Effects of UV-A rays on the corneal epithelial surface after topical application of riboflavin solutions: an electron microscope study

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Material reported in this article has been presented at the American Academy of Ophthalmology Annual meeting, October 2010, Chicago (P0073).

Abstract

Purpose: to evaluate the effect of different riboflavin solutions on the corneal epithelium when ultraviolet-A (UV-A) irradiation is used during trans-epithelial cross-linking (TE-CXL).

Design: experimental study.

Participants and Controls: Three groups of five corneas tested, a fourth group of three corneas used as control.

Methods: the eye-bank human corneas were soaked in vitro with: 1) a balanced salt solution; 2) a solution of riboflavin-dextran 0.1% widely used in TE-CXL; and 3) a solution of riboflavin 0.1%, vitamins and amino acids. The corneas then underwent UVA irradiation.

A fourth group of corneas (control group) did not undergo any treatment. All corneas were subsequently analyzed with electron scanning microscopy.

Main outcome measure: structural and ultra-structural aspect of corneal epithelium Results: after irradiation, the corneal epithelia of the three groups showed: 1) severe damage or even layer destruction; 2) evidence of cellular and inter-cellular damage; 3) evidence of less damage respect to group 2, although microvilli on the surface were less densely distributed compared with non-treated corneas. The epithelium of the control group showed good cellular adhesion; microvilli were well present and displayed with a great density.

Conclusions: the proposed solution of riboflavin, vitamins and amino acids was more effective in protecting the corneal epithelium integrity and the microvilli of the epithelial surface respect to the solution widely used in TE-CXL, as evidenced by electron microscope investigations.

KEYWORDS: epithelium; corneal microvilli; riboflavin; 52 , microscopy, electron scanning; enhancer.

Introduction

The epithelial layer is the first corneal structure irradiated 55 by ultra-violet A rays (UV-A rays) during trans-epithelial corneal cross-linking (TE-CXL), and consequently it absorbes a large amount of radiation. Irradiation-induced damage to epithelial structures is well documented.[1-4] To determine the effectiveness of a proposed riboflavin solution for TE-CXL treatment in protecting the corneal epithelium, we evaluated the effects of UV-A irradiation on the morphology of the surface of the corneal epithelium after soaking with solutions containing riboflavin versus balanced salt solution (BSS).

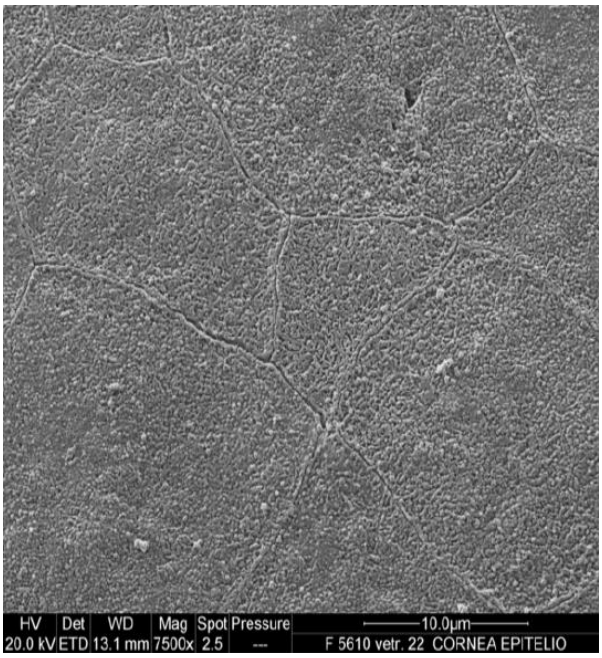

Figure 1 - Scanning electron microscopy of an untreated corneal epithelial surface.

Methods

This research adhered to the tenets of the Declaration of Helsinki. Informed consent was obtained for each cornea; Institutional Review Board (IRB)/Ethics Committee approval was obtained (authorization n. 1269). The corneas used in this study were obtained from our regional eye bank and were not suitable for transplant due to donor marker positivity. They were examined before the study with light microscopy of the endothelial cell layer .Only corneas with good transparency, thickness between 500 and 600 microns and a normal endothelial mosaic were considered.

We studied three groups of five corneas; a fourth group of three corneas was not treated and served as control. We examined in this last group with scanning electron microscopy (SEM, 7500x) the morphology of the microvilli on the surface of epithelial cells (Figure 1).

The three groups were treated as follows: group 1 with a balanced salt solution (BSS); group 2 with a solution of riboflavin-dextran 0.1%, edetate sodium, tromethamine, bihydrate sodium phosphate monobasic and bibasic, widely used in TE-CXL (Ricrolin® TE, Sooft, Italy); and group 3 with a new solution consisting of riboflavin-dextran 0.1 mg/100 ml, D-alpha-tocopheryl polyethylene-glycol 1000 succinate (vitamin E TPGS) 500 mg/100 ml, coenzyme Q 100 mg/100 ml, L-proline 0.1 mg/100 ml, glycine 0.1 mg/100ml, lysine hydrochloride 0.05 mg/100 ml and L-leucine 0.08 mg/100ml. Treatment included instillation of solution on the corneal surface every five minutes (for a total of 30 minutes) before UVA irradiation and every 3 minutes for a total of 30 minutes during irradiation. Radiant energy (370 nm wavelength) was 3 mW cm2 (5.4 J/cm284); it was applied at 5 cm from the corneal surface using a solid-state medical device CBM X linker (CSO, Firenze, Italy), with a 9-mm diameter treatment area. Soon after treatment, we examined the morphology of the superficial epithelial layer and of the microvilli on the epithelial cells of each cornea using scanning electron microscopy (SEM, 7500x).

Results

In group 1 corneas, treatment resulted in the destruction of all epithelial layers thereby exposing the Bowman membrane (Figure 2).

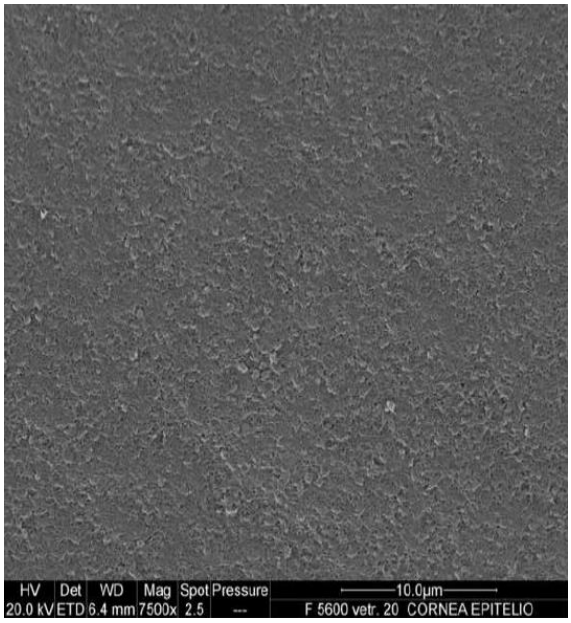

Figure 2 - Scanning electron microscopy of the epithelial surface of a cornea of group1.

In corneas of group 2, there were several cellular gaps in the superficial epithelial layer mostly due to rupture of intercellular tight junctions. In addition, there was a remarkable loss of microvilli and of cytoplasmic nuclei (Figure 3).

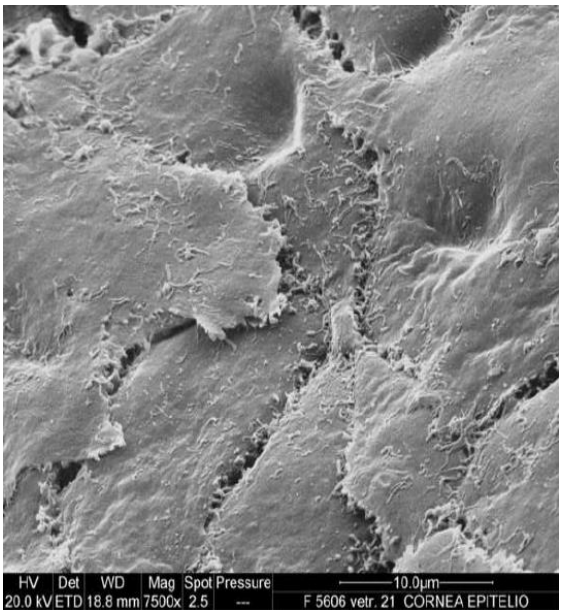

Figure 3 - Scanning electron microscopy of the epithelial surface of a cornea of group 2.

In corneas of group 3, there was less damage to the epithelial layers, the cell nuclei and the inter-cellular tight junctions compared with group 2. Although there was UVA-radiation damage in group 3 corneas, the reduction in microvilli density was less evident than in group 2, and the surviving microvilli appeared morphologically intact (Figure 4).

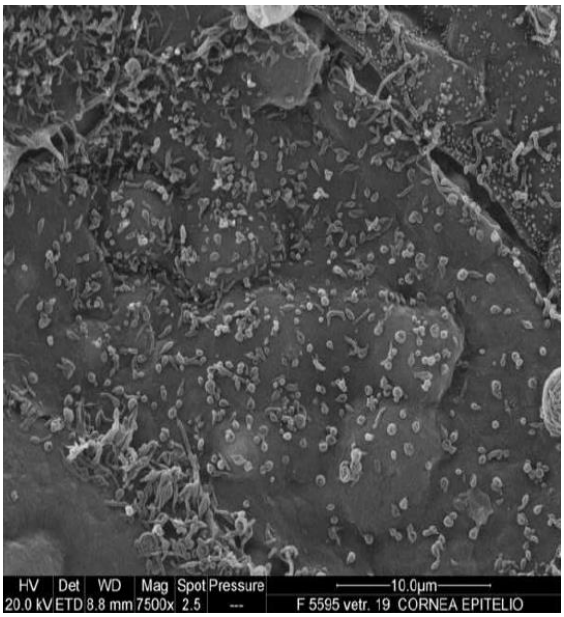

Figure 4 - Scanning electron microscopy of the epithelial surface of a cornea from group 3. The cellular nuclei and the tight junctions are less damaged and cellular gaps fewer than in group 2. Note the significant reduction in microvilli density, although the remaining microvilli are morphologically intact.

Discussion

To our knowledge, this is first study to evaluate the effects of trans-epithelial cross-linking treatment on the morphologic integrity of the corneal epithelial layer, and of microvilli on the surface of epithelial cells. The most reliable methods to evaluate corneal epithelium cells are cytology with the impression technique and examination with the scanning electron microscope [5-7].

Disruption of the corneal epithelial layer after UV irradiation is well described [1-4]. Here we show that the soaking solution used in group 3 was more effective in protecting the corneal epithelium than the standard solution used for TE-CXL[8-12]. Ultra-structural analysis suggest that at least vitamin E, ubiquinone Q10, amino acids such as L-proline, glycine, lysine hydrochloride and L-leucine help to protect the corneal epithelium from the effects of UV-A irradiation. Vitamin E and ubiquinone Q10 [13,14] exert a cytoprotective action. Vitamin E act also on glutathione oxidase and on dismutase peroxide, which are the enzymes involved in the repair of the epithelium [13]. The presence in the new solution of essential or conditionally essential amino acids, improve cytorepairing function [15]. Performing TE-CXL with the standard riboflavin solution, on the contrary, leads to a greater loss of microvilli. This generates instability of the pre-corneal lachrymal film, due to the lack of cellular surface adherence thereby resulting in hitching phenomena [15].

The new soaking solution reported herein results in less damage to the corneal epithelium than the other one. Should these results be confirmed in clinical study, this solution may be a valid alternative to other solutions used in trans-epithelial cross-linking treatments.

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