Ascorbic acid prevents corneal ulceration and perforation following experimental alkali burns

Richard A. Levinson, Christopher A. Paterson, and Roswell R. Pfister*

Depressed aqueous humor glucose and ascorbic acid levels returned to control values within 14 days following a 20 sec., 6 mm. diameter, 1N sodium hydroxide burn of the rabbit cornea. These corneas did not ulcerate or perforate. After a 20 sec., 12 mm. diameter, 1N sodium hydroxide burn, aqueous humor glucose levels returned to normal values, but ascorbic acid levels remained significantly depressed for up to 30 days. These corneas became markedly ulcerated in about 60 per cent of animals and frequently perforated. Following 12 mm. alkali burns, rabbits treated daily with 1.5 Gm. of subcutaneous ascorbic acid rarely developed corneal ulcerations and the corneas did not perforate. It is suggested that exogenous maintenance of adequate aqueous humor levels of ascorbic acid overcomes the relatively scorbutic state of the anterior segment induced by a 12 mm. alkali burn, thereby impairing the development of corneal ulceration and perforation. Elevated aqueous humor levels of ascorbic acid had no influence on corneal epithelial cell migration patterns following alkali burns.

Key words: cornea, alkali burns, ulceration, perforation, ascorbic acid, glucose.

Persistent corneal epithelial defects, corneal ulceration and perforation following alkali burns of the eye are well documented.1-3 It has been suggested that these changes might be the result of a nutritional deficiency in the aqueous humor caused by alkali-induced damage of the ciliary body. Previous investigators have studied the glucose content of the aqueous humor following alkali burns.4-6 However, since ascorbate is known to be actively transported by the ciliary processes into the aqueous humor,7 we felt that aqueous ascorbate levels would provide a more specific parameter by which to judge the viability of the ciliary epithelium.

This paper describes clinical changes in the cornea as they relate to levels of glucose and ascorbate in the aqueous humor following 6 and 12 mm. alkali burns. On the basis of the results, we also investigated the influence of systemically administered ascorbic acid on corneal epithelial defects, ulcers, and perforations after 12 mm. alkali burns. Detailed morphological observations

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on alkali-burned corneas will be published elsewhere.\footnote{1}

**Methods and materials**

Male and female, New Zealand-Dutch strain albino rabbits, weighing between 2.5 and 3.5 kilograms were used in all experiments. The rabbits were anesthetized with sodium pentobarbital (approximately 30 mg. per kilogram) administered via a marginal ear vein.

*Effect of alkali burns on the cornea and aqueous humor chemistry.* Circular lucite wells, 6 or 12 mm. in diameter, (Fig. 1) were placed on the cornea of the proptosed eye and filled with 0.4 ml. of IN sodium hydroxide. After 20 sec. the alkali was aspirated from the well, and the interior of the well and the cornea rinsed for 5 sec. with saline. Saline irrigation was continued for 5 sec. after the well had been removed from the corneal surface. The same area (6 or 12 mm. in diameter) of both eyes of each animal was burned. The eyes were subsequently examined daily, with special attention paid to closure of the epithelial wound, vascularization, ulceration, and perforation.

At specified times, aqueous humor was withdrawn from both eyes of at least three animals in the 6 and 12 mm. alkali-burn groups. Aspiration of aqueous humor was performed once in each eye, under urethane anesthesia. The animal was then killed. The aspirated aqueous humor was divided into two weighed aliquots and analyzed for glucose and ascorbic acid. Ascorbate was determined in 0.1 ml. of aqueous humor, with the 2,6-dichlorophenol-indophenol method of Mindlin and Butler.\footnote{8} Ascorbate level in rabbit plasma was determined in an identical manner, with 2 ml. of fresh plasma. Glucose was determined by the hexokinase method described by Lowry and Passaneau.\footnote{9}

**Influence of parenteral ascorbic acid on corneal changes following 12 mm. alkali burns.** These experiments were performed in a separate series of animals from those described above. Elevation of aqueous humor levels of ascorbic acid was achieved by daily subcutaneous injection of 1.5 Gm. of ascorbic acid as a freshly prepared, neutralized aqueous solution. In a separate series of experiments, this dose of ascorbic acid was shown to achieve maximum elevation of aqueous humor ascorbic acid levels, measured 24 hours after each daily injection.

Ten rabbits were subjected to 12 mm. burns of both eyes and then received no further treatment. A further 10 rabbits were subjected to identical alkali burns, but this group then received daily injections of 1.5 Gm. of ascorbic acid subcutaneously. Both groups were examined daily and photographed at selected times. Ascorbic acid levels were determined in aqueous humor and plasma samples, taken simultaneously, from a few selected animals just prior to their daily injection. One rabbit from each group died of mucoid enteritis unassociated with the treatment schedule. Observations on the 18 surviving animals are reported.

**Results**

The changes in aqueous humor glucose and ascorbate levels as a function of time following 6 and 12 mm. alkali burns are shown in Fig. 2. After a 6 mm. alkali burn,
Fig. 2. Changes in aqueous humor levels of ascorbic acid (A) and glucose (B) at intervals following 6 and 12 mm. burns of the rabbit cornea with 1N NaOH. Each point represents the arithmetic mean of four determinations; the bar gives the standard error of the mean. Separate groups of rabbits were employed for each time interval.
Fig. 3. Typical changes in the corneas of a control (A and B) and ascorbate-treated (C and D) rabbit following 12 mm. alkali burns. A. Control rabbit No. 7 showed severe corneal ulceration in the right eye at 21 days after the burn. The areas in the cornea of greater lucency represent severe ulceration. B. The same control eye at 42 days after the burn showed a large corneal perforation with partial extrusion of the crystalline lens. C. This ascorbate-treated rabbit, No. 2, showed extensive vascularization in the right eye at 17 days after the burn. D. The same eye at 45 days shows a heavily vascularized rosette around the central 3 mm. of the cornea with extensive vascularization centrally. No ulcers appeared in the post-burn course.
glucose levels returned to normal by 14 days, whereas ascorbate levels returned to 80 per cent of control values within 7 days. These corneas demonstrated persistent epithelial defects, occasional peripheral vascularization, but no ulceration or perforation. After a 12 mm. alkali burn, glucose levels in the aqueous humor closely paralleled those following a 6 mm. burn. However, ascorbate levels remained well below control values, even at 28 days. All 12 mm. burned corneas demonstrated acute inflammation, persistent epithelial defects, and corneal vascularization, first seen peripherally in some animals at 6 days. Ulceration, formation of descemetoceles, and subsequent perforation were frequently observed in animals maintained longer than 14 days. There was no correlation between the patterns of epithelial wound closure and changes in aqueous humor chemistry in either 6 or 12 mm. burned eyes.

The observation that perforations occurred only in the 12 mm. burned corneas, where aqueous ascorbate levels were well below normal, led us to investigate the effect of raising aqueous humor ascorbate levels in a group of animals receiving 12 mm. alkali burns. There was a remarkable difference in the ascorbate-treated animals, as is demonstrated in Fig. 3. Whereas 11 out of 18 corneas in the non-ascorbate-treated group progressed to perforation, none of the 18 corneas in the ascorbate-treated group perforated. Three out of the 18 eyes in the ascorbate-treated group showed some ulceration, but this completely reversed with continued ascorbic acid treatment. Aqueous humor and plasma ascorbate levels in selected ascorbate-treated animals were much higher than those in nontreated animals at the same time period (Table I).

Apparently, raised ascorbate levels had little influence on epithelial migration, since the 12 mm. alkali-induced defect followed a pattern identical to that observed in the non-ascorbate-treated animals.

Table I. Ascorbate levels in the aqueous humor following 12 mm. corneal burns with 1N NaOH in control and ascorbate-treated rabbits

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<tr>
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<th>Aqueous ascorbate levels* (mg./100 ml.)</th>
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<tbody>
<tr>
<td></td>
<td>3 days</td>
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<tr>
<td>Nontreated</td>
<td>6.6 ± 1.4</td>
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<tr>
<td>Ascorbate</td>
<td>21.3 ± 0.9</td>
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*Data given are means ± standard error of four determinations for each value. Separate groups of rabbits were used for each time period.

Discussion

In 1969, Brown, Wassermann, and Dunn found no change in the aqueous humor glucose content 7 and 28 days following alkali burns of the cornea and sclera, made with cotton pledgets soaked in 0.5N sodium hydroxide. Pfister, Friend, and Dohlman demonstrated marked reduction in aqueous humor glucose content up to 14 days following 45 sec., 1N sodium hydroxide burns of the anterior half of the rabbit eye. As suggested by the latter authors, the disparity between the two results might be explained by the differing severity of the burns. They postulated that low glucose levels in the aqueous humor might be an indication of an inadequate nutritional supply for reparative processes in the damaged cornea. It should be noted, however, that in the series reported by Brown, Wassermann, and Dunn, a high percentage of the alkali-burned corneas progressed to perforation despite reported normal aqueous humor glucose levels. Since corneal ulcers occurred after the milder burns of these authors, and in the present study despite normal aqueous glucose levels, glucose deficiency cannot be the determining factor causing ulceration.

Perforations did not occur in the 6 mm. alkali-burned eyes where the aqueous ascorbate and glucose levels returned to normal. Although glucose levels returned to normal following a 12 mm. alkali burn, the ascorbate levels remained depressed. It was apparent that under the conditions...
where ascorbate levels remained low, many corneas became ulcerated and subsequently perforated. Since ascorbate levels were used as an index of ciliary body function, there might have been a deficiency in any number of aqueous humor components contributing to the observed corneal changes. However, the finding that massive parenteral supplementation of aqueous humor ascorbate levels entirely prevented perforation in 12 mm. burned animals suggests that a severe alkali burn of the eye results not only in a traumatized but also scorbutic cornea. The maintenance of sufficiently high levels of ascorbic acid in the aqueous humor apparently compensated for the alkali burn-induced scorbutic state, thereby preventing the development of corneal ulceration and subsequent perforation. It is noteworthy that raising aqueous ascorbate levels had no demonstrable influence on epithelial migration rate and subsequent epithelial wound closure. Epithelial migration has previously been shown to be unimpaired in scurvy.

The use of ascorbic acid in the treatment of certain corneal diseases is not a new concept. Lyle and McLean and Summers in 1941 and 1946, respectively, presented cases of corneal ulceration in which the healing process in epithelium and stroma was markedly improved with intravenous ascorbic acid. In 1950, Boyd and Campbell reported a significant acceleration in the healing of deep corneal ulcers in patients receiving large oral doses of ascorbic acid.

Many questions arise from our findings. Is ascorbic acid directly influencing corneal metabolism and enhancing collagen and glycosaminoglycan synthesis? Is it acting as a collagenase inhibitor? Is it influencing neovascularization directly or indirectly? Although no attempt will be made to specifically answer such questions here, it is worthwhile to draw attention to some previous studies in this area. It is known that ascorbic acid is intimately involved in the biosynthesis and maintenance of collagen, and in the biosynthesis of glycosaminoglycans. Ross and Benditt have reviewed the literature dealing with defective wound healing in human beings and animals with scurvy. These same authors contributed morphological findings demonstrating marked differences in the pattern of healing of skin wounds in normal and scorbutic guinea pigs.

In human scurvy, ocular lesions, generally consisting of conjunctival hemorrhages, are rare and seen only in severe cases; no specific corneal lesions have been described. In 1950, Campbell and Ferguson found that superficial epithelial defects in scorbutic guinea pigs healed normally, but that stromal defects healed slower than normal and the healing lesions were structurally weak. However, no corneal changes were observed in scorbutic animals over a prolonged period. Campbell and Ferguson concluded that in the scorbutic state, aqueous humor levels of ascorbic acid are adequate to preserve normal function. Only in times of stress (i.e., wound healing) does the cornea experience a paucity of ascorbate in the aqueous humor.

Corneal ulceration and perforation represent a shift of collagen metabolism toward destruction, probably mediated by the enzyme collagenase. Whether ascorbic acid is an inhibitor of collagenase activity in the cornea has not been determined. Grillo and Gross, however, have indicated that collagenase activity in skin wound healing is not affected by altered ascorbic acid levels.

The precise mechanism whereby adequate aqueous humor levels of ascorbic acid are able to reduce corneal ulceration and perforation following alkali burns of the cornea is presently under investigation.

REFERENCES

2. Hughes, W. F.: Alkali burns of the eye. II.