Prostaglandin-like activity in the aqueous humor following alkali burns

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Following application of 20 µl of 2 N sodium hydroxide to the rabbit cornea, the prostaglandin-like activity in the aqueous humor increased from undetectable levels to around 30 ng per milliliter, a level which was sustained for up to 24 hours. This increase correlated with the previously reported sustained elevation of intraocular pressure following a 20 µl alkali burn. Pretreatment with aspirin or indomethacin virtually abolished the increase in intraocular pressure and elevation of prostaglandin-like activity in the aqueous humor following a 20 µl burn. Marked ocular hypotension following 50 µl and 100 µl sodium hydroxide burns correlated with very low prostaglandin-like activity in the aqueous humor. The low prostaglandin-like activity probably resulted from alkaline hydrolysis of aqueous prostaglandins and massive cellular death in iris and ciliary body tissues.

Key words: alkali burns, intraocular pressure, prostaglandins, aspirin, indomethacin, pH.

It has been shown that application of 20 or 50 µl of 2 N sodium hydroxide to the rabbit or monkey eye results in a biphasic rise in intraocular pressure (IOP). The first, transient part of this response has been shown to result from alkali-induced shrinkage of the outer coat of the eye. The second, sustained rise in IOP was thought to be partially mediated by a release of prostaglandins, since it was almost completely prevented by subconjunctival pretreatment with the prostaglandin antagonist, polyphloretin phosphate (PPP). Furthermore, it was observed that application of 100 µl or more of 2 N sodium hydroxide induced only the initial hypertensive IOP response, the second rise being absent. It was thought that the absence of the second IOP rise after a large alkali application was possibly due either to the direct cellular destructive effect of alkali on the ciliary processes, or very high prostaglandin levels in the aqueous humor.

It was the purpose of this study to determine changes in the prostaglandin levels and pH of the aqueous humor at selected time intervals after alkali burns. In addition, we studied the effect of aspirin and indomethacin on changes in IOP and prostaglandin activity in the aqueous humor after an alkali burn.

Methods and materials

Determination of prostaglandin-like activity in aqueous humor. New Zealand strain albino rabbits (2.5 to 3 kilograms) were anesthetized with 25
per cent urethane (1 to 2 Gm. per kilogram) administered via the marginal ear vein. One eye was burned with a given volume (20, 50, or 100 \( \mu l \)) of 2 N sodium hydroxide, and the other eye used as a control. At specified time intervals, the aqueous humors were aspirated from experimental and control eyes separately, using 1 ml. disposable syringes. Each specimen was immediately transferred into a vial on dry ice. Each pooled sample consisted of aqueous humor specimens from four experimental or four control eyes. The samples were stored at -20° C. and shipped on dry ice to the Institute of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, for extraction and assay for prostaglandin-like activity as previously described.\(^1\)

To determine the relative proportion of prostaglandins of the E and F types, pooled aqueous humor samples were taken from four rabbits one hour after a 20 \( \mu l \) burn. These samples were adjusted to pH 12 by adding 1 N sodium hydroxide dropwise, incubated at 37° C. for one hour, then frozen until assayed. At pH 12, PGE's lose their biological activity, while PGF's remain unaffected.\(^1\)

In a separate series of experiments, rabbits were pretreated with aspirin or indomethacin 1 hour prior to alkali burning of the eye. Indomethacin (50 mg. per kilogram) or aspirin (200 mg. per kilogram) was injected intraperitoneally as a suspension in 3 ml. phosphate buffer (pH 7.4). Aspirin and indomethacin have both been demonstrated to be inhibitors of prostaglandin synthetase.\(^2\)

Preliminary experiments using aspirin administered as a 600 mg. suppository in fasting rabbits were inconclusive. Absorption of the drug across the wall of the rectum is often variable and intraocular levels may not have been adequate. In other experiments with intraperitoneal indomethacin there were instances when the drug was apparently ineffective. Examination of the peri-
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Inadvertent injection and loculation of the drug suspension in the mesentery and bowel wall. The results of these experiments were discarded and subsequently the peritoneal contents were examined after each experiment.

**Measurement of IOP and systemic blood pressure.** These experiments were performed on a separate series of animals from those described above. The manometric techniques employed to measure IOP and blood pressure changes following an alkali burn in anesthetized rabbits were identical to those described previously. Only one eye of each animal was burned with 20 μl of 2 N sodium hydroxide; the contralateral eye served as a control. Rabbits were pretreated with either aspirin or indomethacin 1 hour prior to the alkali burn, as described above.

**Determination of pH changes in aqueous humor.** In a further series of rabbits, the change in aqueous humor pH following topical application of 20, 50, or 100 μl of 2 N sodium hydroxide was continuously monitored by means of a pH microelectrode in the anterior chamber. The anesthetized rabbit was prepared as for measuring IOP changes. After cannulation of the anterior chamber, the system was left open to the reservoir at 20 mm Hg. Using a 21-gauge needle, a beveled tract was made through the peripheral cornea, but the anterior chamber was not penetrated. The pH microelectrode, sealed into the shaft of a 21-gauge stainless-steel needle (Microelectrodes, Inc., Model No. MI-408) was then introduced into the anterior chamber through the previous needle tract. The tap to the reservoir was then closed. The reference electrode (MI-401) was placed in the conjunctival sac of the contralateral eye.

Changes in pH were measured on an Orion Model 410 pH meter and recorded on a Heathkit HSL-15M strip chart recorder. When a steady pH was attained in the experimental eye, the alkali was applied. Aqueous humor pH was recorded continuously for up to three hours. At the end of the experiment, aqueous humor was withdrawn and the pH checked using a Fisher combination pH electrode.

**Results**

Following a 20 μl alkali burn the prostaglandin-like activity in the eye rose to 26.6 ± 10.0 ng per milliliter (mean ± S.E.; three pooled samples) within one hour. At six hours, the level was 40.2 ± 13.6 ng per milliliter (four pooled samples). The sustained elevation of aqueous prostaglandin levels is shown to accompany the sustained rise in IOP following a 20 μl alkali burn (Fig. 1). The prostaglandin-like activity in

the aqueous humor at one hour following a 20 μl burn was principally due to E-type prostaglandins, since alkaline hydrolysis reduced the level from 26.6 ng per milliliter to 5.2 ng per milliliter.

Pretreatment of animals with intraperitoneal aspirin reduced the prostaglandin-like activity in the aqueous humor, collected one hour after a 20 μl burn, from 26.6 to 4.6 ng per milliliter (average of two pooled samples). Similarly, the prostaglandin-like activity in the aqueous humor of indomethacin-pretreated animals was only 1.9 ng per milliliter (average of two pooled samples), one hour after a 20 μl burn. In each case, the prostaglandin-like activity in control eyes was below 1.0 ng per milliliter. Aspirin and indomethacin pretreatment also essentially abolished the
Fig. 3. Aqueous humor pH changes in comparison with IOP changes following application of 20, 50, and 100 µl of 2 N sodium hydroxide to the rabbit cornea. Each pH curve represents the mean of four experiments. IOP curves are taken from previous data. 2
Table I. Prostaglandin-like activity (nanograms per milliliter) in the aqueous humor one and two hours following 20, 50, and 100 μl alkali burns with 2 N sodium hydroxide

<table>
<thead>
<tr>
<th>Volume of 2 N sodium hydroxide</th>
<th>One hour after burn</th>
<th>Two hours after burn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Experimental eye</td>
<td>Control eye</td>
</tr>
<tr>
<td>20 μl (3)</td>
<td>26.6</td>
<td>1.2</td>
</tr>
<tr>
<td>50 μl (2)</td>
<td>6.5</td>
<td>1.4</td>
</tr>
<tr>
<td>100 μl (2)</td>
<td>3.7</td>
<td>0</td>
</tr>
</tbody>
</table>

The data are the mean values obtained from the number of pooled samples indicated in parentheses. Each pooled sample contained the aqueous humor from four eyes. The data for the 20 μl alkali burn are taken from IMJ. 1.

secondary hypertensive response following a 20 μl burn (compare Fig. 1 with Fig. 2).

The prostaglandin-like activity in the aqueous humor one hour and two hours following a 50 or 100 μl alkali burn (Table 1) was only 10 to 25 per cent of that at the same times after a 20 μl burn. In previous experiments, we had shown that two hours following a 50 μl burn, and at one and two hours following a 100 μl burn, the eye is hypotensive (see IOP changes in Fig. 3).

Changes in pH in comparison with IOP changes following 20, 50, and 100 μl alkali burns are depicted in Fig. 3. The sudden rise in IOP following an alkali burn precedes any marked rise in aqueous humor pH; the first IOP peak occurs at around one minute, while the peak pH is attained at four to six minutes (Fig. 3). The rise in aqueous humor pH following a 100 μl burn was greater and more sustained than that following either a 20 μl or 50 μl burn. IOP and systemic blood pressure changes following 20, 50, and 100 μl burns were characteristic of those described previously.

Discussion

We have shown that an alkali burn of the external eye is followed by a rise in prostaglandin-like activity in the aqueous humor. The levels of prostaglandin-like activity attained after a 20 μl alkali burn are generally less than that found in the rabbit aqueous humor during experimental uveitis (45 to 150 ng. per milliliter), but greater than the levels found following paracentesis (4 to 16 ng. per milliliter). The sustained elevation of aqueous humor levels of prostaglandins following a burn with 20 μl of 2 N sodium hydroxide corresponds with the sustained rise in IOP. Similarly, the decrease in prostaglandin-like activity in the aqueous humor of animals pretreated with the prostaglandin synthetase inhibitors, aspirin or indomethacin, correlated with the lack of a second hypertensive response. It would seem, therefore, that the second hypertensive response following an alkali burn is largely due to the synthesis and release of prostaglandins within the eye. The inability of the prostaglandin antagonist, PPP, to completely inhibit the second response following a 20 μl alkali burn might have been due to an insufficient level of drug within the eye.

Recently, it has been suggested that the increase in IOP following chemical trauma is mediated by an axon reflex while the increase in IOP following mechanical trauma is the result of prostaglandin release within the eye. Cole and Unger were unable to demonstrate increased prostaglandin activity in the rabbit aqueous humor following intracameral administration of formaldehyde, and could not inhibit the formaldehyde-induced ocular hypertension with indomethacin. On the basis of these findings they suggested prostaglandins were not directly involved in the response of the eye to chemical irritation. The apparent conflict between our results and those of Cole and Unger might possibly be explained by a direct effect of intra-
cameral formaldehyde in “fixing” membranes of the prostaglandin-producing cells and in affecting aqueous humor outflow routes. Cole and Unger\textsuperscript{6} did not study any other chemical irritants for comparison. However, Jampol, Neufeld, and Sears\textsuperscript{7} reported that aspirin did not block the irritative response to topically applied nitrogen mustard. Furthermore, they demonstrated that an intact sensory innervation was necessary for the irritative response to nitrogen mustard, but not to the mechanical trauma of paracentesis. Also, Obstbaum and Podos\textsuperscript{8} demonstrated that aspirin and indomethacin blocked the ocular hypertensive response following ocular compression. This latter finding is of particular interest in the present study since the initial ocular hypertensive response to alkali may be considered an effective ocular compression resulting from transient shrinkage of the outer coats of the eye.\textsuperscript{9} Whether the alkali-induced ocular compression itself could be the stimulus for the release of prostaglandins is an important question. However, in a number of preliminary experiments (unpublished data), we have demonstrated that even when the initial hypertensive response following an alkali burn is eliminated by manipulation of the reservoir system, the second response still persists, but can be inhibited by aspirin pretreatment. We feel, therefore, that the unique penetrability characteristics of sodium hydroxide set it apart from other chemical irritants. Nitrogen mustard elicits an IOP response via the axon reflex, but probably does not penetrate the aqueous humor to a significant extent. Alkali, on the other hand, penetrates into the aqueous humor and directly stimulates the intraocular tissues, resulting in a release of prostaglandins.

The different levels of prostaglandins in the eye and the different IOP response following 20, 50, and 100 \( \mu l \) of 2 N alkali may be explained by the resultant pH within the eye. It is known that E-type prostaglandins are very unstable in alkaline solution: Karim, Devlin, and Hillier\textsuperscript{10} demonstrated total loss of biological activity of E-type prostaglandins within one hour in solution at pH 10 to 11. In contrast, prostaglandins of the F-type retain their activity in solutions of pH 5 to 11 for up to 183 days. Our results demonstrated that the prostaglandins released into the eye following an alkali burn are principally of the E-type, as has been similarly observed after paracentesis\textsuperscript{11} and experimental ocular inflammation.\textsuperscript{12} Therefore, in the presence of high hydroxyl ion levels, the prostaglandin activity in the aqueous would be rapidly reduced. However, unless the cells producing the prostaglandins are damaged, prostaglandins will continue to be released into the aqueous humor. This is probably the case following a 20 \( \mu l \) burn, when the pH peaks at 10 but is down to about pH 8.5 by two hours. The relatively shortlived pH peak may be insufficient to cause irreversible cellular damage, although some prostaglandin hydrolysis could occur. Following a 50 \( \mu l \) burn, the pH peaks at 11.9 and falls only to 10.1 at two hours. In this case, the peak pH may cause more prostaglandin hydrolysis and more cellular damage. A second hypertensive curve is seen, but this is shortlived, perhaps because of cellular damage preventing prostaglandin synthesis and release. Reduction in aqueous humor formation due to damage of the ciliary epithelium and vascular thrombosis\textsuperscript{13} might result in the subsequent hypotension. Following a 100 \( \mu l \) burn, the prolonged maintenance of a pH of 12 may be so traumatic that rapid cellular death, especially in the ciliary epithelium, and alkaline hydrolysis of any released E-type prostaglandins occurs before a second hypertensive phase can be generated. Again, due to reduced aqueous humor formation, the eye might be expected to become hypotensive.

The remaining prostaglandin activity following 50 and 100 \( \mu l \) burns may be F-type prostaglandins. It was perhaps fortuitous that the level of prostaglandin-like activity in the eye one hour following a 50
and 100 μl alkali burn was almost identical to that in the sample of aqueous which was subjected to alkaline hydrolysis.

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REFERENCES