Original Article

Variation in Central Corneal Thickness during Open- or Closed-Eye Riboflavin Instillation for Corneal Collagen Cross-linking

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Abstract

Purpose: To assess the effect of having an open or closed eye on the variation in central corneal thickness during riboflavin instillation for corneal collagen cross-linking (CXL).

Methods: Thirty eyes of 15 New Zealand White rabbits underwent an in vivo anterior segment optical coherence tomography (OCT) examination at 0, 10, 20, and 30 min after riboflavin instillation on the de-epithelialized corneal surface. Each eye of every rabbit was randomly placed into one of two different treatment groups (open-eye or closed-eye) during the instillation; the examinations were performed one after the other. After instillation for 30 min, the changes in the corneal stroma and anterior chamber were observed by slit lamp.

Results: A significant decrease in the central corneal thickness (CCT) was demonstrated during riboflavin instillation; the variations were smaller in the measurements performed with the eye closed than with the eye open (81.36±15.13 μm and 129.20 ±12.05 μm respectively). Both methods turned the corneal stroma and anterior chamber yellow.

Conclusion: Keeping the eye closed during riboflavin instillation reduced the decrease in the CCT. The same yellow change in the corneal stroma and anterior chamber occurred, but the exposure time of the ocular surface was shorter. Therefore, keeping the eye closed was a more effective and safer method than keeping the eye open. (***Eye Science 2013***; ***28*:*185–189***)

Keywords: central corneal thickness (CCT); keratoconus; corneal collagen cross-linking (CXL); eyelid

Keratoconus, a progressive degeneration of the cornea, has an incidence of 1 in 2000 and often affects young patients; this condition has been one of the most common indications for keratoplasty[1–3]. Riboflavin/ultraviolet-A (UVA) induced corneal collagen crosslinking (CXL) is the first treatment available that stabilizes the keratoconic process by impacting the pathogenetic cause; it was first used in the 1990s at Dresden University, Germany[1,4]. After placement in the corneal stroma, the riboflavin, which is a photosensitizer, is then irradiated by UVA; this generates reactive oxygen species that induce the formation of chemical covalent bonds that bridge the amino groups of collagen fibrils. This treatment leads to an increase in the biomechanical stabilization and stiffness of the cornea and can be used in keratoconus, post-refractive surgery keratectasia, keratitis, and bullous keratopathy, as well as other conditions[3,5].

Classical CXL requires removal of the corneal epithelium to allow adequate penetration of the riboflavin (molecular weight 376.36 g/mol) into the corneal stroma[6–8]. A 0.1% riboflavin solution in 20% dextran is instilled on the corneal surface for 30 min, and then the yellow coloration of the anterior chamber is examined by slit lamp to ensure sufficient penetration of the riboflavin into the cornea. Corneal pachymetry is measured[3,9–11]. and if the corneal thickness is more than 400 um, then UVA irradiation is applied at 370 nm and 3 mW/cm^2 for 30 min. A thinner cornea has a higher risk of damage[12–13]. Recently, all research has confirmed a statistically significant decrease in the central corneal thickness (CCT) during riboflavin instillation; most research has also shown a possible continual decrease in the CCT during UVA irradiation[14–19]. Therefore, to ensure

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a corneal thickness of more than 400 μm, it is important to minimize the extent of decrease in the CCT, especially in patients with a critical CCT. The effect of the various riboflavin eyedrop compositions on CCT has been reported[18], but the effect on the CCT variation by having the eye open or closed is unclear. Raiskup and Spoerl[3] first pointed out that the lid speculum should be removed during the saturation period but their reasoning was not explained.

This study compared the *in vivo* variations in the CCT during riboflavin instillation when the eye was either open or closed. The amount of riboflavin penetration was examined by a slit lamp. The decrease in the CCT was significantly smaller with the eye closed than with the eye open, although both methods allowed an appropriate amount of riboflavin saturation in the cornea. This study showed that application with the eye closed was safer.

Materials and methods

Study subjects

Fifteen New Zealand white rabbits without eye disease, weighing between 2.0 and 2.5 kg, were used in the experiments. One eye of every rabbit was placed into either the open-eye or closed-eye treatment group; these experiments were performed one after the other. The central 8 mm of epithelium was removed and a 0.1% riboflavin solution in 20% dextran was instilled on the corneal surface for 30 min. An optical coherence tomography (OCT) of the anterior segment was performed at 0, 10, 20, and 30 min after the riboflavin instillation. The yellow changes in the corneal stroma and anterior chamber were observed using a slit lamp. The rabbits were killed postoperatively. All animal procedures were approved by the ethics committee and conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research.

The open-eye group

General anesthesia was induced by an intramuscular injection of a mixture of xylazine hydrochloride (1.5 ml) and ketamine (2 ml) at a dose of 0.3 ml/kg. The rabbits were placed on one side and the eye facing upward was held open with a blepharostat; the central 8 mm of the corneal epithelium was debrided with a spatula. The riboflavin solution was instilled onto the corneal surface every three min for 30 min.

The closed-eye group

The management of the general anesthesia and position in this group was the same as that in the open-eye group. The blepharostat was removed immediately after the epithelial debridement. The riboflavin solution was instilled on the corneal surface every three minutes for 30 min. The eyelids were held open during the riboflavin instillation and gently closed in between instillations.

Anterior segment OCT determination

At every time point, the mean value of three continuous determinations was calculated. The riboflavin film on the corneal surface was not included in the CCT.

Anterior segment photography

The yellow changes of the corneal stroma and anterior chamber after riboflavin instillation were observed and recorded using photography.

Statistical analysis

Matched pair design Student t tests were used to compare the CCT values. Results with a P value less than 0.01 were considered statistically significant. Data analyses were performed using SPSS 16.0 software.

Results

A significant decrease in the CCT was demonstrated during riboflavin instillation; it changed in

Table 1 Central corneal thickness variations during riboflavin instillation(μm)

Group	De-epithelialized cornea	10 min after instillation	20 min after instillation	30 min after instillation	Change	Change rate(%)
Group with eye open	340.58±16.92	217.02±38.19	211.62±29.41	211.38±22.65	129.20±12.05	38.06±4.16
Group with eye closed	341.73±18.05	283.60±31.33	264.51±28.65	260.38±24.76	81.36±15.13	23.89±4.72
t	0.280	9.614	9.293	7.828	10.865	10.040
P	0.7834	0.0000	0.0000	0.0000	0.0000	0.0000

thickness by 1/4 to 1/3. The variations were smaller with the eye closed than with the eye open (81.36 ± 15.13 μm and 129.20 ± 12.05 μm, respectively) (Table 1, Figure 1). The corneal stroma and anterior chamber both turned yellow with each method of instillation (Figure 2). The OCT revealed a higher gray level of the corneal stroma with the eye closed than with the eye open in most cases (Figure 3).

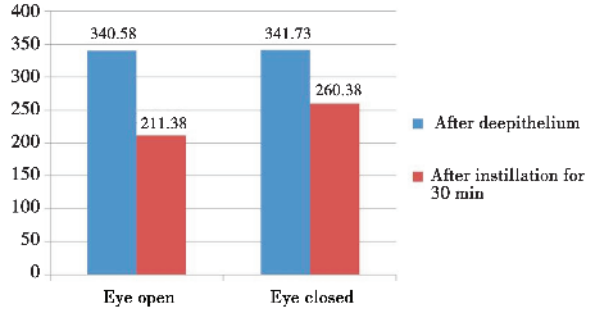

Figure 1 Variations in CCT after riboflavin instillation with the eye open or closed

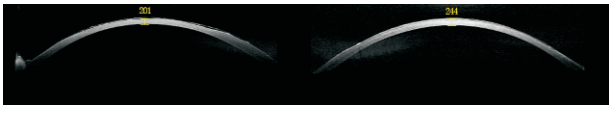

Figure 2 OCT image at 30 min after riboflavin instillation. Left: The open-eye group; Right: The closed-eye group

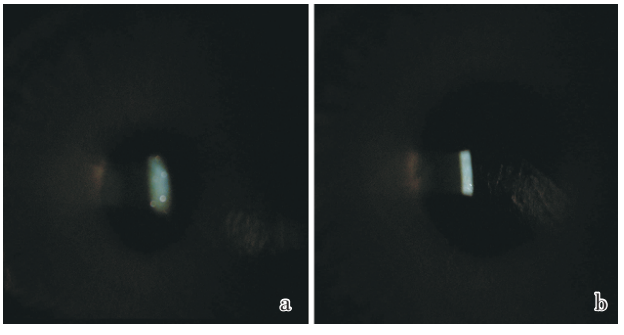

Figure 3 Yellow change in the corneal stroma and anterior chamber at 30 min after riboflavin instillation. a: The open-eye group; b: The closed-eye group

Discussion

Several methods are used to measure the corneal thickness, including anterior segment OCT, ultrasound, and ORA. We selected OCT for this study, in part because OCT measurements had the best repeatability of all the methods available. Another reason was that the use of OCT can lessen any discomfort and possible risk of infection from direct contact with the de-epithelialized corneal surface. OCT also allowed identification of the different corneal layers without washing away the riboflavin film. The status was real and simple to obtain. The washing process, which would be necessary if performing an ultrasound examination, might have had an additional influence on the corneal thickness[20–23]. The OCT examination time was about 10s, so any CCT effect of this short time with the eye open was expected to be small and negligible in the group with the eye closed.

Keeping the eye open caused the ocular surface to dry out easily and had a different effect on the conjunctiva, cornea, and tear film. It also increased the risk of infection in the de-epithelialized cornea. The ocular surface of patients with keratoconus tends to be abnormal and fragile[24], and ocular surface injury during 30 min of UVA irradiation is often unavoidable[25]. If the amount of time the eye is open during riboflavin instillation can be decreased, this could help protect the ocular surface.

This study confirmed that the necessary riboflavin concentration in the corneal stroma was reached after 30 min in the closed-eye group; this allowed for effective cross-linking. The decrease in the CCT was also smaller in the group with the eye closed; thus, the potential for inner ocular injury was smaller in this group. Some patients who had a critical corneal thickness were able to undergo UVA irradiation without the use of a hypo-osmolar riboflavin solution on the swollen cornea.

The CCT thickness of both groups at 10, 20, and 30 min were statistically different, with the change in CCT occurring mainly during the first 10 min, with little change in the remaining 20 min in either group, which indicated that most of the CCT change occurred at the early stage. The effect of eyelid state on CCT occurred within the first 10 min and its influence lasted for 30 min.

Possible reasons were explored to explain the differences in the CCT variations between the two methods. One reason was that the water in the cornea could have evaporated while the eye was open after removal of the epithelium because the epithelium is an important barrier for corneal stability. Another reason might be that the high osmolarity of 20%

dextran may have decreased the CCT after epithelium removal. In the open-eye group, this high viscosity could have caused the riboflavin solution to aggregate in the central portion of the de-epithelialized corneal surface, since dextran has a high capacity for water absorption. In contrast, in the closed-eye group, the riboflavin solution was spread in the conjuctival sac when the eyelids were closed. The riboflavin film was therefore thinner in this group than in the open-eye group, so the ability to absorb water was lower. Even so, similar yellow changes were observed in the closed-eye group, indicating that a sufficient riboflavin concentration was achieved in the corneal stroma with the eye closed. This could be attributed to eyelid pressure, which may have facilitated riboflavin penetration. The results of this study were consistent with previous reports on corneal pachymetry during CXL. Nevertheless, the change in the CCT during riboflavin instillation was amazing. The great extent of corneal thickness change drew attention to the importance of paying particular attention to the possibility of intraocular injury. Even with the closed-eye method, a corneal pachymetry examination was required before the UVA irradiation.

The relationship of the corneal gray degree on OCT with the corneal riboflavin concentration has not been previously examined. In this study, we found that the corneal gray degree on OCT was increased after the riboflavin instillation. As is the case for most instruments that use optical principles, the examination results from OCT are affected by factors such as the illuminating light, position, and bias light. While several factors caused interference, the high repeatability of these examination results still suggested that the corneal gray degree on OCT was related to the riboflavin concentration in the corneal stroma. Therefore, the closed-eye method might possibly be more conducive to riboflavin penetration into the corneal stroma, based on the gray degree on OCT.

This study had some limitations. The experimental subjects were rabbits, and the rabbit cornea differs in structure and is significantly thinner than the human cornea. This means that the percentage change in the CCT during riboflavin instillation may be higher in rabbits than it is in humans. Furthermore, if high performance liquid chromatography (HPLC) had been used to determine and compare the corneal riboflavin concentrations after instillation with the eye-open and eye-closed methods, the difference in efficacy might have been more accurate.

In conclusion, use of the closed-eye method during riboflavin instillation in CXL reduces the decrease in the CCT while giving the same yellow coloration change in the corneal stroma and anterior chamber, and reducing the exposure time of the ocular surface. Therefore, the closed-eye method was more effective and safer than the open-eye method.

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