Epithelial abrasion precipitates stromal ulceration in the vitamin A–deficient rat cornea

Deborah D. Sendele, Kenneth R. Kenyon, George Wolf, and Laila A. Hanninen

Although the role of vitamin A deficiency in the development of xerophthalmia is well established, there is still some question as to whether the deficiency alone is sufficient cause for the development of keratomalacia. This article describes the clinical, histologic, and microbiologic changes occurring in eyes of vitamin A–deficient rats when keratomalacia-like stromal ulceration is induced by epithelial injury alone. The corneal epithelia of 21 severely vitamin A–deficient rats and 11 pair-fed controls were totally removed either by scraping or by n-heptanol. At 96 hr after epithelial removal, 93% of the deficient animals showed extensive epithelial defects and stromal ulceration. Histologically, an intense acute inflammatory response and abundant bacterial forms were consistently evident. Staphylococcus aureus and Streptococcus fecalis were the most frequent pathogens cultured from these ulcerating eyes. In contrast, the control corneas showed essentially complete re-epithelialization, with no ulceration, minimal inflammatory reaction, and an absence of morphologically demonstrable bacteria. Bacterial cultures from the control eyes showed abundant Pasteurella, with pathogens also present. These observations suggest that abnormal epithelial recovery, acute inflammation, and bacterial infection may be important factors for the development of keratomalacia-like corneal ulceration in experimental vitamin A deficiency. (INVEST OPHTHALMOL VIS SCI 23:64-72, 1982.)

Key words: stromal ulceration, vitamin A deficiency, epithelial abrasion, corneal epithelium, keratomalacia

Keratomalacia is a leading cause of blindness in underdeveloped nations. In 1921, Wason1 first described the ocular findings in vitamin A–deficient rats and concluded that the changes in the cornea are secondary to bacterial invasion. Others, such as Mori,2 thought that dryness due to lacrimal gland involvement was responsible for the corneal and conjunctival findings seen in these animals. Many of the children who suffer from...
keratomalacia have nutritional deficiencies in addition to a lowered resistance to infection. The role of vitamin A deficiency in the development of xerophthalmia is well established. However, vitamin A deficiency alone without protein depletion or trauma is thought to be insufficient to produce keratomalacia. Seng et al. recently reported that a mild thermal trauma induced collagenase release with the development of corneal ulceration in vitamin A-deficient rats, whereas the identical trauma produced no stromal lesions in pair-fed controls. These results suggest that vitamin A deficiency alone might be insufficient to cause corneal ulceration but that these corneas are at a greater risk to ulcerate if exposed to environmental or other injury.

The purpose of this study was twofold: first, to evaluate whether epithelial trauma alone could precipitate stromal ulceration in vitamin A-deficient rats, and second, to determine the roles of infection and inflammation in the development of such ulceration.

Materials and methods

Weanling Holtzman albino rats were put on a vitamin A-deficient diet for 8 to 12 weeks; pair-fed control rats were given the same diet with weekly vitamin A supplementation (200 μg of retinyl acetate). The food intake of the control group was restricted to that of the test group.

After 12 to 16 weeks of dietary deficiency, 21 severely vitamin A-deficient rats (20% weight loss after plateau) and 11 pair-fed controls were anesthetized by an intraperitoneal injection of 0.5% chloral hydrate solution per 100 gm body weight (3.6% solution for deficient animals, 7% for controls). The corneas were then totally de-epithelialized either by scraping with a Bard-Parker blade or by swabbing with n-heptanol. A drop of Richardson’s (methylene blue-azure II) solution was then placed on the eye to determine that epithelial removal was complete. Erythromycin ointment was applied once after de-epithelialization. Slit-lamp examination and external photography were performed at 48 and 96 hr after epithelium removal to determine epithelial defect size and stromal ulcer depth. On about half the animals, immediately before epithelial debridement and again immediately before enucleation, bacterial cultures were obtained by swabbing the corneal surface. Fig. 1. Top, Typical lusterless and irregular surface appearance of a vitamin A-deficient cornea. Middle, Severe ulceration in vitamin A-deficient cornea 96 hr after epithelial scraping. Bottom, Healing epithelial defect (margin at arrow) of a control cornea 48 hr after complete epithelial scraping; there is no evidence of corneal ulceration.

surface with a cotton applicator and inoculating blood agar, chocolate agar, Sabouraud medium, and meat broth. All animals were sacrificed at 96 hr and, the eyes were immediately enucleated and placed in Karnovsky's glutaraldehyde-formaldehyde solution. Routine preparation for phase-contrast, transmission electron microscopy (EM), and scanning EM was performed.

**Results**

Prior to de-epithelialization, ocular changes of xerophthalmia were seen in the vitamin A-deficient rats—a lusterless cornea, punctate epithelial keratopathy, and keratinization (Fig. 1). The control group had no ocular changes. Forty-eight hours after either mechanical or heptanol de-epithelialization, the eyes of the deficient rats exhibited extensive epithelial defects, with underlying stromal haze; the control eyes showed clear corneas (Fig. 1), with uniform healing to 50% of the original defect size. At 96 hr, slit-lamp examination of the deficient animals demonstrated stromal ulceration in 93% (39/42) of eyes; about 10% (4/42) showed perforation (Fig. 1). No difference was evident between eyes de-epithelialized mechanically or by heptanol. The 22 control eyes showed nearly complete re-epithelialization and no ulceration.

Histologically, light microscopy of deficient corneas revealed persistent epithelial defects, ulcerated stromas infiltrated by numerous polymorphonuclear neutrophils (PMNs), and masses of bacteria adherent to the corneal surface and within the stroma (Fig. 2). The anterior chamber contained many inflammatory cells (Fig. 3). Scanning EM of these corneas confirmed the extensive epithelial defects, demonstrated the loss of surface microvilli in the remaining epithelial cells, and often showed bacterial forms enmeshed within the ulcerating stromas (Figs. 2 and 3). By transmission EM, the deficient corneas revealed disorganization of the epithelium and stroma (Fig. 2). In areas of severe ulceration, numerous degranulated PMNs, often containing phagocytosed bacteria and extracellular debris, were present (Figs. 4 and 5). The stromal collagen fibrils appeared to have been degraded, leaving amorphous fibrillar debris (Fig. 5). A particularly prominent finding was the abundance of bacteria present within the remnants of the epithelial layer and stroma (Fig. 3).

In contrast, light microscopy and scanning EM of control corneas revealed an intact epithelium, with normal epithelial surface structures, minimal stromal inflammation, and an absence of bacteria. By transmission EM, these corneas demonstrated an intact but seemingly loosely adherent epithelial cell layer, normal-appearing keratocytes, and rare, quiescent PMNs. The stromal collagen also appeared ultrastructurally normal. No bacteria were present within the epithelium or stroma. Descemet's membrane, endothelium, and anterior chamber were also unremarkable.

The bacteriologic findings before and 96 hr after epithelial removal are summarized in Table I. Several bacterial species were cultured from both the deficient and the control groups, but the distribution of species, particularly of pathogens, differed.

**Discussion**

These results indicate that epithelial trauma in vitamin A-deficient rats can precipitate
Fig. 2. For legend see facing page.
Fig. 3. For legend see facing page.
Table I. Bacteriologic evaluation of corneal cultures from vitamin A-deficient and control rats

<table>
<thead>
<tr>
<th>Organism</th>
<th>Vitamin A-deficient</th>
<th>Control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Before epithelial removal (n = 22)</td>
<td>96 hr after removal (n = 14)*</td>
</tr>
<tr>
<td>Pasturella pneumotropica</td>
<td>16 (73%)</td>
<td>3 (21%)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>11 (50%)</td>
<td>8 (57%)</td>
</tr>
<tr>
<td>Hemolytic Streptococcus</td>
<td>11 (50%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>4 (18%)</td>
<td>5 (36%)</td>
</tr>
<tr>
<td>Streptococcus fecalis</td>
<td>2 (9%)</td>
<td>6 (43%)</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>2 (9%)</td>
<td>1 (7%)</td>
</tr>
<tr>
<td>Morganella morganii</td>
<td>1 (5%)</td>
<td>None</td>
</tr>
<tr>
<td>Enterobacter species</td>
<td>None</td>
<td>None</td>
</tr>
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*After anesthesia, four vitamin-A deficient animals and one control animal died.

stromal ulceration. Ninety-three percent of the deficient corneas ulcerated after epithelial removal, compared with none of the controls. The deficient eyes had not only a markedly increased acute inflammatory response but also showed many bacteria present in the corneal stroma. The latter morphologic finding was confirmed by postscraping bacteriologic findings of a higher prevalence of several pathogens in the ulcerating corneas of the deficient animals than in the controls. Thus inflammatory cells and bacteria are shown to have a role in the development of keratomalacia-like ulceration in this experimental model, since there was not only an increased number of pathogens in the ulcerating corneas but also consistent morphologic evidence of bacteria within the stroma.

The importance of the acute inflammatory cells, particularly the PMNs, in the pathogenesis of human corneal stromal melting has been reported. Kenyon et al., using thermal- or alkali-induced corneal melting in the rabbit, found that a tissue adhesive greatly reduced stromal PMN infiltration and subsequent ulceration. Fogle et al. arrested progressive corneal melting in humans with the use of this tissue adhesive, again postulating that the adhesive provides a barrier to regenerating epithelium and tear fluid, thereby preventing the stromal invasion of PMNs and their subsequent stromal damage. Thus the possible pathogenesis of experimental keratomalacia may involve an acute stromal inflammatory response in the presence of a persistent epithelial defect, as was found in the deficient animals. Support for this theory is provided by Seng and co-workers, who prevented stromal PMN infiltration and ulceration in thermally burned, vitamin A-deficient rats by the application of the same cyanoacrylate adhesive. Recently, Smolin and Okumoto have shown decreased epithelial regeneration and wound healing in vitamin A-deficient animals. Such a prolonged epithelial defect would not only allow the influx
Fig. 4. Transmission electron micrograph of a vitamin A-deficient cornea 96 hr after epithelial scraping. Numerous PMNs (P) are present within the degraded stroma (asterisk) and appear to be actively phagocytizing bacteria (B) and other extracellular material. (×13,857.)

of inflammatory cells from tears but also would diminish the primary barrier to corneal superinfection.

It is well known that in vitamin A deficiency there is a generalized depression of the immunologic defense mechanisms, with frequent secondary ocular bacterial infections as a possible contributing factor to the development of keratomalacia. In the initial clinicopathologic ultrastructural studies of human keratomalacia, Sommer et al. have found not only an acute inflammatory response with PMNs but also many bacterial forms in the ulcerated corneas of severely vitamin A-deficient children. In further support of the role of bacterial infection in the development of keratomalacia, Carter-Dawson et al. and Beeson et al. have shown decreased susceptibility to inflammation, neovascularization, and ulceration in vitamin A-deficient rats placed in a germ-free environment.

Thus, as previously found by Seng and as-
sociates vitamin A deficiency appears to be a necessary but not a sufficient factor in the development of keratomalacia. In addition, this ulceration may be facilitated by some external insult such as epithelial trauma. Both the human and animal studies suggest that the factors of abnormal epithelial recovery, acute inflammation, and bacterial infection may be important for the development of keratomalacia in severe vitamin A deficiency. We hypothesize that decreased wound healing in the vitamin A-deficient cornea may lead to a persistent epithelial defect, allowing the influx of bacteria and PMNs, with a resultant corneal ulceration. In contrast, control eyes receiving epithelial trauma would heal rapidly, thus preventing bacterial and inflammatory invasion and subsequent ulceration.

REFERENCES
2. Mori S: Primary changes in eyes of rats which result from deficiency of fat soluble A in diet. JAMA 79:197, 1922.


