The healing of linear nonperforating wounds in rabbit corneas of different ages

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Linear nonperforating incisions were made in the corneas of 2-week-old and 2-year-old rabbits. The resulting wounds were examined by light microscopy and transmission and scanning electron microscopy. A corneal incision of a 2-week-old rabbit produced a wide gaping wound caused by retraction of the cut stromal lamellae away from the incision. The wound became wider with time as the developing eye enlarged and the cut lamellae retracted further. Polymorphonuclear leukocytes, presumably from the tear film, penetrated into the wound area before it was covered over by the sliding epithelium. Most of the leukocytes disappeared by 3 days after wounding. Three to six layers of fibroblasts appeared beneath the epithelial plug. The tissue eventually rebuilt approximately one third of the corneal depth lost to the wound. The stroma of the wounded region did not return to its normal width, but the epithelium was thicker than that of the unwounded cornea. An incision in a 2-year-old rabbit cornea produced a narrow V-shaped wound that did not change shape with time. This wound was repaired by fibroblasts resulting in collagenous repair tissue being the same depth as the normal stroma. There appears to be no evidence for wide gaping wounds in humans in the literature, as was found in this study in rabbits. (INVEST OPHTHALMOL VIS SCI 23:660-665, 1982.)

Key words: corneal wound, collagen, rabbit, leukocytes

Corneal wounds are common due to the exposed position of the eye. Because of the importance of corneal wounds, a number of studies have been made on the adult rabbit as a model, involving either perforating or nonperforating corneal wounds. Considering that children have a higher rate of corneal injuries than adults, it is somewhat surprising that the studies on rabbit corneal injuries have used nothing but adult animals. The following investigation describes the striking difference in the healing of linear, nonperforating corneal wounds in juvenile and adult rabbits.

Materials and methods

Eighteen 2-week-old and four 2-year-old New Zealand White rabbits were anesthetized with ether or with intravenous injections of sodium pentobarbital (Nembutal; Abbott Laboratories, North Chicago, Ill.). A drop of proparacaine hydrochloride (Ophthaine; E. R. Squibb, Princeton, N. J.) was placed in each eye, and a linear nonperforating corneal incision was made with a razor blade fragment held in a Storz knife breaker. The wound was made across the center of the cornea from 12 to 6 o'clock and examined in a Haag-Streit slit lamp at various times after the incisions were made. The depth of the incisions varied from zero at the edges of the cut to 40% to 80% of the corneal depth in the center. The rabbits were sacrificed with an overdose of Nembutal, and the eyes were enucleated and placed in 2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.2, for 30 min. The

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cornea of each eye was excised at the limbus and fixed for another hour. Some of the corneas were dehydrated and embedded in Paraplast, sectioned on a rotary microtome, and stained with hematoxylin and eosin. Other corneas were postfixed with 1% osmium tetroxide in 0.1M phosphate buffer, pH 7.2, dehydrated in a series of ethanol concentrations, and embedded in an Epon 812 formulation. Sections (0.5 to 1.0 μm) were made on a LKB Ultratome and stained with toluidine blue (1%) in 1% aqueous sodium tetraborate. Although no transmission electron micrographs are presented, thin sections of corneas were made as above, stained with Reynolds lead citrate, and examined in a Philips EM 300 electron microscope.

For scanning electron microscopy some corneas were washed in distilled water after glutaraldehyde fixation, dehydrated in ethanol, and dried in a Ladd critical-point dryer with carbon dioxide. The corneas were coated with gold in a sputter coater (Polaron) and examined with an AMR 1000A scanning electron microscope.

Fig. 1. Section of a 2-year-old rabbit cornea with an incision that penetrated about 60% of the cornea. The cornea was fixed 7 days after wounding. An epithelial plug (E) is still present, although fibroblasts (F) have formed new stromal tissue in the posterior part of the wound. (Bar = 100 μm.)

Fig. 2. Section of a 2-week-old rabbit cornea with an incision that penetrated about 60% of the cornea. The cornea was fixed within 10 min of wounding. (Bar = 100 μm.)

Fig. 3. Section of a 2-week-old rabbit cornea with an incision that penetrated about 50% of the cornea. The cornea was fixed 2 hr after wounding. Polymorphonuclear leukocytes (L) are present on the surface of the incision and have begun to penetrate into the stroma lamellae. The epithelium (E) has begun to slide over the wound. No polymorphonuclear leukocytes are found in the part of the stroma that is intact. (Bar = 100 μm.)
Fig. 4. Scanning electron micrograph of a 2-week-old rabbit cornea that was wounded 6 hr before sacrifice. The epithelium (E) has partially covered the surface of the wound. (Bar = 1 mm.)

Results

Linear nonperforating incisions of 2-year-old rabbit corneas resulted in a wound shaped like a narrow V when viewed in cross section (Fig. 1). Within 12 hr the epithelium had moved over the wound and covered the exposed stroma, completely filling the wound with an epithelial plug within 24 hr. Polymorphonuclear leukocytes penetrated into the exposed stroma before it was covered by epithelium. Fibroblasts accumulated in the incision area, forming collagenous scar tissue (Fig. 1) in the cleft as the epithelial plug receded.

Linear, deep nonperforating incisions of 2-week-old rabbit corneas resulted in large gaping wounds that widened rapidly in comparison to those in 2-year-old rabbit corneas. Examination with the slit lamp showed that the edges of the wound were opaque, apparently due to the swelling of the cut collagenous lamellae. Sections prepared from such wounds 10 min after wounding (Fig. 2) showed that the cut stromal lamellae had pulled back from the incision, with the cut lamellae closest to the anterior surface pulling back the most. Two hours after the incision was made, the wound had gaped more and the epithelium began to move over the surface of the wound (Fig. 3). At this time polymorphonuclear leukocytes were seen on the surface, and immediately under, the cut stromal lamellae. No leukocytes were seen in the intact part of the stroma.

Six-hour-old wounds were wider than 2-hour-old wounds, presumably because of further retraction of the cut stromal lamellae (Figs. 4 and 5). By this time the epithelial movement had covered the ends of the wound, while the center of the wound was only partly covered (Fig. 4). The polymorphonuclear leukocytes had disappeared 3 days after the incision (Fig. 6). By this time fibroblasts accumulated under the surface of the epithelial plug. Eventually three to six layers of migrating fibroblasts were under the surface of the epithelial plug. One month after the incision the fibroblasts under the epithelial plug had formed collagenous lamellae, with each lamella of approximately the same appearance and width as the intact stromal lamellae. However, the injured portion of the stroma did not recover to the depth found in the uninjured portion. As a result, the stroma was thinner in the injured area (although the epithelium was thicker). In addition, as the animal aged and the eye became larger, the wound was not filled with collagen but appeared to widen by the sliding of the cut lamellae over the intact lamellae,
resulting in a greater area for the epithelium to cover.

Although a linear nonperforating incision of the cornea of a 2-week-old rabbit resulted in a wide gaping wound, the healed tissue was clearer than that in the wounds of 2-year-old rabbits. Initially after wounding, the area was opaque due to the swelling of the damaged stromal lamellae. Within 7 days, however, the opacity was gone and it was difficult to see the wound, even with the slit lamp. Apparently the relatively small amount of scar tissue formed under the epithelial plug does not result in much disruption of the normal stromal organization.

In some of the animals the incision extended to, or near, the limbus. When this occurred, vascularization of the wound usually occurred, proceeding from the limbus to the center of the cornea. Vascularization resulted in a greatly increased cellular population (largely fibroblasts) and formation of substantial scar tissue in the wound.

Discussion

The repair of linear nonperforating incisions of adult rabbit corneas has been previously studied, with results in agreement with ours. Such wounds in juvenile rabbit corneas apparently have not been investigated. The results presented here show a considerable difference in the response to linear incisions in adult and juvenile rabbit corneas. The anterior stromal lamellae in the juvenile rabbits appear to be under tension so that they rapidly retract when cut, resulting in a wide wound. The subsequent widening of the wound may be due to the growth of the developing eye, with the cut lamellae sliding back over the intact posterior lamellae. The younger and smaller the eye when wounded, the wider the eventual corneal wound. In contrast, in the adult eye the lamellae do not slide over one another and the wound remains narrow.

The contraction of the anterior stromal lamellae in linear nonperforating incisions of 2-week-old rabbit corneas may be due to two basic causes: either the collagen in the stromal lamellae are under tension, causing the lamellae to retract when cut, or there is a minor component of the stromal lamellae that pulls the collagen along with it when it retracts. If the first proposal was true, then the lamellae should appear relatively straight in sections perpendicular to the corneal surface. However, if the collagenous lamellae are not contractile and are being retracted passively by a minor lamellae component, then the stromal lamellae should have a wavelike appearance after being pulled back; in some sections the anterior stromal lamellae (i.e., those that have contracted the most) have a
Fig. 6. Section of a cornea that received an incision when the rabbit was 2 weeks old. The cornea was fixed 3 days after wounding. The wound has widened with the epithelial plug filling the wound. (Bar = 100 μm.)

crenulated appearance. These sections, however, also have been dehydrated during preparation, which might influence the appearance of the lamellae. Interestingly, the underlying intact lamellae do not have a crenulated appearance, suggesting that the appearance of the cut lamellae is not artifactual. The component of the stroma that might cause the collagen in the lamellae to constrict is not known. There are, however, groups of electron-dense fibrils, of a smaller diameter than collagen, in the stroma that might serve as likely candidates for such a contractile protein. These fibrils resemble the precursors of elastin, but their functions or origin is unknown.

The failure of mature rabbit corneas to gape after linear incisions is indicative of an aging phenomenon in the outer covering of the eye. Whether this is due to a change in the collagen, elastin, glycoprotein, or some other molecule in the cornea is not known.

The appearance of polymorphonuclear leukocytes 2 hr after wounding in the 2-week-old rabbit corneas is similar to the results obtained with wounds in adult rabbit corneas, although the appearance of the first leukocytes after corneal wounding has been reported to be as late as 5 to 6 hr. After the epithelium has closed over the wound there appears to be no further increase in the number of leukocytes in the wounded area, reinforcing the postulate that the leukocytes are derived from the tear film, at least when the wounded area is some distance from the limbus. The disappearance of most of the leukocytes by 3 hr after wounding agrees with their lifetime of approximately 2 days, as suggested by Rhodin.

To our knowledge there has been no report of wide gaping corneal wounds in humans similar to those reported in 2-week-old rabbits.

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