Inhibition of prostaglandin-mediated ocular inflammatory responses by 4-biphenylacetic acid. EDWARD L. TOLMAN, RUTH PARTRIDGE, TERRY O. MYERS, AND JAY E. BIRNBAUM.

4-Biphenylacetic acid (BPAA), a prostaglandin-synthesis inhibitor, was tested for its effects on prostaglandin-related, laboratory models of ocular inflammation. Topically applied, BPAA inhibited arachidonic acid, but not prostaglandin E\textsubscript{2}–induced increases in rabbit intraocular pressure (IOP). BPAA inhibited the IOP response to alkali burn and altered IOP changes following paracentesis. In vitro, BPAA inhibited prostaglandin production from arachidonic acid in cell-free preparations of rabbit uvea. It is suggested that BPAA may be useful for the therapy of ocular inflammatory disease.

4-Biphenylacetic acid (BPAA), previously reported to inhibit prostaglandin (PG) synthesis in homogenates of guinea pig lung,\textsuperscript{1} was tested for its effects on experimentally induced, PG-mediated eye inflammations and on PG synthesis by subcellular preparations of eye tissue. The observed relationship between above normal levels of PG's and the acute ocular inflammatory response\textsuperscript{2} and the inhibition of ocular PG production by other nonsteroidal anti-inflammatory agents\textsuperscript{3} suggested the potential utility of such compounds in the therapy of ocular inflammatory diseases. The present study was designed to extend the findings with BPAA to several models of ocular inflammation and to investigate the effects of BPAA on PG synthesis in eye tissue.

**Methods.** Female New Zealand white rabbits, weighing 2 to 2.5 kg, were held immobile while intraocular pressure (IOP) levels were measured and recorded on an Applamatic tonometer (Bausch & Lomb, Inc., Rochester, N. Y.) calibrated for this species. Rabbits were allowed a 30 min. acclimatation period before experimentation, one eye of each rabbit was used per experiment, and eyes were anesthetized with topical proparacaine hydrochloride (E. R. Squibb & Co., Princeton, N. J.) prior to each IOP measurement. Baseline IOP levels were established for each animal by averaging the last three recordings made at 15 min. intervals during the 1 hr. prior to application of drug or vehicle. BPAA was applied, where noted, as 0.05 ml. of 0.05 to 5.0 per cent light mineral oil solution; PGE\textsubscript{2} (Ono Pharmaceutical Co., Ltd., Osaka, Japan) as 0.005 ml. of a 1 mg./ml. equimolar Na\textsubscript{2}CO\textsubscript{3} solution; and NaOH, as 0.02 ml. of a 2N solution. The drug or its vehicle was applied topically over the corneal surface 10 min. prior to chemical or mechanical challenge and subsequent IOP readings begun 15 min. after challenge. The maximum response to topical arachidonic acid or PGE\textsubscript{2} occurred 15 to 45 min. after application and was quantitated by subtracting the average baseline level from the highest IOP value recorded within the 15 to 45 min. postchallenge period.

**Arachidonic acid** (Analabs Inc., North Haven, Conn.) was administered topically as 2 drops of a 2 per cent mineral oil solution; PGE\textsubscript{2} (Ono Pharmaceutical Co., Ltd., Osaka, Japan) as 0.005 ml. of a 1 mg./ml. equimolar Na\textsubscript{2}CO\textsubscript{3} solution; and NaOH, as 0.02 ml. of a 2N solution. Paracentesis was performed on awake rabbits after application of topical anesthetic and involved withdrawal with a 30-gauge needle of 0.02 ml. of aqueous humor from the anterior segment. Care was taken not to cause undue damage to

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Average increase (15-45 min. post-challenge) in IOP (mm. Hg.) ± 1 S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineral oil vehicle</td>
<td>5</td>
<td>3.5 ± 1.3</td>
</tr>
<tr>
<td>2% arachidonic acid:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ vehicle</td>
<td>20</td>
<td>10.2 ± 1.1*</td>
</tr>
<tr>
<td>+ BPAA 5%</td>
<td>11</td>
<td>3.4 ± 1.0*</td>
</tr>
<tr>
<td>+ BPAA 2.5%</td>
<td>7</td>
<td>2.4 ± 0.66*</td>
</tr>
<tr>
<td>+ BPAA 1%</td>
<td>11</td>
<td>3.5 ± 0.54*</td>
</tr>
<tr>
<td>+ BPAA 0.5%</td>
<td>12</td>
<td>6.0 ± 1.0*</td>
</tr>
<tr>
<td>+ BPAA 0.1%</td>
<td>12</td>
<td>6.3 ± 0.88*</td>
</tr>
<tr>
<td>+ BPAA 0.05%</td>
<td>4</td>
<td>14.1 ± 3.1*</td>
</tr>
</tbody>
</table>

\*Differs from arachidonic acid alone by p < 0.05.
surrounding eye tissues and rabbits were sacrificed as soon as possible after paracentesis.

The effect of BPAA on eye tissue PG synthesis was determined by incubating low-speed (800 x g) supernatants of rabbit iris (Pel-Freez Bio-Animals, Inc., Rogers, Ark.) homogenates with arachidonic acid for 30 min. at 37°C. The final concentrations were approximately 2 mg. of tissue protein, 10 μg of arachidonic acid, 50 μg of reduced glutathione, and 5 μg of hydroquinone per milliliter of modified Bucher's medium. The PG-like activity produced in the presence and absence of BPAA was measured by bioassay as previously described.1

Aqueous humor protein content was analyzed by the method of Lowry and associates4 with bovine serum albumin used as standard. PG levels were estimated with a commercially available radioimmunoassay kit (Clinical Assays, Inc., Cambridge, Mass.). Statistical analysis of the differences between means was done by the Student t test and significance was assigned to differences having p values of 0.05 or less.

Results. A maximally effective dose of arachidonic acid, i.e., 2 drops of a 2 per cent mineral oil solution, increased IOP levels by about 10 mm. Hg (Table 1). This was significantly above the change observed following application of the vehicle itself. Pretreatment of the eyes with topically applied 0.1 to 5.0 per cent suspensions of BPAA significantly inhibited the response to arachidonic acid, whereas a 0.05 per cent suspension was without effect. Similar inhibitory effects were observed when BPAA was applied as an aqueous (equimolar Na₂CO₃) solution.

BPAA did not affect the increases in IOP levels induced by topically applied PGE₁. The average maximum IOP increase without BPAA pretreatment was 11 mm. Hg following PGE₁, whereas that when BPAA was applied as a 5 per cent mineral oil suspension was 14 mm. Hg. The difference was not significant.

The time course of the IOP response to arachidonic acid with and without BPAA pretreatment is shown in Fig. 1. At the time of maximal response to challenge, the increase in IOP was significantly inhibited by a maximally effective dose of BPAA. As shown in Fig. 2, topical application of 2N NaOH also markedly raised IOP levels. The effect of this alkali burn had been shown to be due to an increase in aqueous humor PG concentrations.5 Again, pretreatment with a 5 per cent suspension of BPAA markedly inhibited the IOP response to NaOH.

Secondary increased aqueous humor PG levels had been shown to follow paracentesis of aliquots of aqueous humor.6 There was, as expected, a precipitous drop in IOP levels, to about 6 mm.

Fig. 1. Effect of topical BPAA pretreatment on rabbit IOP increases induced by topical arachidonic acid. Each point is the mean of 11 to 16 observations. *Statistically significant differences between corresponding points.
Hg, following paracentesis of 0.2 ml. of aqueous humor, but in eyes receiving no pretreatment, IOP levels returned to normal or slightly above (25 mm. Hg) baseline levels (22 mm. Hg) within 15 min. of challenge. In eyes pretreated with BPAA, IOP levels remained extremely low (6 to 10 mm. Hg) and only gradually returned to baseline levels about 3 hours after challenge. These data suggested that disruption of the blood-aqueous barrier, a known consequence of paracentesis, was prevented by BPAA pretreatment, perhaps a result of inhibited PG synthesis.

When aqueous humor was analyzed 30 min. after application of arachidonic acid in the absence of any pretreatment, aqueous humor protein concentrations were increased significantly about 5-fold over those in normal, control eyes (2.9 ± 0.6 mg./ml. vs. 0.6 ± 0.1 mg./ml.). This increase was completely inhibited by pretreatment with 5 per cent BPAA (protein concentration, 0.5 ± 0.02 mg./ml.). Aqueous humor PG levels in BPAA-pretreated eyes were about one-half those in eyes challenged with arachidonic acid and not pretreated (0.30 ± 0.03 ng./ml. vs. 0.70 ± 0.10 ng./ml.; n=4).

Finally, BPAA added in vitro inhibited the production of bioassayable PG-like activity from arachidonic acid by cell-free preparations of rabbit iris. The inhibition was dose-related and an approximate IC₅₀ range of 1 to 2 μg/ml. or 5 to 10 μM was observed. Complete (100 per cent) inhibition occurred at BPAA concentrations of 5 μg/ml. and above.

**Discussion.** The data obtained in the present study indicated that BPAA markedly inhibited the ocular effects of both chemically and mechanically induced trauma. These included changes in IOP levels and aqueous humor composition. The mechanism of action of BPAA appeared to be the inhibition of PG synthesis from both endogenously and exogenously supplied substrate material. This conclusion was based on the following observations: BPAA inhibited the IOP-raising effects of arachidonic acid, but not those of PGE₁; aqueous humor PG concentrations of eyes challenged with arachidonic acid were greatly reduced by pretreatment with BPAA; and BPAA directly inhibited PG production by eye tissue in vitro. The inhibitory effects of BPAA on IOP responses to alkali burn and paracentesis further supported this conclusion since both means of trauma were shown to be mediated through the increased production of ocular PG's.

The mechanism of PG-mediated increases in IOP had been attributed to the breakdown of the blood-aqueous barrier, which was observed functionally as an increase in passage of protein-containing fluid through interstices of the non-pigmented ciliary epithelial cells and accumulation of high protein amounts in fluids of the anterior segment of the eye. This effect of increased vascular permeability by PG's had been observed previously for other tissues. By reducing the production of PG's in the eye from arachidonic acid, BPAA appeared to prevent the breakdown of the blood-aqueous barrier after the various challenges. It was of interest to observe the slow "refilling" of the anterior chamber after paracentesis in BPAA-pretreated eyes. This in-
dicated maintenance of aqueous-blood barrier integrity by BPAA via its inhibition of PG production from endogenous substrate.

The following observations indicated the potential clinical significance of a potent ocular PG-synthesis inhibitor like BPAA. PG's applied topically or intracameraly, produced the signs and symptoms of acute ocular inflammation, including miosis, hyperemia, and ocular hypertension. PG levels in aqueous humor extracts from patients with acute anterior uveitis or animals with experimentally induced uveitis were significantly higher than those in eye fluid extracts from their normal counterparts. Clinical treatment of acute anterior uveitis returned aqueous humor PG levels to normal. Other non-steroidal anti-inflammatory agents, which inhibit PG synthesis, had been observed to reverse the signs of experimentally induced acute ocular inflammation and were suggested for clinical trial for this property.

In preliminary studies, BPAA reversed the inflammatory symptoms in the eyes of rabbits with immunogenically induced anterior uveitis. The effectiveness of topically applied BPAA acid in the model systems discussed in detail in the present study and in the uveitis model indicated its potential utility in the treatment of ocular inflammatory diseases.

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REFERENCES


Pilocarpine was administered to a selected group of ocular hypertensive subjects in the form of a synthetic biosoluble matrix inserted into the conjunctival cul-de-sac. Satisfactory lowering of the intraocular pressure resulted, with a minimum of subject intolerance. The decreased pressure response was significant in some cases for greater than 24 hours. Drug delivery by soluble inserts offers promise as a convenient and effective mode of therapy.

Table I. The effect of pilocarpine inserts on intraocular pressure

<table>
<thead>
<tr>
<th>Pilocarpine dose (mg.)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>8</th>
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<tbody>
<tr>
<td>0.5</td>
<td>0.88 ± 1.09</td>
<td>4.13 ± 1.65*</td>
<td>2.63 ± 1.28</td>
<td>6.25 ± 2.48*</td>
<td>1.13 ± 0.64</td>
<td>5.63 ± 1.64</td>
</tr>
<tr>
<td>1.0</td>
<td>0.88 ± 1.20</td>
<td>3.38 ± 1.39*</td>
<td>2.13 ± 1.92</td>
<td>2.38 ± 1.31</td>
<td>4.25 ± 1.35*</td>
<td>5.00 ± 1.401</td>
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<tr>
<td>1.5</td>
<td>1.71 ± 0.64</td>
<td>5.00 ± 1.21†</td>
<td>7.29 ± 1.36†</td>
<td>3.00 ± 1.83*</td>
<td>8.14 ± 0.96†</td>
<td>5.43 ± 1.251</td>
</tr>
<tr>
<td>2.0</td>
<td>0.50 ± 1.65</td>
<td>1.88 ± 1.71</td>
<td>2.63 ± 1.53</td>
<td>2.00 ± 2.07</td>
<td>2.63 ± 1.02*</td>
<td>6.13 ± 0.771</td>
</tr>
</tbody>
</table>

Mean and standard error for the lowering of the IOP with time for varying dosages of pilocarpine inserts. Each value was obta before and after insertion of a pilocarpine insert. Each value is the averaged response for six subjects.

*Statistically significant, p <0.05.
†Statistically significant, p <0.01.