The effect of intravitreal and topical prostaglandins on intraocular inflammation

P. S. Kulkarni and B. D. Srinivasan

We investigated the effects of prostaglandins (PG) E\(_2\), PGD\(_2\), PGI\(_2\), and its metabolites 6-keto-PGE\(_1\), and 6-keto-PGF\(_{1\alpha}\), and U46619 (stable analogue of the PG endoperoxide, PGH\(_2\)) administered either intravitreally or topically on intraocular pressure (IOP), pupil diameter, aqueous protein, and the entry of polymorphonuclear cells (PMNs) in the aqueous. PGE\(_2\), 6-keto-PGE\(_1\), U46619, and PGI\(_2\) increased IOP after either intravitreal or topical administration in a dose-dependent manner, 6-keto-PGE\(_1\) was the most potent in increasing IOP. U46619 and PGI\(_2\) increased IOP when administered intravitreally; however, these agents also increased IOP of the contralateral control eye. High doses of 6-keto-PGE\(_1\) and PGI\(_2\) but not 6-keto-PGF\(_{1\alpha}\) or PGE\(_2\) increased the IOP of both experimental and contralateral eyes, suggesting that this effect may be due to the entry of these agents into the systemic or intraorbital circulation or to stimulation of neuronal pathways. Intravitreal administration of 6-keto-PGE\(_1\), PGE\(_2\), and PGI\(_2\) increased protein content of the aqueous, with 6-keto-PGE\(_1\) significantly more potent than other PGs. Topically applied PGE\(_2\) and 6-keto-PGE\(_1\) also increased protein content of the aqueous at doses that elevated IOP. However, topical 6-keto-PGE\(_{1\alpha}\) at doses that increased IOP did not increase protein content of the aqueous. In contrast, PGD\(_2\) increased the IOP in both eyes; however, it significantly increased aqueous protein content of the experimental eye, indicating that increase in protein content of the aqueous and increase in IOP are not necessarily associated. None of the PGs tested in this study had any effect on pupil diameter or PMN entry into the aqueous. Therefore the classic signs of intraocular inflammation, i.e., increase in IOP, increase in protein content of the aqueous, miosis, and PMN entry into aqueous, are not necessarily associated and sequential, and PGs do not induce all signs of inflammation. (INVEST OPHTHALMOL VIS SCI 23:383-392, 1982.)

Key words: 6-keto-PGE\(_1\), PGI\(_2\), PGD\(_2\), intraocular pressure, rabbit aqueous, intraocular inflammation

Arachidonic acid (AA) is metabolized by a cyclooxygenase enzyme into unstable cyclic prostaglandin (PC) endoperoxides,\(^1\) which are then enzymatically converted to biologically active compounds such as PGE\(_2\), PGF\(_{2\alpha}\), PGI\(_2\), PGD\(_2\), and thromboxane-A\(_2\) (TxA\(_2\)). PGI\(_2\) (half-life 10 min)\(^2\) and TxA\(_2\) (half-life 30 sec)\(^3\) are labile substances in aqueous medium and spontaneously break down to the stable compounds, 6-keto-PGF\(_{1\alpha}\) and TxB\(_2\), respectively.\(^4\) PGI\(_2\) is also enzymatically converted to 6-keto-PGE\(_1\) by the rabbit liver.\(^5\) PGI\(_2\) and TxA\(_2\) are natural biologic antagonists. For example, PGI\(_2\), which is synthesized mainly in vascular tissues, is a potent vasodilator,\(^2\) a potent platelet antiaggregating agent,\(^2\) and a stimulator of adenyl cyclase.\(^6\) In contrast, TxA\(_2\), which is synthesized mainly by plate-
PGs of the E and F types and their precursor, AA, when administered intracamerally or topically, cause miosis, increase in intraocular protein content, and entry of white cells into the aqueous and tear fluid. Ocular tissues, such as the iris-ciliary body and the conjunctiva of rabbits, synthesize all cyclooxygenase products, and cyclooxygenase inhibitors such as indomethacin and aspirin inhibit experimental intraocular inflammation induced by various stimuli. On the basis of these observations, Eakins concluded that PGs could at least be one of the mediators of certain types of ocular inflammation.

In this study, we assessed the effect of PGE₂, PGI₂, 6-keto-PGF₁₀, and PGD₂ administered intravitreally or topically on IOP, pupillary diameter, aqueous protein content, and the entry of white cells in the aqueous. We also studied the effects of 6-keto-PGE₁ and U-46619, a stable analogue of the PG endoperoxide, PGH₂, which is a platelet-aggregating and vasoconstricting agent.

**Methods**

Male albino rabbits weighing about 2.5 kg were used in these studies. The rabbit eye was proptosed and a drop of 2% proparacaine hydrochloride (Alcon Laboratories, Fort Worth, Tex.) was applied topically to the eye. PGE₂, PGI₂, 6-keto-PGF₁₀, and PGD₂ administered intravitreally or topically on IOP, pupillary diameter, aqueous protein content, and the entry of white cells in the aqueous. We also studied the effects of 6-keto-PGE₁ and U-46619, a stable analogue of the PG endoperoxide, PGH₂, which is a platelet-aggregating and vasoconstricting agent.

Intravitreal administration of PGE₂ at the 10 μg dose significantly increased IOP within 15 min after administration, with the effect lasting about 80 to 100 min (p < 0.05). The low dose of PGE₂ (0.1 μg) was found to be slightly hypotensive (p < 0.05) at 30 min. Fifteen minutes after intravitreal administration of a known PG antagonist, N-0164 (5 μg), PGE₂ at a dose of 1 μg (which alone had no effect on IOP) decreased IOP significantly. The PG antagonist N-0164 alone did not produce this response.

**Results**

**Effect of intravitreal and topical administration of PGs on intraocular pressure**

**PGE₂.** Fig. 1 shows the effect of various doses of intravitreally or topically administered PGE₂ on IOP at different time intervals. Intravitreal administration of PGE₂ at the 10 μg dose significantly increased IOP within 15 min after administration, with the effect lasting about 80 to 100 min (p < 0.05). The low dose of PGE₂ (0.1 μg) was found to be slightly hypotensive (p < 0.05) at 30 min. Fifteen minutes after intravitreal administration of a known PG antagonist, N-0164 (5 μg), PGE₂ at a dose of 1 μg (which alone had no effect on IOP) decreased IOP significantly. The PG antagonist N-0164 alone did not produce this response.

PGE₂ applied topically in various doses increased the IOP of the experimental eye in a dose-dependent manner. PGE₂ at the 1 and 10 μg doses significantly increased IOP within 15 min and this effect was sustained for up to 45 min. The lowest dose (0.1 μg) tested did not significantly increase IOP, but it slightly
decreased IOP (p < 0.05) at approximately 100 min after PGE2 application.

6-Keto-PGE1. Fig. 2 illustrates that intravitreal 6-keto-PGE1 in doses of 0.1, 1, and 10 μg significantly increased IOP within 15 to 20 min of administration. This increase in IOP lasted up to 80 to 100 min. Interestingly, the highest dose (10 μg) of 6-keto-PGE1 tested also slightly increased the IOP of contralateral saline-treated eyes. When the PG antagonist N-0164 (5 μg) was injected intravitreally 15 min prior to the administration of intravitreal 6-keto-PGE1, it significantly inhibited the hypertensive response induced by 6-keto-PGE1 (1 μg).

Topically applied 6-keto-PGE1 (1 and 10 μg doses) increased IOP significantly, however, low doses (0.01 and 0.1 μg) did not alter IOP. PGI2 (prostacyclin). This unstable compound was administered intravitreally in 0.01, 0.1, 1, and 10 μg doses. The higher doses of PGI2 increased IOP in the experimental eye. However, the IOP of the contralateral eye treated with Tris buffer (pH 8.5) was also increased. Although there was no significant difference between the IOP of the control and experimental eyes at the start of the experiment, the IOP in both was increased from the baseline by intravitreal PGI2 (Fig. 3).

Topically applied PGI2 increased the IOP of the experimental eye in a dose-dependent manner. PGI2 at 10 and 100 μg doses increased IOP significantly; however, at the higher dose (100 μg), PGI2 also increased the IOP of the contralateral eye treated with Tris buffer (pH 8.5). Topical (n = 20) or intravitreal (n = 5) Tris buffer (pH 8.5) alone in other control experiments did not affect IOP. All doses tested induced pronounced conjunctival hyperemia and edema, and surprisingly, this effect of PGI2 lasted up to 3 hr.

6-Keto-PGF1α. Fig. 4 illustrates that intravitreal administration of 10 μg of 6-keto-PGF1α significantly increased IOP within 15 min and maintained this effect for 180 min. A lower dose (1 μg) also increased IOP of the experimental eye in 45 min; however, this increase in IOP lasted a very short period. Only the 10 μg dose of 6-keto-PGF1α applied topically increased IOP significantly within 30 min.

Stable PG-endoperoxide analogue U-46619.
Fig. 2. Effect of intravitreally and topically administered 6-keto-PGE₁ on IOP. Vertical line on each point (mean) represents the S.E.M., and n represents the number of experiments. Asterisks, p < 0.05 level of significance compared with IOP of the experimental and contralateral control eyes.

Fig. 5 shows that stable PG endoperoxide administered intravitreally increased the IOP of the experimental eye and of the contralateral control eye that received only saline. After N-0164 administration, U-46619 had less effect in raising IOP of the experimental eye, while there was no increase in IOP of the contralateral saline-treated eye.

U-46619 applied topically in doses of 0.1, 1, and 10 µg also increased the IOP of the experimental eye and contralateral saline-treated eye, especially at the 1 and 10 µg doses.

PGD₂ and PGF₂α. Fig. 6 shows that various doses of PGD₂ applied topically or administrated intravitreally increased IOP in both experimental and contralateral eyes from the base level. PGF₂α, when administered intravitreally or topically (10 µg), had no significant effect on IOP (not shown).

Effect of intravitreal and topical administration of PGs on the protein content of aqueous. The effects of intravitreal 6-keto-PGE₁, PGE₂, PGI₂, 6-keto-PGF₁₀, PGD₂, and the stable PG endoperoxide on the release of protein into the rabbit aqueous are shown in Table I. 6-Keto-PGE₁ very significantly increased the protein content of the aqueous of the experimental eye at 1 and 10 µg doses. PGE₂ also significantly increased the protein content of the aqueous at a dose of 10 µg, but it was considerably less potent than 6-keto-PGE₁. PGI₂ (0.1, 1, and 10 µg) increased the protein content in the experimental eyes in a dose-dependent manner. However, PGI₂ at 1 and 10 µg also increased the protein content of the aqueous of the contralateral eye. 6-Keto-PGF₁₀ increased the protein content of the aqueous of both eyes only at the 10 µg dose. The stable PG endoperoxide increased the protein concentration when administered at 1 and 10 µg doses, whereas PGD₂ significantly increased aqueous protein at the 10 µg dose. When N-0164 (5 µg) was administrated intravitreally 5 min prior to the injection of either 6-keto-PGE₁ (10 µg), PGE₂ (10 µg), or U44619 (10 µg), the normally observed increase in protein content of the rabbit aque-
Fig. 3. Effect of intravitreally and topically administered PGI₂ on IOP. Vertical line on each point (mean) represents the S.E.M., and n represents the number of experiments. Asterisks, p < 0.05 level of significance compared with IOP of the experimental and contralateral control eyes.

Fig. 4. Effect of intravitreally and topically administered 6-keto-PGF₁₀ on IOP. Vertical line on each point (mean) represents the S.E.M., and n represents the number of experiments. Asterisks, p < 0.05 level of significance compared with IOP of the experimental and contralateral control eyes.

uous induced by these PGs was inhibited.

Table II summarizes the effect of topically applied PGs on the aqueous protein content. 6-Keto-PGE₁ and PGE₂ increased the protein content at 1 and 10 μg very significantly. However, PGE₂ was less potent than 6-keto-PGE₁ at a dose of 1 μg. PGI₂ increased the protein content of the aqueous of both eyes only at doses of 10 and 100 μg, whereas 6-keto-PGF₁₀ and the stable endoperoxide had no effect on the protein content of the aqueous at all of the doses tested (0.1, 1, and 10 μg). Paradoxically, PGD₂ at 0.1, 1, and 10 μg doses significantly increased the protein of the aqueous in the experimental eye.

Effects of PGs on the pupil diameter and PMNs released in the aqueous. There was no change in the pupillary diameter after intravitreal or topical administration of any of the PGs tested. In addition, all PGs administered
Stable PG Endoperoxide Analog (Intravitreal)

- Control  
- Exp

10 μg  

n = 8

0.01 μg  

n = 6

1 μg + 5 μg n = 6  

n = 6

0.1 μg  

n = 7

Stable PG Endoperoxide Analog (Topical)

- Control  
- Exp

10 μg  

n = 6

1 μg  

n = 6

0.1 μg  

n = 6

Fig. 5. Effect of intravitreally and topically administered U-46619 (stable PG-endoperoxide analogue) on IOP. Vertical line on each point (mean) represents the S.E.M., and n represents the number of experiments. Asterisks, p < 0.05 level of significance compared with IOP of the experimental and contralateral control eyes.

PGD₂ (Intravitreal)

- Control  
- Exp

10 μg  

n = 8

0.1 μg  

n = 7

PGD₂ (Topical)

- Control  
- Exp

10 μg  

n = 12

0.1 μg  

n = 6

Fig. 6. Effect of intravitreally and topically administered PGD₂ on IOP. Vertical line on each point (mean) represents the S.E.M., and n represents the number of experiments. Asterisks, p < 0.05 level of significance compared with IOP of the experimental and contralateral control eyes.

either topically or intravitreally did not release PMNs into the aqueous.

Discussion

PGs of the E and F types cause the well-recognized signs of intraocular inflammation when administered intracameraly or applied topically (see Eakins, ref. 8). In the present study, we demonstrate that PGs of the E type as well as some of the other newly discovered PGs increase the IOP and protein content of the aqueous but do not cause miosis or the release of PMNs into the aqueous.

Camras et al.²⁰ demonstrated that topical or intracameral PGE₂ administration
Table I. Aqueous protein (mg/ml) after intravitreal applications

<table>
<thead>
<tr>
<th>Doses</th>
<th>6-Keto-PGE₁</th>
<th>PGE₂</th>
<th>PGI₂</th>
<th>6-Keto-PGF₁₀⁻⁶</th>
<th>U44619</th>
<th>PGD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01 µg</td>
<td>50 ± 5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>40 ± 8</td>
<td>(n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1 µg</td>
<td>10 ± 5</td>
<td>50 ± 5</td>
<td>93 ± 20*</td>
<td>51 ± 3</td>
<td>46 ± 5</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>Control</td>
<td>8 ± 4</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>1 µg</td>
<td>2055 ± 280*</td>
<td>129 ± 65</td>
<td>266 ± 56*</td>
<td>53 ± 3</td>
<td>229 ± 65</td>
<td>61 ± 29</td>
</tr>
<tr>
<td>Control</td>
<td>245 ± 22</td>
<td>110 ± 85</td>
<td>129 ± 68</td>
<td>51 ± 3</td>
<td>216 ± 85</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>10 µg</td>
<td>2053 ± 174*</td>
<td>885 ± 212*</td>
<td>381 ± 142</td>
<td>371 ± 192</td>
<td>885 ± 212*</td>
<td>266 ± 56*</td>
</tr>
<tr>
<td>Control</td>
<td>472 ± 106</td>
<td>196 ± 56</td>
<td>207 ± 57</td>
<td>210 ± 97</td>
<td>196 ± 56</td>
<td>50 ± 5</td>
</tr>
<tr>
<td>10 µg + 5 µg N-0164</td>
<td>297 ± 44†</td>
<td>394 ± 61†</td>
<td>ND</td>
<td>ND</td>
<td>476 ± 48†</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>180 ± 51</td>
<td>185 ± 20</td>
<td>(n = 6)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND = not determined.

*P < 0.05 level of significance compared with the protein content of the experimental and contralateral control aqueous.

caused a biphasic IOP response, i.e., an initial rise in IOP followed by (3 or 4 hr later) prolonged hypotension. We observed only an elevation of IOP by higher doses of PGE₂, and a lower dose of this drug somewhat lowered the IOP. Since our IOP measurements were done up to 3 hr after PGE₂ application, it is possible that higher doses of PGE₂ might have lowered the IOP 4 or 5 hr after administration. It is also possible that the hypertensive and hypotensive effects of PGE₂ are dose related. 6-Keto-PGE₁ and PGI₂ also significantly increased IOP, and 6-keto-PGE₁ was the most potent of all PGs tested in this study. Interestingly, the highest dose of topical PGI₂ (100 µg) was very significantly ocular hypertensive.
in both the experimental eye and the contralateral Tris buffer–treated eye, suggesting that the hypertensive effects were due to the entrance of PGI₂ into the systemic circulation. Some PGs such as 6-keto-PGE₁, PGD₂, and U46619, especially at high doses, also increased the IOP of both experimental and contralateral eyes. It is possible that these PGs, being very stable, induced a hypertensive effect in the contralateral eye by entering into the systemic circulation. Recently, Foster et al.²¹ demonstrated the connection between the two internal ophthalmic arteries in the rabbit, it is possible that PGs entered into the contralateral eye by such local pathways. Another possibility is that this effect is due to stimulation by PGs of neuronal pathways such as the trigeminal nerve.

Since PGI₂ is very unstable, its effect on IOP may have been due to either its spontaneous breakdown product, 6-keto-PGF₁₀. or its metabolic product, 6-keto-PGE₁. The effect of PGI₂ cannot be due to 6-keto-PGF₁₀ because only high doses (10 μg) of this compound increased IOP. Furthermore, the higher doses of PGI₂ and 6-keto-PGE₁, but not 6-keto-PGF₁₀, increased the IOP of the contralateral eye. It is possible then that the PGI₂ effect may be due to its conversion into 6-keto-PGE₁. In this regard, although it is known that rabbit liver can synthesize 6-keto-PGE₁ from PGI₂,⁵ it is not yet known whether rabbit ocular tissue(s) can synthesize 6-keto-PGE₁ from PGI₂. 6-Keto-PGE₁ very significantly increased IOP and was 10 to 15 times more potent than PGI₂; the highest intravitreal dose of 6-keto-PGE₁ and PGI₂ also increased the IOP of the contralateral control eye. Since intravitreal 6-keto-PGE₁ was much more potent in elevating IOP than PGI₂, it is unlikely that the hypertensive effect of PGI₂ was the result of its conversion to 6-keto-PGE₁. Furthermore, topical 6-keto-PGE₁ was also more potent than PGI₂ in increasing the IOP, suggesting that topically applied PGI₂ was not converted to 6-keto-PGE₁ in the rabbit eye.

6-Keto-PGE₁ at doses that increased IOP was significantly more effective than other PGs in increasing the protein content of the aqueous. Topical PGI₂ at doses that increased IOP (except the highest dose of 100 μg) did not significantly increase the protein content of the aqueous, presumably because topical PGI₂ is not converted to 6-keto-PGE₁. Interestingly, a higher dose of topical 6-keto-PGF₁₀, although it significantly increased IOP, did not increase the protein content in the aqueous. In contrast, PGD₂ raised IOP in both eyes but significantly increased the protein content of the aqueous in only the experimental eye. These results demonstrate that a PG-induced rise in IOP and aqueous protein content are not necessarily associated.

The effect of PGs on IOP and aqueous protein appears to be specific because a known specific PG receptor antagonist, N-0164,¹⁹ inhibited their actions. Furthermore, since PGE₂ at a dose that did not alter the IOP response was found to be significantly hypertensive after N-0164 treatment, it is likely that N-0164 selectively inhibits hypertensive responses induced by some PGs. It is possible that PG-induced hypertensive and hypotensive IOP responses may be mediated by two separate receptors. Further investigation with various PG antagonists is required to illustrate such a phenomenon.

In this study we did not observe any alteration of the rabbit pupil diameter by any of the PGs tested. However, previous reports²²–²⁴ have indicated that intracameral injections of PGE-like substances caused miosis. This discrepancy may be caused by the presence of a lesser amount of PG in the anterior chamber after topical or intravitreal administration compared with that achieved by intracameral injections.

Finally, we did not observe PMNs in the aqueous up to 5 hr after intravitreal or topical administration of all PGs tested, although we recently showed that low doses of PGs of the E type induced PMN release into the tear fluid within 5 hr of corneal denudation in rabbits.⁹ Therefore it appears that PGE acts differently on the conjunctival vasculature than on the vasculature of the iris–ciliary body. In the present experiments, it is possible that PGs
induce PMN release into the aqueous within 24 hr of topical or intravitreal administration. However, this is highly unlikely because the half-life of intravitreally administered PGs is about 3 hr. Other evidence indicates that lipoxygenase products but not cyclooxygenase products are mediators of chemotaxis for PMNs. Indications that different PGs, depending on their intraocular concentration, may produce one or more signs of intraocular inflammation. However, none of these PGs induces PMN chemotaxis in this model. These results also indicate that the parameters of ocular inflammation described by Eakins may not be sequential and can be studied individually by the use of various drugs.

We thank Dr. J. Pike of The Upjohn Co., Kalamazoo, Mich., for the generous gift of prostaglandins, Dr. Eric Nelson of the Nelson Research and Development Co., Irvine, Calif., for kindly providing N-0164, and Ms. Carol Brodowicz and Mrs. Carolyn Rudeck for their excellent technical assistance. We also thank Ann S. Zaragoza for typing this manuscript.

REFERENCES