Dynamic Changes of the Corneal Epithelium and Stroma after Alkali Burning

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Summary: Dynamic changes of rat corneal epithelium and stroma after alkali burning were observed with the electron microscope. The neovascularization process in the stroma was almost the same as that observed after burning by heat.

Reversible changes of the epithelial cells occurred throughout the course of wound healing. Approximately 10 days after burning, cells in deeper layers of the epithelium showed temporary changes such as increased in number of mitochondria and formation of lamellar cytoplasmic infoldings. These changes gradually disappeared while the reconstruction of regular arrangement of the collagen lamellae in the stroma was established. As it is widely accepted that such infoldings play a role in active transport, dynamic changes of the epithelial cells in the present study indicate that reabsorption of the corneal edema is accomplished by both the corneal endothelium and the corneal epithelium.

Key words: rat cornea — epithelial cells — alkali burning — lamellar infoldings — neovascularization

Introduction

Recently, there have been a number of studies on the fine structural changes in corneal wound healing (Matsuda and Smelser, 1973; Kuwabara et al., 1976; Henriquez et al., 1976; Kenyon et al., 1977). However, the role of the corneal epithelium in wound healing has not received much attention. The present morphological investigation was performed to clarify the fine structural changes of the corneal epithelium in alkali burned cornea and the role of these changes.

Materials and Methods

Adult albino rats were used for the present investigation. The animals were anesthetized with ether and corneas were burned with one drop of 0.1 N sodium hydroxide for 1 minute. The corneas were then washed with saline. The animals were sacrificed 5 minutes to 50 days after burning of the cornea. Radial segments of the cornea were fixed in 3.5% glutaraldehyde in 0.1M cacodylate buffer at 4°C, refixed in 2% osmium tetroxide in the same buffer, dehydrated by acetone, and embedded in epoxy resin. The sections were made with a Porter-Blum MT-1 ultramicrotome.
They were stained with toluidine blue in 1% phosphate buffer for the light microscopic observations, and stained with uranyl acetate and lead citrate for the electron microscopic observations. The specimens were observed with a Hitachi H-500 type electron microscope.

Results and Discussion

Observation five minutes after alkali burning of the corneal epithelium showed disappearance of the epithelial surface cells and flatness of wing cells and basal cells. However, the epithelial basement membrane on the surface of the stroma was intact. Edema in the corneal stroma was observed and the regular arrangement of collagen lamellae was greatly disturbed. Dilatation of limbal vessels was also evident.

One day after burning, new formation of vessels had occurred in the limbal region. These new vessels were formed in association with pre-existing vessels in the limbal plexus. Abundant young fibroblasts aggregated in the limbal region as a mesenchymal reaction and were transformed to the endothelial cells of these new vessels.

After ten days, the newly formed vessels had anastomosed to each other and complicated capillary networks were established throughout the corneal stroma (Fig. 1). These neovascularization processes of corneal stroma were quite similar to those observed after the application of heat (Yoshizuka et al., 1979).

In this report (1979), we propose the hypothesis that the aggregation of mast cells from the limbal region in the corneal stroma is a causative factor of corneal neovascularization. It is widely accepted that corneal edema is a necessary condition of corneal neovascularization (Cogan, 1949). Furthermore, the onset of corneal edema has been thought to be caused by the contraction of limbal veins (Majno et al., 1961; Majno et al., 1969).

In our previous report, we suggest that corneal edema may be induced by venous contraction after release of biogenic amines from mast cells. However, in the present experiment, severe corneal edema has occurred without a remarkable invasion of mast cells. This may be contradictory to our previous observations. Since the corneal endothelium is lost immediately after alkali burning, it is thought that edema depends mainly on the inflow of aqueous humor into the tissue. Thus, heat and alkali burning seem to cause corneal edema in quite different ways. For this reason, corneal edema after alkali burning may not require abundant invasion of mast cells, while invasion of mast cells, is necessary for edema after heat burning.

The corneal epithelial cells are regenerated by mitotic proliferation of the basal cells. Ten days after alkali burning, when the capillary network has been established in the stroma, unique morphological changes were observed in deeper cell layers of the corneal epithelium. These included an increase in number of mitochondria and formation of lamellar infoldings. However, ultrastructure of the basement membrane and half-desmosomes of the basal cells appear unchanged (Figs. 2 and 3). Basal and lateral infoldings of the cell cytoplasm play an important role in the active transport of water and ions. It is well known that regulation of water transport in the cornea is done mainly by the endothelium. However, such infoldings of the epithelial cells may also play an important role in the absorption of the corneal edema in cooperation with corneal endothelium.

Furthermore, we also observed that the edema in the superficial layer of the stroma as shown in Fig. 2 was much less than that at deeper levels as shown in Fig. 4. Thus, it seems likely that such a difference also depends on the active trans-
port role of epithelial infoldings. It is interesting to note that these epithelial infoldings gradually disappeared during the process of reconstruction of the corneal stroma.

Several authors suggest a sliding movement theory of the corneal epithelial cells (Robb and Kuwabara, 1962; LaTessa and Ross, 1964; Kuwabara et al., 1976). Kuwabara et al. (1976) demonstrated the fine structure of such sliding epithelial cells, which possess abundant cytoplasmic projections and extend into the tissue defects in an amebic fashion. Cells possessing basal and lateral infoldings which we observed are, however, quite different from those described by these previous authors because their sliding cells are not associated with half-desmosomes and basement membrane.

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References

Fig. 1. Capillary network in the corneal stroma is shown 10 days after alkali burning. \( \times 560 \)

Fig. 2. Formation of lamellar infoldings in the deeper cell layers of the corneal epithelium 10 days after burning. \( \times 4200 \)
Fig. 3. Higher magnification of the lamellar infoldings and the increased number of mitochondria 10 days after burning. Arrows indicate the basement membrane and the half-desmosomes. ×7600

Fig. 4. Severe edema in the deeper part of the stroma 10 days after burning. ×4300