The influence of parenteral ascorbate on the strength of corneal wounds

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Rabbits receiving subcutaneous ascorbate after corneal wounding showed significant elevation of aqueous humor ascorbate levels but no enhancement of wound breaking strength when compared to controls. In a second group of rabbits, perilimbal alkali burning reduced aqueous humor ascorbate levels one-half to one-third normal. In these periliminally burned eyes with wounds in clear cornea, subcutaneous ascorbate significantly raised the aqueous humor ascorbate level and enhanced breaking wound strength compared to controls. We conclude that parenterally administered ascorbate has no salutory effect on the breaking strength of corneal wounds in the normal rabbit eye. In contrast, subcutaneous ascorbate has a very favorable effect on the breaking strengths of corneal wounds in those eyes with depressed aqueous humor ascorbate.

Key words: cornea, vitamin C, corneal wound healing, corneal burns, rabbits

Corneal wound healing is inhibited by a variety of conditions, including topical corticosteroids,\textsuperscript{1, 2} beta irradiation,\textsuperscript{3} immunosuppressive agents,\textsuperscript{4} antiviral medications,\textsuperscript{5} and epithelial denudation.\textsuperscript{6} Only soft contact lens therapy has been reported to increase the strength of limbal wounds.\textsuperscript{7} No drug treatment is known that can accelerate the healing of corneal stromal wounds.

Ascorbic acid is extremely important for the production of collagen in the healing of wounds.\textsuperscript{8, 9} It functions as a cofactor in the hydroxylation of proline\textsuperscript{10} and may be required for the maturation of mononuclear cells into repair fibroblasts.\textsuperscript{11} Corneal requirements for ascorbic acid are provided by the aqueous humor, which contains about 20 times the concentration of this vitamin in the plasma; maintenance of such high levels is a function of membrane transport in ciliary epithelium. Ocular alkali burns damage this concentrating mechanism thereby decreasing ascorbic acid in the aqueous humor.\textsuperscript{12} Preliminary experiments revealed that perilimbal alkali burning alone could suppress aqueous humor ascorbate levels while leaving the cornea clear.

The experiments in this paper address the following questions. Will an excess of ascorbic acid in the aqueous humor accelerate corneal wound healing in a normal eye? Is the strength of a corneal wound diminished by lower concentrations of ascorbic acid in the aqueous humor? In the latter instance, does the subcutaneous injection of ascorbate improve the corneal wound strength?

Materials and methods

Group I. Perforating corneal lacerations in normal eyes. Forty-three New Zealand albino

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rabbits, weighing 3.5 to 4.5 kg, were anesthetized with intravenous pentobarbital. The pupils were dilated with 2 drops of 10% phenylephrine and the corneas were anesthetized topically with 2 drops of 0.5% tetracaine. Razor blade, knife, and scissors were used to make a vertical 7 mm linear perforating laceration in all the corneas. Each wound was approximated with three 10-0 interrupted nylon sutures placed with the aid of an operating microscope (Fig. 1). Erythromycin (0.5%) ointment was applied to the eye postoperatively.

**Group II. Perforating corneal lacerations in periliminally burned eyes.** In a subsequent experiment, 48 New Zealand albino rabbits, weighing 3.5 to 4.5 kg, were anesthetized with intravenous pentobarbital. Each eye was proptosed and then pressed through a small hole in thin plastic drape back to the equator. A 12 mm round plastic guard was then placed over the cornea to protect it from burning. This left a ring of sclera from the limbus to the equator of the eye on which 3 ml of IN sodium hydroxide were spread by a medicine dropper for 20 sec (Fig. 2). Excess sodium hydroxide was irrigated away with 5 ml of 0.9% saline. The cornea remained clear after the burning procedure. Immediately after the burn procedure, a perforating corneal laceration was performed in each eye and repaired as described above.

**Ascorbic acid subcutaneous injections.** Immediately after corneal surgery, the rabbits were alternately assigned to the experimental or control group. At this time and once daily thereafter, each experimental rabbit was injected subcutaneously with ascorbic acid (0.5 gm/kg body weight), and controls were given 10 ml subcutaneous injections of 0.45% saline.

**Postoperative care.** All eyes were treated twice daily with 0.5% erythromycin ointment. Wounds were examined weekly for integrity, width of scar, loose sutures, and for onset of infection, edema, and vascularization. During the examinations, any loose sutures were removed after topical application of 0.5% tetracaine. Eyes were excluded from...
Mean Tensile Strength vs. Time (Unburned Animals)

N=8 N=9

Mean Tensile Strength vs. Time

N=6 N=5

N=6 N=5

N=6 N=6

N=7 N=5

N=6 N=6

N=6 N=9

10 14 21 28 42

Time (Days)

400

300

200

100

40

400

300

200

100

40

Mean Tensile Strength

Experimental

Control

Fig. 3. There was no statistically significant difference (p > 0.1) in the tensile strength of corneal wounds in normal animals with or without ascorbate treatment.

the study before the tensiometer measurements if they showed overt corneal, conjunctival, or intraocular infection, moderate wound infiltration, moderate anterior wound separation, moderate corneal edema at the lips of the wound, or wound vascularization. During all examinations, the investigator was masked from the identity of each animal.

Blood, aqueous humor, and tissue samples. At designated times from 20 to 22 hr after the final injections, animals were deeply anesthetized. A specially designed handle armed with two parallel number 15 scalpel blades was used to make 5 mm parallel tracks perpendicular to and centered on the wound in each eye. The tracks extended from limbus to limbus but did not perforate the eye. Aqueous humor was then obtained by paracentesis, and the strip of cornea containing the wound was excised. Any remaining sutures were cut. Care was taken to minimize mechanical stress to the corneal wound during removal. Blood was then obtained by cardiac puncture. Aqueous humor and plasma samples were immediately deproteinized with cold 40% trichloracetic acid and the samples were assayed for ascorbic acid.13 14

Breaking strength determination. Each rectangular corneal strip was gently maneuvered into specially designed clamps and hung vertically by attaching one clamp to a fixed mount. A lightweight bottle was attached to the clamp at the free end of the corneal strip. Water was allowed to flow at a constant rate through tubing into the lightweight bottle until the weight was sufficient to break the wound. The total weight of the bottle, contained water, clamps, and enclosed tissue was then recorded as the measure of breaking strength. All measurements were double masked.

Results

Group I. Perforating corneal laceration in normal eye. In the control animals there were 32 normally healing wounds, one infected wound, nine wound dehiscences, one vascularized wound, and one anterior synechia. In the animals receiving ascorbate there were 35 normally healing wounds, four infected wounds, two wound dehiscences, and one vascularized wound. Results are given only from the normally healing wounds. There was no significant difference (p > 0.1) in the breaking strength of corneal wounds from the ascorbate-treated animals and the control group (Fig. 3). Although the mean breaking strength of both the ascorbate-treated and control animals was lower at 28 days than at 21 days, this difference was not statistically significant (p > 0.05). Fig. 4, A and B, illustrate the significantly higher aqueous humor and plasma levels of ascorbic acid in the experimental animals compared to the control animals (p < 0.05).

Group II. Perforating corneal lacerations in periliminally burned eyes. The corneas in both of these subgroups were clear and free of any evidence of alkali injury. In the control animals there were 28 normally healing wounds, three infections, and 13 dehiscences. The animals receiving ascorbate had 25 normally healing wounds, four infections, 13 dehiscences, and four with moderate corneal edema. Results are derived from only the normally healing wounds. The breaking strengths of corneal wounds from the ascorbate-treated and the control groups were similar at the 7- and 10-day periods (Fig. 5). There is a statistically significant increase in the corneal wound strength of ascorbate-
treated animals beginning at 14 days (p < 0.038) and continuing at 21, 28, and 35 days (p < 0.01).

Fig. 6, A, shows the significantly depressed aqueous humor ascorbic acid level in the control animals compared to the normal, or greater than normal, values in the ascorbate-treated animals throughout the experiment (range p < 0.01 to p < 0.001). Fig. 6, B, shows the significantly elevated plasma ascorbic acid in the treated group compared to the control group (range p < 0.01 to p < 0.001).

**Discussion**

Subcutaneous administration of ascorbic acid in rabbits with corneal lacerations significantly increased aqueous humor levels of ascorbic acid above those in control animals. Such elevation was evident even 20 hr after subcutaneous injections of ascorbic acid and thus one must conclude that the levels were even greater during the preceding time period. Despite the marked elevation of aqueous humor levels of ascorbate in the normal animal eyes, the breaking strength of corneal wounds in the treated group was not greater than that in the control animals.

The inability of supranormal levels of ascorbic acid to improve corneal wound strength in rabbit eyes is not entirely unexpected. Veen-Baigent et al. showed that, in guinea pigs, various parameters of skin wound healing reached a maximum level even when tissue levels had only been half-saturated by ascorbic acid intake. Excessive intakes did not enhance wound healing. One might conclude that the normal rabbit cornea has access to a more than adequate supply of ascorbic acid to support corneal wound healing; hence no enhance-
ment can be achieved by supplementation.

After the perilimbal alkali burn, the animals not receiving subcutaneous ascorbic acid had markedly reduced levels of ascorbic acid in the aqueous humor. The rabbits that received ascorbic acid injections showed a significant increase in the level of this vitamin in the aqueous humor. This increase correlated with an increase in the ultimate strength of corneal wounds when compared to the untreated groups of rabbits. Although statistically significant, the ascorbic acid levels in the aqueous humor of these two groups may not appear to be sufficiently great to account for the breaking strength differences observed. To explain this, it must be remembered that the ascorbate injection occurred 20 to 22 hr previously and that the recorded concentrations represent the lowest level of aqueous humor ascorbic acid during any 24 hr period.

It is not surprising that no statistical differences could be found in corneal wound strength between these two groups 7 to 10 days after the injury. This period includes the acute inflammatory response and the subsequent phagocytic phase. Only after 3 or 4 days do prominent numbers of monocytes and lymphocytes appear with mature fibroblasts that appear to be actively producing collagen. Hydroxyproline, a convenient marker for new collagen, appears in increasing concentrations from the fourth to sixth day. Argyrophilic fibers and native collagen can only be identified in fresh wounds on the fifth or sixth day after injury. We conclude therefore that the tissue events in the wound prior to 7 days are attended by relatively little collagen production and that hence the wound possesses little tensile strength.

The strength of these corneal wounds depends on the presence of bridging collagen fibers and the degree of crosslinking. After 10 days, the consistently lower strength of the wounds in the control animals implies that collagen production and possibly crosslinking are curtailed when the ascorbic acid level in the aqueous humor is halved.

Nevertheless, when animals in our study are supplemented with ascorbate, wound strength is statistically greater, suggesting a larger amount of newly synthesized collagen in the wound. Supplemental ascorbate is able to overcome burn-induced alterations and deficiencies of aqueous humor components that might be unfavorable for wound healing. One other study showed that when the availability of ascorbic acid to the cornea was decreased the process of wound healing was compromised. Boyd reported that the healing of corneal wounds in aphakic animal eyes, where the corneal and aqueous humor levels of ascorbic acid are usually low, is generally slower than that in the contralateral phakic eye. Furthermore, the number of relapses during healing was greater in the aphakic eye than in the phakic eye. Our results support this concept.

Exogenous ascorbate has also been shown to reduce the incidence of alkali burn–induced corneal ulcers in the rabbit, another situation where the anterior segment levels of ascorbic acid are greatly diminished. The mechanism of this effect is thought to be the reversal of tissue scurbutus. We believe
Ascorbate effect on corneal wounds

Fig. 6. Ascorbic acid level of aqueous humor (A) and plasma (B) in animals receiving perilimbal burns was significantly higher in the ascorbate treated animals compared to the control animals.

that ascorbate exerts its favorable effects on the alkali-burned eye and on the strength of corneal wounds in periliminally burned eyes by enhancing collagen production.

The dietary dependence of humans on exogenous sources of ascorbic acid leads to specific clinical implications, especially in perforating ocular wounds. It is rare for ophthalmologists to have comprehensive knowledge of the nutritional status of patients with perforating ocular trauma or of patients in whom surgery is planned. This study suggests that an evaluation of the nutritional status of individuals after such trauma or before anticipated surgery, especially in the chronic ill, alcoholics, cancer victims, and even the elderly might be rewarding.

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REFERENCES


