influencing the concentration of insulin in aqueous humor are the plasma insulin concentration, the permeability of the blood-aqueous barrier, and the aqueous humor turnover rate; it is conceivable that the blood-aqueous barrier may have the capability of degrading insulin and that this also could influence the insulin concentration in aqueous humor.

It is not yet known whether or not insulin influences the lens metabolism under physiologic conditions. The presence of insulin receptor sites in the lens suggests that such an influence might exist. Appropriate studies of various metabolic effects of insulin on the lens in vitro at insulin concentrations known to occur physiologically would be expected to help in assessing the effects of insulin on lens metabolism.

From the Division of Research, Scott and White Memorial Hospital, Scott, Sherwood and Brindley Foundation, Temple, Texas. Supported by NIH Research Grant EY 00404, awarded by the National Eye Institute. Submitted for publication May 1, 1980. Reprint requests: Dr. J. B. Coulter, III, Scott and White Memorial Hospital, 2401 South Thirty-First Street, Temple, Texas 76508.

Key words: insulin concentration, aqueous humor, paracentesis, blood-aqueous barrier, lens, diabetic cataract

REFERENCES


The efficacy of ascorbate treatment after severe experimental alkali burns depends upon the route of administration. Roswell R. Pfiester, Christopher A. Paterson,* John W. Spiers,*, and Sonia Anderson Hughes.

Rabbit eyes were subjected to severe alkali burns (35 sec, 12 mm, 1N sodium hydroxide). In one experiment, rabbits in the treated group received a daily subcutaneous injection of neutralized ascorbic acid solution (0.5 gm/kg body weight), while control animals received no treatment. At the termination of the experiment (30 days), 11 of 16 eyes (68.8%) in the control group had ulcerated or formed descemetocytes, and in the experimental (treated) group, 15 of 20 eyes (75%) had ulcerated, formed descemetocytes, or perforated. In a second experiment, burned rabbits received topical 10% ascorbic acid while control eyes were given the vehicle only. At the termination of the experiment (34 days), 16 of 20 eyes (80%) in the control group had ulcerated or perforated, compared to five of 18 eyes (27.8%) in the ascorbate treated groups. The failure of systemic administration of ascorbic acid to prevent corneal ulceration could be explained on the basis of inadequate penetration of ascorbic acid into the anterior segment of severely burned rabbit eyes. On the other hand, immediate topical treatment of identically burned rabbit eyes achieved greatly elevated aqueous humor ascorbate levels and provided substantial protection from corneal ulceration and perforation.

After 20 sec, 12 mm, 1N sodium hydroxide burns of the rabbit eye, the level of ascorbic acid in the aqueous remains persistently depressed. It has been established that the incidence of corneal ulceration and perforation induced by such burns can be significantly reduced by elevating the aqueous humor ascorbate levels by subcutaneous injection or by topical administration of ascorbic acid. This beneficial effect was considered to be the result of restoring corneal levels of ascorbic acid, which is essential for collagen synthesis.

The purpose of this study was to determine whether parenteral and topical administration of ascorbic acid would each be effective in reducing...
Table I. Parenteral ascorbate treatment: clinical observations and ascorbic acid levels in aqueous humor and plasma

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 16)</th>
<th>Experimental (n = 20)</th>
<th>p value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No ulcer</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2. Ulcers</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3. Descemetocoele or perforations</td>
<td>5</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4. Ulcers invaded by vascularization</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5. Items 2 to 4 combined</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>6. Plasma ascorbic acid (mg/dl)</td>
<td>0.6–1</td>
<td>3–6.5</td>
<td>&gt;0.5</td>
</tr>
<tr>
<td>7. Aqueous humor ascorbic acid (mg/dl)</td>
<td>4.69 ± 0.71</td>
<td>10.14 ± 0.58</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Clinical observations and aqueous ascorbic acid levels made at 30 days.
†Range of values taken through the experiment.

Table II. Topical ascorbate treatment: clinical observations and ascorbic acid levels in aqueous humor and plasma

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 20)</th>
<th>Experimental (n = 18)</th>
<th>p value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. No ulcer</td>
<td>4</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2. No ulcer with total vascularization</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3. Ulcers</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4. Descemetocoeles</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5. Perforations</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6. Items 3 to 5 combined</td>
<td>15</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>7. Plasma ascorbic acid (mg/dl)</td>
<td>0.78 ± 0.09</td>
<td>1.59 ± 0.11</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>8. Aqueous humor ascorbic acid (mg/dl)</td>
<td>5.41 ± 2.00</td>
<td>22.20 ± 4.54</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

the incidence of ulceration and perforation in more severely burned rabbit eyes.

Materials and methods

General considerations. New Zealand Dutch strain albino rabbits, weighing between 2.0 and 2.5 kg, were used in all the experiments. All ascorbic acid determinations were made with the method of Maickel⁴ as modified by Zannoni et al.⁵

Alkali burns. Rabbits were anesthetized with intramuscular ketamine hydrochloride (12 mg/kg) and xylazine (7.5 mg/kg) supplemented by 0.5% proparacaine applied topically to the cornea. Each eye was proptosed, and a 12 mm plastic well was centered on the cornea. Sodium hydroxide (IN, 0.4 ml) was pipetted into the well and allowed to remain in contact with the cornea for 35 sec, rather than 20 sec as previously used.¹² After 35 sec, the alkali was aspirated from the plastic well, and the interior of the well and the cornea thoroughly irrigated with normal saline.

Parenteral treatment with ascorbic acid. Immediately after burning, rabbits were placed in the experimental group (10 rabbits) or the control group (10 rabbits). Only animals in the experimental group received a subcutaneous injection of neutralized 15% ascorbic acid solution (0.5 gm/kg body weight) within 3 hr of the burn and once daily thereafter. This dose of ascorbic acid has been shown to achieve maximally elevated levels of ascorbic acid in the aqueous humor. All eyes received 0.5% erythromycin ointment daily as a prophylactic measure.

The eyes were examined three times a week, with special focus on ulceration, descemetocoele formation, perforation, and corneal vascularization. Significant changes were examined under a portable slit lamp and photographed.

At the termination of the experiment (30 days), blood samples were drawn, and aqueous humor was aspirated from all eyes that had not perforated. These samples, taken 20 to 24 hr after the last subcutaneous injection, were analyzed for ascorbic acid content.

Topical treatment with ascorbic acid. The ascorbic acid–dropping solution was 10% ascorbic acid in hydroxyethylcellulose with polyvinylpyrrolidone, thimerosal, and EDTA (Adsorbotear; Burton, Parsons & Co., Inc., Washington, D. C.) adjusted to pH 7.2 with 10N sodium hydroxide. The dropping solutions were prepared freshly.
every 14 days and kept refrigerated when not in use. The solutions were analyzed for ascorbic acid content weekly to ensure that the appropriate concentration was being used.

Rabbits were then assigned to the experimental group (11 rabbits) or the control group (10 rabbits).

Two hours after burning, rabbits in the experimental group were given 2 drops of the 10% ascorbate in Adsorbotear in both eyes. The eyes of rabbits in the control group received 2 drops of Adsorbotear alone. The dropping technique of Fraunfelder was used. In both groups, the appropriate dropping preparation was applied to each eye at hourly intervals from 8:00 A.M. to 9:00 P.M. for the duration of the study (35 days). Rabbits were head-restrained during the 14 hr daytime dropping period and returned to separate cages with food and water at night. Examinations and antibiotic prophylaxis were identical to those described in the previous experiment.

Animals were killed on days 34 and 35 of the experiment between the hours of 1:00 and 3:00 P.M. For the determination of aqueous humor ascorbate levels, drops begun at 8:00 A.M. were continued in the usual manner until the time of termination. Ascorbic acid determinations were made on all aqueous humor and blood samples.

**Results**

**General considerations.** The clinical course of these more severely alkali burned rabbit corneas was virtually identical to that described previously for corneas undergoing 20 sec, 1N sodium hydroxide burns. The only difference was that a greater number of corneas progressed to ulceration after more severe burns; this aspect is discussed later.

**Parenteral treatment.** During the period of the experiment, two animals in the control group died. The data presented therefore are drawn from 16 control eyes and 20 experimental eyes. No eyes were infected.

The clinical findings in this study are summarized in Table I. Clearly, the overall pictures in both the control and experimental group are very similar. In the control group, 11 of 16 eyes (68.8%) ulcerated or formed descemetoceles. In the experimental group, 15 of 20 eyes (75%) ulcerated, formed descemetoceles, or perforated. In the control group, four eyes ulcerated, but the ulcers were subsequently invaded by vascularization and healed; in the experimental group, five eyes showed this behavior.

All animals receiving daily subcutaneous injections of ascorbic acid manifested levels of ascorbic acid in the plasma significantly greater than those in the control, untreated rabbits (Table I). Similarly, the mean level of ascorbic acid in the aqueous humor of treated rabbits was greater than that in controls (Table I).

**Topical treatment.** Two rabbits in the experimental group died prior to day 17 and were excluded from the study. Thus data are drawn from 20 control eyes and 18 experimental eyes. No eyes were infected.

The clinical data are summarized in Table II. In the control group, 16 of 20 eyes (80%) ulcerated, formed descemetoceles, or perforated as compared to five of 18 eyes (27.8%) in the ascorbate-treated group. There were seven perforations in the control group vs. only one perforation in the ascorbate-treated group. Four ulcers in the treated group included one anterior stromal ulcer, two midstromal ulcers, and one descemetocele. In contrast, the control group showed two anterior stromal ulcers, two midstromal ulcers, two posterior stromal ulcers, and three descemetoceles. Three ascorbate-treated corneas became totally vascularized as opposed to none in the control group. All 18 eyes in the control group developed clinical band keratopathy. Only three unilateral bands were observed in the topically ascorbate-treated animals.

At the end of the experiment, the mean aqueous humor ascorbate level in the control group was significantly lower than that in the ascorbate-treated group. The plasma ascorbic acid levels were also statistically different. These data are given in Table II.

**Discussion.** In previous studies, the incidence of ulceration following 20 sec, 12 mm, 1N sodium hydroxide burns to the cornea was between 47% and 60% in untreated rabbits. Under these burn conditions, both subcutaneous and topical administration of ascorbic acid significantly reduced the incidence of subsequent corneal ulceration. By an increase in the time of the burn from 20 to 35 sec, the incidence of ulceration in control group animals was increased to between 69% and 80%; the number of ulcers progressing to perforations was also increased. The present study shows that under the conditions of a more severe burn, parenteral administration of ascorbic acid does not reduce the incidence of corneal ulceration but that topical administration continues to be effective in this respect.

The apparent failure of systemic ascorbic acid therapy can be readily explained in terms of the relative penetration of ascorbic acid into the aque-
ous humor after subcutaneous administration in the 20 vs. 35 sec alkali burns. It has been shown previously that following a 20 sec burn, subcutaneous administration of ascorbic acid (0.5 gm/kg) raised the aqueous humor level of ascorbic acid from about 6 mg/dl to a “near normal” level of 20 mg/dl even 24 hr after the last injection. It was suggested that raising the level of aqueous humor ascorbic acid to at least 15 mg/dl was necessary to exert a beneficial effect upon the alkali-burned rabbit cornea. In the present study, after 35 sec burns, subcutaneous administration of ascorbic acid raised the mean level of ascorbic acid in the aqueous humor (taken 24 hr after injection) to only 10 mg/dl despite adequate levels of ascorbic acid in the plasma. Such a result indicates that the 35 sec burn inflicts greater trauma to the ciliary processes, thereby reducing the transfer of exogenously administered ascorbic acid from blood to aqueous humor.

In contrast, despite the increased severity of the alkali burn, immediate topical ascorbate treatment greatly increased anterior segment levels of ascorbic acid and significantly reduced the incidence of corneal ulceration and perforation. Clearly, topical administration of ascorbic acid obviates the difficulties encountered in the transfer of parenterally administered ascorbic acid across the blood-aqueous barrier.

We can conclude therefore that the systemic route of administration, as singular therapy for alkali burns of human eyes, might not be satisfactory because one cannot readily evaluate the severity of the burn in terms of its effect on the transfer of ascorbic acid from blood into the anterior segment. Thus ascorbic acid is being administered both orally and topically in an ongoing clinical trial to evaluate the effects of ascorbic acid on corneal alkali burns.

Secretarial assistance was given by Kathy Fleck.

From the Combined Program in Ophthalmology, University of Alabama in Birmingham—Eye Foundation Hospital, Birmingham, Ala., and *Department of Ophthalmology, University of Colorado Health Sciences, Denver. Supported by U.S.P.H.S. Consortium Grant EY 02018 from the National Eye Institute and by Ellen Gregg Ingalls Eye Research Institute. Submitted for publication March 31, 1980. Reprint requests: Roswell R. Pfister, M.D., 1720 8th Avenue, South, Birmingham, Ala. 35233.

Key words: alkali burns, vitamin C, cornea and corneal burns, topical administration, parenteral administration, rabbits

References

Utility of the Arden grating test in glaucoma screening: high false-positive rate in normals over 50 years of age. Samuel Sokol, Alice Domar, and Anne Moskowitz.

The Arden grating test was administered to 64 subjects between 6 and 82 years of age without ocular pathology, to 20 glaucoma patients, and to 21 ocular hypertensives. The results show an age effect, with significantly higher scores (lower sensitivity) in normal subjects over 50 years of age. In addition, there was no significant difference in performance between age-matched normals, glaucoma patients, and ocular hypertensives on the Arden gratings. The results show a high percentage of false-positives in older normal subjects. The Arden gratings should be used cautiously when one is testing patients over 50 years of age.

Arden and Jacobson reported in this Journal that a series of photographs of sinusoidal gratings could be used as a quick, simple screening test for glaucoma. According to the authors, this test makes "a very sharp distinction . . . between normal and glaucomatous eyes." Moreover, the grating test scores were considered to be independent of age. The purpose of the present study was to further evaluate the diagnostic value of the Arden plates. Our results do not support the original conclusions of Arden and Jacobson.

The test consists of seven plates: a screening