Effect of topical steroids on the healing of corneal endothelium

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The central portions of the cornea of adult rabbits were injured with a cryocautery and the rate of endothelial healing was compared between untreated and topical dexamethasone-treated corneas. Regenerating cells were labeled with daily injections of tritiated thymidine until the fifth day, when healing occurred. Control eyes showed 90 per cent label, but the number of labeled cells was less in steroid-treated corneas. The circular area of injured endothelium showed a slower healing rate in the first three days of steroid treatment. From then on, the progression of healing was similar to that of the control eyes. When the healing endothelium was studied with the scanning electron microscope, endothelial cells with a predominant fibroblastic shape were found in untreated corneas, while steroid-treated eyes showed fibroblastic and flattened cells with large cytoplasm.

Key words: corneal endothelium, corneal healing, endothelial healing, cryocautery.

It has been demonstrated that the rate of wound healing of corneal stroma treated with topical, subconjunctival, or systemic steroids is notably decreased, even when therapeutic doses are used.1-17 The various techniques or laboratory methods employed to arrive at these conclusions have included histologic, histochemical, historadiographic, and tensile strength studies. Some of these techniques have also been employed in studies of healing of steroid-treated epithelial wounds.1-3, 5, 6 The normal healing of injured endothelial cells has been profusely studied; however, little has been reported on their healing pattern when treated with topical steroids.2, 18 Clinically, it has been assumed that steroids have little or no effect on endothelial cell regeneration, a fact supported by the observation of improvement of corneal edema in some cases of traumatic injuries or bullous keratopathy.

The purpose of this investigation was to determine the effect of topical dexamethasone on the healing rate of non-penetrating freezing injuries of the corneal endothelium.
Methods

Twenty (20) adult albino rabbits (2.5 to 3 kilograms) obtained from the same dealer and of similar age were used for this study. The rabbits were anesthetized with intravenous sodium pentobarbital (30 mg. per kilogram). Six millimeter and 7.5 mm. diameter copper probes filled with dry ice (−79° C.) were applied to the central area of both corneas for one minute. Twenty four hours later, and daily for the next seven days, the anterior chamber was evacuated with a 30-gauge needle inserted at the limbus, and 0.2 microcuries of thymidine tritium (New England Nuclear Corporation, Sp.A 36.6 c per millimole) diluted in 0.1 c.c. of Eagle's basal medium were injected through the same needle for endothelial cell labeling.10 The rabbits were divided in two groups of 10 animals—Groups A and B (20 eyes each).

Group A. This was the control group which received an injection of H\textsuperscript{3}-thymidine once a day for seven days.

Group B. These animals received H\textsuperscript{3}-thymidine once a day and 0.1 per cent dexamethasone, one drop four times a day, for seven days. Drops were given immediately before the thymidine injection. Eyes were examined daily with a slit lamp in order to determine the degree of ocular inflammation and corneal edema. These observations were rated as follows: inflammation—1 to 3 plus (ciliary flush, fibrin or cells in anterior chamber, iritis). Corneal edema: 1 to 3 plus.

Histology—Every day starting on Day 2 after freezing, one pair of rabbits (A and B) were killed one hour after the treatment with dexamethasone and H\textsuperscript{3}-thymidine. Three corneas from each group with a rim of sclera were carefully dissected and fixed in Carnoy's solution for 24 hours. Flat preparations of corneal endothelium and Descemet's membrane were then prepared for histology and autoradiographs.20 The remaining cornea was fixed in glutaraldehyde for electron microscopy.

Autoradiography—Kodak stripping film AR-10 was used following the method of Pelc.21 The film was exposed for two to four weeks, developed, fixed, stained with Harris hematoxylin, and mounted in balsam.

Determination of endothelial regeneration. Radioautographs were examined under 2.5 × magnification. Ink dots were placed at the edge of the unhealed or acellular area in four or six meridians. The spaces between dots were measured with the microscope micrometer and the distance recorded in millimeters. These readings were averaged for each preparation examined and the unhealed area subtracted from the original injury (7 mm. for a 6 mm. probe). The true injury was 1.0 to 1.2 mm. larger due to the expansion of the freezing area during the probe application. The amount of labeling indicates the degree of DNA synthesis during the healing process which, as shown in previous studies, is decreased by steroids.13 The labeled area initially seen as a circle or band of labeled cells was measured in six areas with the micrometric vernier in each radioautograph and the results averaged. The number of labeled cells was counted in six areas under a 40 × objective and averaged.

Scanning electron microscopy (SEM). Corneas obtained together with those for radioautography. (Groups A and B) were fixed in cold 4 per cent glutaraldehyde for eight to 18 hours, at Days 2, 3, 4, and 5, rinsed in distilled water, and placed in 2 per cent osmium for 30 minutes. Specimens were processed through graded alcohols, dried in a freeze-drying machine,22 and coated with gold-palladium. Photographs were taken in a scanning electron microscope (Cambridge 'stereoscan') at 10 or 20 KV.

Results

Corneal edema and ocular inflammation of similar degrees was present in both groups of corneas on Day 1 after treatment. Ocular inflammation decreased in steroid-treated eyes, but edema was more pronounced centrally than in untreated corneas. These showed, on the fifth day, moderate ocular inflammation and diffuse corneal edema. Flat preparations of untreated corneas on Days 4 and 5 showed large numbers of leukocytes on the endothelium, while few were present in treated corneas.

Forty-eight hours after freezing with a 6 mm. diameter cryocautery and 24 hours after labeling, untreated corneas (Group A) showed a ring-shaped band of densely labeled cells. The width of the labeled area and the number of labeled cells was smaller in corneas treated with dexamethasone. In the control corneas, the ring-shaped band surrounding an acellular central area had approximately 1 mm. to 1.5 mm. width, whereas the steroid-treated corneas showed a labeled area of about 0.75 mm. in width after one day of treatment (Figs. 1 and 2). The labeled area included the 1 mm. area of injured endothelium beyond the 6 mm. original frozen lesion.

Four days after freezing, or three days after thymidine injection, Group A corneas

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had labeled endothelial cells almost completely covering the wounded area, but the frozen area of Group B (steroid) still had a 1 to 1.5 mm. central area devoid of endothelial cells with few labeled cells. In two additional days, however, (six days after freezing) labeled endothelial cells covered the central area.

The average number of labeled cells in Group A was twice that of Group B (Fig. 3) indicating that steroids had decreased the uptake of the tritium thymidine and possibly the mitotic rate by a similar value.

The rate of healing of the circular endothelial lesion was estimated in hematoxylin-stained radioautographs after cell counts had been made. Measurements showed that on Day 2, the injured area of untreated eyes had healed by 45 per cent and almost by 80 per cent on the third day. The steroid-treated corneas showed only 20 per cent healing on the second day and 55 per cent on the third day. Both groups showed 100 per cent healing on the sixth day (Fig. 4). It was observed, therefore, that steroid-treated corneas lagged somewhat in healing the endothelial injury as compared with the control corneas. These experiments were repeated with a 7 mm. probe with similar results, but the healing period lasted one more day in each group.

The healing area of untreated corneas when examined with the scanning electron microscope, showed that most of the regenerating endothelial cells had an elon-
gated or fibroblastic shape and were closely packed to each other (Fig. 5). Two types of fibroblast-like cells were seen adjacent to the edge of the injury. Some had a rather smooth surface while others had a profuse number of cytoplasmic excrescences and prolongations (Figs. 6 and 7). Toward the periphery of the injured area, already regenerated, these cells flattened out and became rounded or polygonal. This aspect was present centrally at the end of the observation period (eight days) in at least 80 per cent of the cells. Only one layer of cells was observed after healing.

Group B endothelial cells, on the other hand, had only 40 per cent of fibroblast-like cells, not packed together as in the control group, and a predominance of flattened cells with spread out cytoplasm (Figs. 8, 9, and 10).

On the fifth day, untreated eyes showed a discrete (1+) diffuse corneal edema over the previously injured area. The treated eyes, on the other hand, showed a more pronounced edema (2 to 3+) localized over the central area. Flat endothelial preparations of untreated corneas demonstrated a large number of white cells in the endothelial surface, whereas, very few were present in Group B corneas. This indicated that the edema may have been due to some extent to the presence of white cells in the endothelium. The number of white cells seen in flat preparations was not similar to that seen in SEM photographs. Possibly, a number of these cells came out of the cornea during tissue processing.

Discussion

The pattern of endothelial healing after a freezing injury is similar to that observed in flat preparations of the corneal stroma. Repopulation of a 6.5 to 7 mm. endothelial injury in a young and healthy animal, takes five to seven days to be completed. At the end of this period of time, the cornea is again clear, but the endothelial pattern is not yet similar to that of the uninjured cornea. The new cells are sometimes large and irregular, with one or more giant nuclei, or small with irregular hexagonal or elongated borders. These cells may, in the course of several weeks, rearrange themselves and continue the process of arrangement, but giant or multinucleated cells may persist. The present studies demonstrate that when steroids are used topically for several days following a nonpenetrating injury, a decrease in the rate of healing occurs in the first four to five days, then the lesion heals at about the same rate as the untreated corneas. Healing of the endothelium apparently occurs in the same way as the corneal stroma: by mitosis and by migration. The decreased uptake of tritiated thymidine by steroid-treated corneas (as much as 45 per cent), suggests that the mitotic rate of these corneas was inhibited and that healing was compensated by spreading of cells around the injury.

The differences in label density per cell between the two groups would suggest that steroids interfered with the uptake of the isotope, as they probably do with the synthesis of DNA. This would be supported by the decreased number of labeled cells per field (25 vs. 18). However, it could be argued that the decreased label could be due to leakage of the isotope from the anterior chamber. The steroid effect on the morphology of regenerating cells was also similar to that previously observed in the stroma: steroid-treated cells tend to acquire a rounded, flattened shape, whereas untreated cells have a fibroblastic shape and appear packed together when observed in a microscopic field in the area of active healing. The inflammatory component was minimal in steroid-treated eyes. These corneas usually showed edema localized to the area devoid of endothelial cells toward the end of the observation period; the untreated eyes, on the other hand, gave a more diffuse edema which could be caused by leukocytes observed between or under endothelial cells. This study does not eliminate the possibility that some of the fibroblasts or fibro-
Fig. 5. Scanning electron microphotographs of central area of control cornea four days after freezing (SEM, ×500).

Fig. 6. Electron microphotograph of healing endothelium near the central area. Elongated and fibroblastic endothelial cells are oriented toward the center of the cornea. Scattered red cells and inflammatory cells are seen in the endothelial surface. Four day stage, control. (SEM, ×1,000).

Fig. 7. High-power electron microphotograph of one of the fibroblastic endothelial cells seen in Fig. 6. These cells eventually flatten out and acquire the shape of normal endothelium (SEM, ×3,000).

Fig. 8. Endothelial surface of a cornea treated with dexamethasone for four days. Endothelial cells are migrating toward the acellular central area (*) with rare inflammatory cells on the surface. Descemet's membrane (D) (SEM, ×500).

Fig. 9. High-power view of central area of previous picture showing some fibroblastic cells (f) and flattened endothelial cells (E). Descemet's membrane (D) (SEM, ×2,000).

Fig. 10. Appearance of some of the flattened endothelial cells frequently seen at the edge of regenerating area in steroid-treated corneas. These two cells are joined at one point, the cytoplasm has thick cytoplasmic excrescences on the advancing edge, and fine filamentous prolongations above and behind the nucleus. Nucleus (N) Descemet's membrane (D) (SEM, ×5,000).
blastic cells in the endothelium may arise from the iris or ciliary body. This source of fibroblastic contribution to a corneal injury was demonstrated by Baum.24

As shown in these experiments, topical steroids in moderate amounts seem to retard, but do not stop endothelial healing in the young and healthy endothelium. It is possible that in situations where excessive proliferation of the endothelium is likely to occur in response to a persistent injury or inflammation, such as in a graft rejection, steroids may regulate the pattern of healing and the formation of retrograft membranes may be delayed or inhibited.

**Drugs.** Dexamethasone—Decadron 0.1 per cent.

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**REFERENCES**