Prostaglandin D₂ reduces intraocular pressure

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SUMMARY When PGD₂ was topically applied to the rabbit eye a reduction of intraocular pressure (IOP) was observed within 30 min. The IOP reduction lasted throughout the observation period of 7 hours. No increase of IOP was observed during the period. The effect of PGD₂ was dose-dependent (0.4 µg to 250 µg), and the minimum effective dose was estimated to be 2.0 µg. The application of PGD₂ did not affect the pupil diameter. No sign of inflammatory response was observed by PGD₂ application, that is, no hyperaemia, no flare, no irritation, and no increase of protein content in the aqueous humour. These results suggest that PGD₂ or its analogues may be useful for treating glaucoma.

Prostaglandins (PGs) are formed in the eye^{1,2} following various kinds of stimulation and are thought to mediate inflammatory responses.³ Among several types of PGs the PGE and F series have been extensively studied in relation to intraocular pressure (IOP).⁴⁻⁹ Early studies showed that these compounds are hypertensive.⁴ Recently, however, topical application of them was found to eventually lower the IOP in many animal species.⁵⁻⁷ Yet higher doses induce an initial increase of the IOP in rabbits⁵ and monkeys^{6,7} and also cause aqueous flare in rabbits⁶ and cats.⁷

More recently the effects of PGF_{2α}⁸ and its isopropyl ester⁹ on the human eye have been examined; and these PGs were found, as in animal eyes, to reduce the IOP. However, several side effects such as initial hypertension from higher doses, conjunctival hyperaemia, headache, and ocular smarting were noted. Thus the clinical usefulness of these PGs as hypotensive agents is limited, as has already been pointed out.⁸

During the course of biochemical experiments on PG synthesis in ocular tissues we found that PGD₂ is the major PG formed in the rat ocular system.¹⁰ This PG has been also reported to be synthesised in the rabbit eye.¹² The effect of PGD₂ on IOP has,

however, not been fully studied. We now report that topically applied PGD₂ is effective in reducing the IOP without side effects in rabbit eyes.

Material and methods

Albino rabbits (weighing 2.0-2.5 kg at the start of experiment) were used. The animals were restrained in metal rabbit holders, and the IOP was measured with an Alcon applanation pneumatonograph after the topical administration of a local anaesthetic (0.4% oxybuprocaine). PGs were kindly supplied by Ono Central Research Institute (Osaka, Japan). They were dissolved in 50 µl of 100 mM potassium phosphate buffer (pH 7.3) and applied topically to one eye. For the maximum dose of 250 µg the PGD₂-sodium salt was prepared by addition of an equimolar amount of Na₂CO₃ dissolved in a minimum volume of water to PGD₂, and then the pH and concentration were adjusted with a buffer. The other eye received the same amount of the buffer alone. For the long-term experiment (over 7 h) PG was administered between 11 and 12 am. For the measurement of protein content in the aqueous humour one eye was designated as the control and the other was treated with PGs. Aqueous humour was then collected once from each eye with a needle. Protein content was determined according to Lowry *et al.*¹¹ with bovine serum albumin as a standard.

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Fig. 1 Effects of PGD₂, PGE₂, and PGF_{2α} on the IOP in rabbits. PGs (50 µg) were applied topically at 0 time (indicated by arrows). Each point shows the mean IOP, and the bars show the SEM.

Results

Fig. 1 shows the effect of topical application of 50 µg of PGD₂, PGE₂, or PGF_{2α} on IOP. The effects of PGE₂ and PGF_{2α} at this dose were biphasic, as have been reported by several authors,^{5,6} that is, application of either of these PGs resulted in an initial increase followed by a reduction in IOP. PGD₂ on the other hand did not cause any increase but only reduced IOP. The reduction of IOP by PGD₂ was observed within 30 min (but not at 15 min) and lasted throughout the observation period of 7 h. By 24 h, however, the IOP had returned to nearly its original level. The IOP at 24 h was 17.6±2.3 mmHg for the control and 17.7±2.0 mmHg for the treated eyes (n=9) (mean±SEM).

For four to seven hours after administration all three PGs reduced the IOP. As shown in Table 1, the mean IOP reduction during this period was the

Table 1 Comparison of hypotensive potency among PGD₂, E₂, and F_{2α}

	n	Control IOP (mmHg)	Difference in IOP (mmHg)	R*
PGD ₂	48	17.0±0.03	4.6±0.03	0.64±0.008
PGE ₂	32	17.2±0.09	7.1±0.13	0.97±0.033
PGF _{2α}	16	18.9±0.07	7.3±0.06	0.84±0.005

Data presented in this table were obtained during the hypotensive phase (4–7 h) after topical applications of 50 µg of the PGs. Means±SEM are given.

*R was calculated from the following equation: $R = (IOP_c - IOP_t) / (IOP_c - ESVP)$, where IOP_c and IOP_t are IOP in the control and in the treated eye respectively.

The episcleral venous pressure (ESVP) has been reported to be 10 mmHg.¹²

highest for PGF_{2α}, followed by PGE₂ and PGD₂. However, since it is generally known that the initial IOP level has a significant influence on the magnitude of IOP reduction, we calculated R values to estimate the relative hypotensive effectiveness (Table 1). Judging from the R values, we considered PGE₂ to be the most effective agent for reducing IOP, followed by PGF_{2α} and PGD₂ under these conditions. The effect of PGD₂ on IOP was dose-dependent. No hypertensive phase was observed even with the maximum PGD₂ dose of 250 µg. The hypotensive effect reached nearly its maximum with 10 µg of PGD₂ (Fig. 2). The minimum dose effective in inducing a significant IOP reduction was judged to be

Fig. 2 Effects of different doses of PGD₂ on IOP. Each point shows mean difference of IOP (IOP of control eye minus IOP of PGD₂ treated eye) obtained between 0.5 and 7 hrs after PGD₂ application. Bars indicate SEM. Statistical significance was determined by Duncan's multiple range test.

Table 2 Effects of PGs on protein content of the aqueous humour

	n	Protein content (g/l)	
		Control eye	Treated eye
Control	3	0.62±0.04	0.69±0.09
PGD ₂	3	0.67±0.09	0.58±0.04
PGE ₂	3	0.58±0.04	23.07±1.54
PGF _{2α}	4	0.63±0.08	2.61±0.94

The aqueous humour was collected 1 h after topical application of 50 µg of PG (buffer alone for the control group). Means±SEM are given.

2.0 µg. Interestingly, even after the maximum dose of PGD₂ (250 µg) was given, the IOP was reduced only to about 13 mmHg. On the other hand 50 µg of PGE₂ or PGF_{2α} reduced the IOP to 9–10 mmHg. The pupillary diameter was not affected even by 250 µg of PGD₂ for at least four hours after application.

No hyperaemia or flare was noted by slit-lamp examination during four hours after the application of up to 250 µg of PGD₂. However, PGE₂ and PGF_{2α} (50 µg) caused strong conjunctival and iridal hyperaemia and irritation (eyes tightly closed) within 30 min after the application. These inflammatory responses lasted for at least four hours. Flare was also noted during one to two hours after the application of PGE₂.

The effect of PGD₂ on the protein content in the aqueous humour was also different from that of PGE₂ or F_{2α}. At one hour after application PGD₂ (50 µg) did not induce any elevation of the aqueous protein, while PGE₂ and F_{2α} caused a 40- and 4-fold elevation respectively (Table 2). PGD₂ failed to increase the aqueous protein not only at 1 h but also at later times after its application. Protein contents at 2, 4, and 6 h were 0.72±0.08 mg/ml (mean±SEM, n=4), 0.65±0.08 (n=4), and 0.59±0.03 (n=4), respectively, and did not differ from the control level.

Discussion

The effect of PGD₂ on IOP is distinct from that of PGE₂ or PGF_{2α}. While 50 µg of PGE₂ or PGE_{2α} caused an initial elevation of IOP, PGD₂ (up to 250 µg) reduced IOP without any elevation phase. The effect of PGD₂ is also totally different from that of the other PGs in terms of inflammatory response. PGD₂ (up to 250 µg) caused no sign of inflammation, that is, no hyperaemia, no flare, and no irritation. At the dose of 50 µg PGD₂ did not cause an increase in the protein content of the aqueous humour, while the other two PGs induced a marked increase. Thus the action of PGD₂ on the eye appears to differ from that of the other two.

The hypertensive and inflammatory responses to

PGE₂ or F_{2α} have been shown to be dose-dependent,^{5,7} and lower doses of these two PGs have been reported to cause less inflammation and no hypertension.^{5,7} In this experiment we have shown that the relative hypotensive potency of the three PGs is PGE₂>PGF_{2α}>PGD₂. Therefore it is conceivable that PGD₂, being a weak agent, did not cause the initial IOP elevation or any inflammatory response. However, as our data show, the hypotensive effect of PGD₂ was saturated at about 10 µg, and a 25-fold increase in the PGD₂ dose did not induce any hypertensive or inflammatory response.

Our observations on the effects of PGD₂ on IOP and inflammatory responses differ from those reported by Kulkarni and Srinivasan,¹³ who also used rabbits. They reported that either topical or intravitreal application of PGD₂ (10 µg) slightly raised the IOP of both eyes. In their experiment a five- to six-fold increase in the aqueous protein at three hours after application was also observed, but we did not find any such increase six hours after PGD₂ application. Although only a single dose experiment, PGD₂ (100 µg) topically applied to cats eyes is reported to decrease IOP.¹⁴ A reduction in IOP by 10 to 50 µg of PGD₃ (an equivalent product from eicosapentaenoic acid to PGD₂ from arachidonic acid) has also been reported in rabbits.¹⁵

Species differences in PG-mediated responses are well documented. Therefore the results obtained in this study cannot necessarily be extended to the human eye. However, since PGD₂ was found to be effective in reducing the IOP without causing any sign of inflammatory response, it is worth examining PGD₂ or its analogues as therapeutic agents for the treatment of glaucoma in man.

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