The ocular hypertensive response following experimental acid burns in the rabbit eye

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Following application of 50 μl of 2N hydrochloric acid to the rabbit cornea, the intraocular pressure rapidly increases and remains markedly elevated for up to 3 hr. The initial rapid increase in intraocular pressure appears to be the result of acid-induced shrinkage of the outer collagenous coats of the eye. The sustained rise in intraocular pressure is mediated in part by prostaglandin release. Increased prostaglandin-like activity, determined in the aqueous after an acid burn, was greatly inhibited by pretreatment of rabbits with indomethacin and to a much lesser extent by pretreatment with imidazole. Both indomethacin and imidazole essentially abolished the sustained elevation of intraocular pressure after an acid burn. Analysis of changes in pH and protein level in the aqueous implies that the stimulus for prostaglandin release within the eye is the penetration of hydrogen ions into the aqueous humor, with resultant intraocultra trauma.

Key words: hydrochloric acid, intraocular pressure, protein, pH, prostaglandins, indomethacin, imidazole

A number of chemicals applied topically to the eye result in an increase in intraocular pressure.1 The characteristics of the pressure rise vary with the chemical used. Following topical application of 2N sodium hydroxide in the rabbit and monkey eye, there is a biphasic pressure response.2-5 It has been shown that the first response is due to shrinkage of the outer coats of the eye.3-5 The second response appears to be mediated, to a large extent, by the intraocular release of prostaglandins.6 Chiang et al.2 reported that following a hydrochloric acid burn of the rabbit eye, the ocular pressure response was slower than after that produced by alkali and was not biphasic. The purpose of this study was to examine the characteristics of the ocular hypertensive response following hydrochloric acid burns.

Materials and methods

New Zealand Dutch strain albino rabbits of either sex (2.5 to 3.5 kg), anesthetized with 25% urethane administered via a marginal ear vein, were used in all experiments. Burns were made with 50 μl of 2N hydrochloric acid delivered to the cornea from an automatic pipet.

Intraocular pressure measurements. The pro-
Fig. 1. Intraocular pressure changes in control (open circles) and experimental (filled circles) rabbit eyes following application of 50 μl of 2N hydrochloric acid to the experimental eye. The graph shown is the mean of six experiments. The vertical bars represent the S.E.M. of intraocular pressure change at a given time. Where bars are absent, the S.E.M. is contained within the size of the circle. Aqueous humor pH, protein, and prostaglandin data relating to this experiment are given in Table I.

The intraocular pressure response to the acid was examined under a number of different experimental conditions. To determine whether lid squeezing and/or pooling of acid in the conjunctival cul-de-sac altered the intraocular pressure response, the eyelids and nictitating membrane were excised from both eyes prior to cannulation of the anterior chamber. To further examine the possible influence of pooling of acid within the conjunctival sac, the acid was blotted and then carefully flushed from the external surface of the eye 2 or 15 min following the initial application of hydrochloric acid. The unburned control eye was flushed in a similar fashion.

To examine the possible role of shrinkage of the outer coats of the eye, intraocular pressure measurements were made in dead eyes in situ and following enucleation. The precise procedures for these experiments have been described previously.

Changes in intraocular pressure following the application of hydrochloric acid were also determined in rabbits pretreated for 1 hr with intraperitoneal indomethacin (50 mg/kg) or imidazole (200 mg/kg).

Prostaglandin-like activity in the aqueous humor. Separate groups of rabbits were used in these experiments. One eye of each animal was burned with 2N hydrochloric acid. At a specified time after the burn, aqueous humor was aspirated from each eye with a 1 ml disposable syringe, and transferred to a vial on Dry Ice. Each vial contained aqueous humor from either four control or four...
Fig. 2B. Effect of excising the eyelids prior to applying the acid (n = 7).

Fig. 2C. Effect of blotting and rinsing 2 min after the acid burn in eyes with the eyelids removed (n = 3).

experimental eyes. The samples were stored at -20° C and then shipped, on Dry Ice, to Dr. Kenneth Eakins, in New York, for extraction and bioassay to determine prostaglandin-like activity. Assays were performed on aqueous humor samples obtained in a number of different experimental conditions (see Results).

**pH and protein determinations.** In further series of animals, aqueous humor was withdrawn from acid-burned and control eyes at specified times, and the pH was determined using a microcombination pH electrode. An aliquot of the same aqueous sample was assayed for protein by the method described by Lowry et al. Determinations were made on aqueous humor samples obtained in a number of different experimental conditions (see Results).

**Results**

When 50 μl of 2N hydrochloric acid was applied topically to the cornea and allowed to pool between the eyelids and in the conjunctival cul-de-sac, the intraocular pressure rose rapidly and remained elevated for over 3 hr (Fig. 1). The intraocular pressure response appeared to be slightly biphasic. Data obtained from separate experiments, but employing identical conditions, showed that the pH of the aqueous humor fell, the protein levels were elevated, and prostaglandin-like activity was still apparently increasing at 2 hr (Table I).

Blotting and lavaging the eye with saline 2 min after an acid burn of the intact eye did not affect the rapid initial rise in intraocular pressure but prevented both the maintained elevation of intraocular pressure (Fig. 2A) and the development of an acidic aqueous humor (Table II). However, the protein content of the aqueous humor was increased with respect to control levels (Table II).

Complete excision of the eyelids and nictitating membrane (Fig. 2B) (which greatly reduces the opportunity of acid to pool around the eye) prior to burning resulted in intraocular responses similar to those seen in
Fig. 3. Changes in intraocular pressure following an acid burn in (a) the dead eye in situ (n = 5) and (b) the enucleated eye (n = 2).

Table I. Protein concentration, pH, and prostaglandin-like activity in aqueous humor from experimental (acid-burned) and control eyes*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>N</th>
<th>pH</th>
<th>Protein (mg/dl)</th>
<th>PG-like activity (ng/ml)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Expt'l.</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>7.5 ± 0.04</td>
<td>4.7 ± 0.90</td>
<td>4</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>7.5 ± 0.06</td>
<td>6.6 ± 0.06</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>7.6 ± 0.03</td>
<td>6.4 ± 0.20</td>
<td>4</td>
</tr>
<tr>
<td>120</td>
<td>4</td>
<td>7.5 ± 0.03</td>
<td>6.2 ± 0.70</td>
<td>4</td>
</tr>
</tbody>
</table>

*Values given are mean ± S.E.M.; N = number of determinations.
†Assay was performed on 4 pooled aqueous humor samples.

Table II. Protein concentration and pH in aqueous humor from control and acid-burned eyes (with lids intact) which were blotted at 2 min after the burn*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>N</th>
<th>pH</th>
<th>Protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Expt'l.</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>7.6 ± 0.10</td>
<td>7.5 ± 0.06</td>
</tr>
<tr>
<td>60</td>
<td>4</td>
<td>7.6 ± 0.10</td>
<td>7.5 ± 0.05</td>
</tr>
<tr>
<td>120</td>
<td>4</td>
<td>7.7 ± 0.10</td>
<td>7.8 ± 0.10</td>
</tr>
</tbody>
</table>

*Values are mean ± S.E.M.; N = number of observations.

eyes with intact eyelids, blotted at 2 min. Blotting and rinsing the eye in experiments where the eyelids had been removed resulted in an even faster decline in intraocular pressure immediately following the initial rapid ocular hypertensive response (Fig. 2C).

As in the alkali-burned rabbit eye, it appeared as if the initial rapid rise in intraocular pressure following the acid burn might be due primarily to shrinkage of the outer coats of the globe. The presence of the initial intraocular pressure response alone in the dead eye in situ (Fig. 3, a) and in the enucleated eye (Fig. 3, b) supports this concept.

To examine the characteristics of the maintained increase in intraocular pressure observed in the intact, unblotted eye, we first determined the influence of blotting and lavaging at 15 min, rather than 2 min, after the burn. In contrast to the marked influence
IOP and ocular acid burns

Fig. 4. Changes in intraocular pressure following an acid burn in the intact eye, blotted 15 min after the burn (n = 3). Filled circles represent experimental eyes, and open circles represent the control eyes.

Table III. Prostaglandin-like activity and protein concentration in aqueous humor from control and acid burned eyes in indomethacin- or imidazole-pretreated rabbits

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein (mg/dl)</th>
<th>PG-like activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Control</td>
</tr>
<tr>
<td>Indomethacin (50 mg/kg)</td>
<td>6</td>
<td>196 ± 63</td>
</tr>
<tr>
<td>Imidazole (200 mg/kg)</td>
<td>4</td>
<td>351 ± 84</td>
</tr>
<tr>
<td>None (values from Table I)</td>
<td>4</td>
<td>96 ± 31</td>
</tr>
</tbody>
</table>

* Aqueous humor samples were aspirated from the anterior chamber at 2 hr after the acid burn. Values given are mean ± S.E.M.; N = number of determinations.

1 Assay was performed on 4 pooled aqueous humor samples.

of blotting and lavaging at 2 min on the intraocular response to an acid burn, blotting and lavaging 15 min after a burn in the eye with intact eyelids resulted in only a gradual fall in intraocular pressure back toward the initial level (Fig. 4). From a previous experiment (Table I), we know that at 15 min after an acid burn, the aqueous humor is markedly acidic. Therefore rinsing the eye at 15 min is apparently able to dilute the acid remaining on the exterior of the eye, although any intraocular damage initiated by the penetration of hydrogen ions into the aqueous is not greatly affected by external flushing of the eye.

Pretreatment of rabbits with indomethacin 1 hr before the acid burn essentially abolished the maintained elevation of intraocular pressure that was observed in untreated rabbits (Fig. 5A). The protein level in the aqueous humor was reduced, and prostaglandin-like activity was undetectable 2 hr after the burn (Table III).

Imidazole pretreatment also abolished the sustained rise in intraocular pressure following an acid burn (Fig. 5B). However, 2 hr after the burn in imidazole-treated rabbits, the protein level was only moderately reduced compared to that in untreated animals. The prostaglandin-like activity at 2 hr re-
Figs. 5A and 5B. Changes in intraocular pressure following an acid burn in rabbits pretreated with either indomethacin or imidazole. Closed circles represent experimental eyes, and open circles represent control eyes. Aqueous humor, prostaglandin, and protein data relating to these experiments are given in Table III. Fig. 5A. Indomethacin-pretreated rabbits (n = 8).

Fig. 5B. Imidazole-pretreated rabbits (n = 3).

remained significantly elevated above control levels. Interestingly, in imidazole-pretreated rabbits, the protein and prostaglandin-like activity at 2 hr appeared to be elevated in the unburned control eyes (Table III).

Discussion

Application of 2N hydrochloric acid to the rabbit eye causes an immediate rise in intraocular pressure similar to that seen following an alkali burn. This pressure rise is sustained for longer than 3 hr under conditions where the eyelids are intact and the acid is not blotted or flushed from the eye. If the sustained elevation of intraocular pressure is in some way prevented or inhibited, only the initial, transient rise in intraocular pressure is seen. These observations indicate that the initial elevation of intraocular pressure is almost entirely due to a direct chemical effect of 2N hydrochloric acid on the outer collagenous coats of the eye. It has previously been found that both strongly alkaline and strongly acid solutions cause rapid shortening of collagen fibers due to breakdown of intermolecular forces of the protein. In the eye, such changes in the outer collagenous coats lead to a transient increase in intraocular pressure.

The findings of this study suggest that the sustained increase in intraocular pressure is mediated largely by prostaglandin release within the eye. As has been observed following several types of ocular trauma, the level of prostaglandin-like activity in the aqueous humor following an acid burn was markedly increased. Furthermore, pretreatment with indomethacin essentially abolished the sustained elevation of intraocular pressure and led to undetectable levels of prostaglandin-like activity in the aqueous humor. Indomethacin, a potent inhibitor of prostaglandin biosynthesis, has previously been shown to prevent the rise in intraocular pressure and
reduce the increased aqueous humor protein levels induced by a variety of inflammatory stimuli.\textsuperscript{11}

The sustained elevation of intraocular pressure following an acid burn was also abolished by imidazole. However, this compound did not entirely prevent the appearance of prostaglandin-like activity in the aqueous. This finding is in keeping with the observation of Zink et al.\textsuperscript{13} that imidazole given intraperitoneally inhibits the elevation of intraocular pressure resulting from topically administered PGE\textsubscript{1}. In addition, imidazole inhibits the rise in intraocular pressure elicited by topical nitrogen mustard, a response that appears not to be mediated by prostaglandins and is not prevented by aspirin-like drugs.\textsuperscript{13, 14} Imidazole has also been shown to prevent breakdown of the blood-aqueous barrier induced by arachidonic acid, infrared irradiation of the iris, endotoxin, \(\alpha\)-melanocyte stimulating hormone, and paracentesis.\textsuperscript{15, 16}

The mechanism and site of action of imidazole in the eye are not presently known. Unpublished data of Eakins et al. suggest that high concentrations of imidazole in vitro might inhibit prostaglandin synthesis to some extent. Such an effect was not clearly evident from the present data. One interesting observation made in the imidazole-treated animals was that the aqueous humor protein content and prostaglandin-like activity were significantly elevated in the control, unburned eye. The significance of this finding is obscure at the present time.

The intraocular pressure increase following trauma of the eye is most probably due to alteration in the permeability characteristics of the blood-aqueous barrier, and thus the level of protein in the aqueous humor can be used as an index of the integrity of that barrier. In the acid-burned, but otherwise undisturbed, rabbit eye, the protein level in the aqueous humor is markedly increased. This increase is diminished in animals pretreated with either indomethacin or imidazole.

The relationship between pH changes in the eye and protein levels in the aqueous humor is of significance. Under circumstances where the acid is allowed to pool around the eye (eyelids intact), hydrogen ions penetrate into the eye, as evidenced by the fall in aqueous humor pH. The acidity results in intraocular damage with subsequent prostaglandin release, an increase in the aqueous humor protein level, and sustained elevation of intraocular pressure. When the acid is unable to pool around the eye (eyelids removed) or when the acid is manually blotted from the eye at 2 min after application, the pH of the aqueous remains essentially unchanged, the initial transient pressure rise is seen, but no sustained rise in intraocular pressure is evident. This indicates that the degree of hydrogen ion penetration is relatively low, although the protein level of the aqueous humor is moderately elevated above control levels. These observations would seem to indicate that for a prostaglandin-mediated pressure rise to occur and be sustained, hydrogen ions must penetrate into the eye in sufficient quantity to inflict intraocular trauma. Blotting acid from the eye at 15 min is clearly done too late to prevent some of the intraocular damage due to hydrogen ion penetration.

The moderate breakdown of the blood-aqueous barrier resulting from brief (2 min) exposure of the cornea to acid might have been mediated by a neural pathway such as has been previously suggested.\textsuperscript{14} However, in the present case of an acid burn, the situation is complicated. If a neural pathway were stimulated by the brief contact of acid with the cornea and this did result in a breakdown of the blood-aqueous barrier, any associated elevation of intraocular pressure would be masked by the enormous transient change in pressure caused by acid-induced shrinkage of the outer coats of the eye. Additionally, the transient shrinkage of the coats of the eye could be considered equivalent to ocular compression, which itself might be expected to incite release of prostaglandins.\textsuperscript{18}

In conclusion, the intraocular pressure response of the rabbit eye to an acid burn is very similar to that following an alkali burn.
The rapid initial rise in intraocular pressure is due to transient shrinkage of the outer coats of the eye, and the more sustained pressure rise is, in part, due to intraocular prostaglandin synthesis and release. The generation of a sustained elevation of intraocular pressure is dependent on penetration of hydrogen ions into the eye.

REFERENCES
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