Corneal epithelial regeneration and adhesion following acid burns in the rhesus monkey

Lawrence W. Hirst, Jerry A. Fogle,* Kenneth R. Kenyon, and Walter J. Stark

For study of corneal adhesion after chemical injury, sulfuric acid was used to burn the central and peripheral corneal epithelium in rhesus monkeys. The adhesion properties of the regenerating epithelium were sequentially followed by clinical assessment and by serial corneal biopsy specimens examined by transmission electron microscopy. The peripheral burns healed uneventfully, but the central corneal burns resulted in persistently loose adhesion for more than 8 weeks. The pathogenesis of the defective epithelial adhesion appeared to be initially the persistence of the original basement membrane that could not be utilized by regenerating epithelium. Later, poor adhesion was related to the slow rate of basement membrane regeneration and also to fragmentation of the Bowman’s layer substrate and to mild stromal edema. (INVEST OPHTHALMOL VIS SCI 23:764-773, 1982.)

Key words: corneal epithelium, basement membrane, adhesion, acid, ultrastructure, erosion model

The clinical significance of poor corneal epithelial adhesion and spontaneous epithelial breakdown lies not only in the considerable pain suffered by such patients but also in the frequency with which these problems interfere with proper healing after physical trauma, epithelial herpes infection, chemical burns, and corneal edema. Defects of the ocular surface epithelium also provide access for pathogenic organisms to enter the corneal stroma.

Study of the regenerating epithelial layer after corneal surface damage and the important role of its basement membrane was advanced by the observations of Khodadoust et al.1 in 1968. Numerous studies have since contributed to our knowledge of the function of basement membrane complexes in such conditions as recurrent erosive disorders, anterior corneal dystrophies,2 and chemical and thermal injuries.3-6

Our purpose was to produce an experi-
mental model of poor epithelial adhesion and spontaneous breakdown in the corneal epithelium, using the monkey, the animal whose corneal anatomy is most similar to that of the human.

We elected to use acid as the traumatizing agent in the production of epithelial defects, so as to produce lesions predominantly confined to the anterior cornea. The clinical course of epithelial recovery and adhesion was thereafter correlated with light microscopic and transmission electron microscopic examinations.

Materials and methods

Nine adult rhesus monkeys were sedated with phencyclidine (Sernylan) and the lids were separated with a speculum. One drop of topical proparacaine hydrochloride (0.5%) was applied to each treated eye. A cornea trephine without its piston was held in an Allis clamp and centered on the corneal epithelial surface, with care to obtain firm surface contact without torque.

In group 1, 14 corneas were burned centrally, using a 6.5 mm diameter trephine for containment without involving peripheral cornea (Fig. 1). In group 2, three corneas were burned peripherally in a ring fashion, involving a 1.75 mm–wide zone of the corneal epithelium immediately adjacent to the limbus. This was achieved with a double-barreled trephine (6.5 mm inner diameter, with an outer diameter of 10 mm) (Fig. 2). The outer trephine protected the conjunctiva, while the inner trephine formed a barrier protecting the central 6.5 mm of corneal epithelium from the acid in the peripheral annular well.

A 0.5 ml volume of 47% (18N) sulfuric acid was transferred by pipette to this trephine well. After a 10 sec exposure, the acid was rapidly diluted by vigorous irrigation with balanced salt solution. The opaque area of epithelium was gently debrided with a Bard-Parker blade or a moist cotton-tipped applicator. The monkeys were again sedated for examination at 2 to 3 days after the burns and thereafter at weekly intervals for a minimum of eight observations. Serial slit-lamp examinations were performed, and slit-lamp photographs were taken when possible. At biweekly intervals, superficial corneal biopsies were performed with a 1 mm Elliott biopsy trephine, and a razor-blade knife was used to excise each specimen at the level of the superficial stroma. Four biopsy specimens of each cornea were taken at intervals extending over at least 8 weeks, and these samples were fixed in glutaraldehyde-formalin and examined by light microscopy and transmission electron microscopy.

The adhesion of the epithelium at the surrounding edge of the biopsy site was tested by application of gentle traction with jewelers' forceps under the microscope. Epithelial adhesion was judged as being (1) loose, if the epithelium lifted off the edge of the biopsy site with virtually no traction, or if biomicroscopic examination revealed bullous elevation of the epithelium, or (2) tight, if any detectable traction was necessary before epithelial separation, often with fragmentation, could be achieved.

To study the effect of the acid burn on the underlying corneal stroma and endothelium, an additional three cynomolgus monkeys underwent central corneal burns to both eyes with the method described above. Central clinical specular microscopy was performed prior to the burns and established the presence of a normal endothelial mosaic. Central pachometry with Haag-Streit slit-lamp and Hedbys-Mishima centration attachment was performed 1 day after burn and at 1, 2, 5, and 8 weeks after burn. Eyes were obtained for his-

Fig. 1. Diagram of trephine well and area of central cornea burned.
Fig. 2. Diagram of double-barreled trephine position on cornea and resultant peripheral corneal acid burn.

tologic examination of the corneal endothelium immediately after burn and at 1 day and 1, 2, 5, and 8 weeks after burn. The eyes were placed in glutaraldehyde-formalin, the corneas were excised, and corneal endothelium was examined by scanning and transmission electron microscopy.

Results

Central burns. The closure of the epithelial defects occurred without incident in the 14 eyes with central burns (group 1) over a range of 2 to 10 days after burn, with no episodes of subsequent spontaneous epithelial breakdown. In seven of the 14 eyes there was prolonged poor epithelial adhesion persisting through 7 weeks. In these seven eyes, the apparent initial reestablishment of epithelial adhesion was followed about 2 weeks later by recurrence of poor adhesion for up to 14 weeks of follow-up. The other seven of the 14 central-burn eyes failed to establish firm adhesion at all for a follow-up period of 8 weeks after burn, and three of them remained nonadherent for 17 weeks of follow-up.

Although the stroma appeared biomicroscopically normal and there was no anterior-chamber reaction in 13 of the 14 eyes after an initial 1 to 2 weeks of stromal edema (Figs. 3 and 4), sterile stromal ulceration occurred in one eye of the 14 with intact epithelium.

Histologic and ultrastructural studies of biopsy samples taken immediately after the acid application showed an intact basement membrane, with only minimal epithelial debris remaining adherent (Fig. 5). Bowman’s layer and the stroma appeared unaffected.

In the seven of 14 eyes that failed to rees-
Corneal epithelial regeneration and adhesion

Establish adhesion, the basal cells did not form hemidesmosomal attachment complexes with the underlying original basement membrane (Fig. 6). Also, the basal cells showed no attempt to manufacture new basement membrane until the slow disintegration of the old basement membrane began at about 6 weeks after injury. The new basement membrane produced was in many cases thickened and otherwise aberrant in appearance (Figs. 7 and 8). In some instances, a plane of nonadherence within the zone of aberrant basement membrane was apparent, with resultant separation of the entire layer of overlying epithelium (Fig. 9).

In contradistinction, the seven eyes that...
Fig. 7. Central cornea 16 weeks after acid burn, with nonadhering epithelium showing redundant accumulation of multilaminated new basement-membrane material (asterisks). (×12,000.)

Fig. 8. Central cornea 8 weeks after acid burn; discontinuous segments of new basement membrane and hemidesmosomal complexes (asterisks) over areas of nonadhering fibrillar-granular debris (circle). (×30,000.)
had established tight adhesion at 8 weeks appeared to do so by promptly engaging the original basement membrane in attachments achieved with new hemidesmosomal attachment complexes. In these cases, no evidence of new basement membrane production was observed in any specimen (Fig. 10).

Regardless of whether epithelial reattachment was loose or tight, abnormalities of Bowman’s layer became evident at about 3 to 4 weeks after the burn. The normally dense network of collagen became disrupted by vacuoles, fibroblastic proliferation, and eventual scarring (Figs. 10 and 11). This derangement of Bowman’s membrane usually occurred sooner in the corneas with initially tight adhesion than it did in the corneas with initially loose epithelial adhesion after the burn.

Pachometric examination indicated a substantial increase in corneal thickness from preburn values (mean 0.42 ± 0.01 mm) to 0.55 ± 0.05 mm 1 day after burn and persistently increased thickness of 0.57 ± 0.02 mm at 5 weeks after burn. This corresponds to the scanning and transmission electron microscopic findings of central endothelial vacuolization immediately after acid burn, attenuated endothelium at 1 week after acid burn, and areas of absent endothelium at 5 weeks after burn, with recovery to an attenuated endothelial layer without vacuolation at 8 weeks. Anterior stroma was acellular for the first 2 weeks, but posterior stroma remained normal throughout the 8 weeks of observation.

Peripheral burns. The healing of the peripheral annular burns in the three eyes proceeded promptly and with none of the problems of epithelial nonadhesion that occurred with the central burns. The defect was cov
Fig. 10. Central cornea 10 weeks after acid burn. Inset, Normal-thickness regenerated epithelium (phase-contrast microscopy). Fibrocellular scarring of Bowman's layer and superficial stroma is evident. (PPDA; ×450). Main figure, Normal-appearing, closely apposed epithelial basement membrane and Bowman's layer surface. (×40,000.)

Discussion

The epithelial regeneration in all the central and the peripheral ring burns appeared to follow an uneventful course that was parallel to the course seen in human corneal abrasions. Although the original basement membrane remained in situ after burn, epithelial adhesion was delayed for up to 8 weeks in all central burns. This is in sharp contrast to the rapid utilization of original basement membrane for corneal healing in the rabbit after scraping of the epithelium, and the establishment of firm adhesion within 1 week, as described by Khodadoust et al. We suppose the reason that adhesion may be delayed despite retention of the original basement membrane is that the acid may well alter the character of this original basement membrane, making it less suitable as an attachment site for the regenerating epithelium. The prolonged persistence of this functionally useless basement membrane may account for the delay in secretion of new basement membrane with concomitant delay of epithelial readhesion. A possible contributing cause to the poor epithelial adhesion in this model is the mild but persistent stromal...
edema as a result of corneal endothelial damage. The resultant migration of fluid through the cornea could further jeopardize the formation of stable adhesion complexes for the regenerated epithelium.

The persistent failure of epithelial adherence after the burn was also apparently based on significant changes occurring in Bowman's layer and stroma. Continuing failure to use the new basement membrane for epithelial adherence in the seven eyes (50%) that could not reattach to the old basement membrane, and the subsequent loose adhesion of the seven other eyes that initially did form attachment complexes to the original basement membrane, can possibly be attributed to an inability of the anchoring fibrils of the hemidesmosomal attachment complexes to obtain a firm substratum in the disrupted and changing Bowman's layer. The importance of this infraepithelial bond for achievement of epithelial attachment to the dermis and skin has been well demonstrated by Heaphy and Winkelmann. This plane of possible separation at the level of superficial Bowman's layer...
Fig. 13. Peripheral cornea 3 weeks after peripheral ring burn. Inset, Slightly thinned epithelial layer with normal Bowman's layer. (PPDA; × 450.) Main figure, Normal-appearing interface of epithelium and Bowman's layer, and attachment complexes (circles). (×25,000.)

Fig. 14. Peripheral cornea 6 weeks after peripheral ring burn, with normal-appearing interface of epithelium and Bowman's layer, and attachment complexes with compact Bowman's layer (×25,000.)

has not been appreciated prior to the present study, possibly because experiments undertaken in rabbits demonstrate rapid reattachment to stroma taking place, with no intervening Bowman's layer. Clinical analogies to our findings include demonstration of poor epithelial adhesion in clinical thermokeratoplasty because of abnormal subepithelial basement-membrane material and debris, the regenerating but poorly adhering corneal
Corneal epithelial regeneration and adhesion

Fig. 15. Representation of adhesion zone of corneal epithelium consisting of basal cells, basement membrane complex, and Bowman's layer. The potential plane of separation between epithelium and basement membrane and between basement membrane and Bowman's layer are evident.

epithelium in the diabetic patient after vitrectomy, and the recurrent corneal erosion syndrome.

The peripheral annular burns were induced in such a way that the total area of epithelial surface burned (approximately 39 mm²) was the same as that of the central burns. In the annular-burn series there was rapid epithelial cover within 2 days or less and immediate firm adhesion of the epithelium, with reutilization of existing basement membrane. The speed of epithelial cover was possibly enhanced in the annular series by the greater length of regenerating epithelial margins. Therefore, although the total area to be covered by epithelium was the same as that in the central burns, the available length of adjacent migrating epithelial edges in the ring burns was 55 mm, as compared with the 22 mm circumference in the 6.5 mm central acid burns. The total lack of vascularization, absence of inflammatory infiltrate, and maintenance of a normal Bowman's layer could also be factors in the benign course of this type of burn. Finally, possible protection of corneal endothelium by increased thickness of peripheral cornea could account for the absence of clinically detectable corneal edema within this area at any time and lack of subsequent epithelial adhesion problems contributed to by this edema.

We have developed a model of poor corneal epithelial adhesion in monkeys. Our model demonstrates the separation of epithelium from stroma at two levels. Faulty adhesion at either of the two levels of separation—between epithelial cells and the basement membrane, or within Bowman's layer or an abnormal basement-membrane layer—could result in clinical syndromes of poor epithelial attachment (Fig. 15).

REFERENCES