenolytic enzymes in the CXL group, resulting in improved wound healing. Nevertheless, further investigation is required with respect to these collagen bridges and wound healing. Some case reports have also shown the antimicrobial effect of ultraviolet light associated with riboflavin in the treatment of infectious keratitis. The mechanism is presumably either due to the bactericidal effect of ultraviolet light or to the increased resistance of the collagen fibers of the cornea, which prevents the infectious agent from proliferating<sup>(12,21,22)</sup>.

A statistically significant difference was found between the groups regarding the mean extent of the corneal injury on day 30 following the injury (p=0.021). At this time point the lesion caused by the ocular burn is at a late phase of tissue repair; the effect of collagenolytic enzymes has been largely overcome, and the tissue has undergone regeneration of the fibroblasts, with migration of myofibris to the site and progressive re-epithelization taking place. The associated effects of the increase in resistance to the collagenases in the first few weeks, and the increase in the rigidity of the corneal tissue due to cross-linking, suggests a better recovery in the CXL group compared to the control group. It should be noted that even considering the statistical significance of our results with respect to the mean extent of the corneal injury, when the absolute data is taken into consideration the difference in the mean size of the lesions is close to 1 mm.

The magnitude of this measurement is imperceptible in clinical practice when considering devastating grade IV burns.

Statistically significant results regarding the mean size of the corneal epithelial defect were also found on post-injury days 15 (p=0.008) and 30 (p=0.008). Therefore, we can conclude that the centripetal movement of the corneal epithelial cells that occurs in the regeneration phase of the burned cornea occurred faster in the experimental group compared to the control group. The centripetal movement is associated with an increase in the rigidity of the cornea and the resistance of the tissue to enzymatic degradation through the formation of stronger stroma. This serves as a base for the sliding of the epithelial cells during the recovery phase of the corneal epithelial wound.

Some of the difficulties encountered during this study that merit particular mention refer to the objective measurement of the data obtained from the rabbits, and the manipulation of the animals’ eyes...
when using a hand-held slit lamp. Measuring the greatest horizontal and vertical diameters also proved difficult, since although the lesions were circular and symmetrical throughout their extension, in some cases there were slight discrepancies in re-epithelialization. This occasionally gave rise to asymmetry in the shape of the healing wound. In addition, the sample size used in this study was small (5 rabbits in the CXL group and 5 in the control group). However, it is important to stress that these numbers were agreed upon following an in-depth discussion with the ethics committee for animal research. Importantly, these results suggest that the use of CXL may improve the prognosis of acute corneal alkali burns.

Figure 4. Corneal stroma 30 days after the alkali-burn procedure in the control group. Note the disorganized pattern of collagen fibers and the absence of bridges (arrow) between the stromal fibers. HE staining (A) and trichrome Masson staining (B).

Table 5. Presence or absence of stromal interfibrillar bridges in histological sections of alkali-burned rabbit corneas according to the procedure performed (CXL or none)

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Corneal procedure</th>
<th>Interfibrillar bridges</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CXL</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>CXL</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>CXL</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>CXL</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>CXL</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Control</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Control</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Control</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>No</td>
</tr>
</tbody>
</table>

REFERENCES