Effect of 8-iso Prostaglandin E<sub>2</sub> on Aqueous Humor Dynamics in Monkeys

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Objective: To evaluate the effects of 8-iso prostaglandin E<sub>2</sub> (8-iso PGE<sub>2</sub>; prosta-5,13-dien-1-oic acid, 11,15-dihydroxy-9-oxo-<sup>1213</sup>[5Z,8β-11X,13E,15]-) on the intraocular pressure (IOP), outflow facility, and aqueous humor flow rates in normal monkeys and monkeys with glaucoma.

Methods: The IOP was measured before and as long as 6 hours after the topical application of 8-iso PGE<sub>2</sub> to 1 eye of 6 normal monkeys and to the glaucomatous eye of 8 monkeys with unilateral laser-induced glaucoma. The pupil diameter was measured at the same times as the IOP measurements in the normal monkeys. Topographic outflow facility and fluorophotometric flow rates of aqueous humor were measured in 6 normal monkeys before and after drug treatment.

Results: In normal monkeys, a single dose of 0.1% 8-iso PGE<sub>2</sub> reduced (P<.01) the IOP for 4 hours in the treated eyes with a maximum (mean ± SEM) reduction of 3.2 ± 0.2 mm Hg, compared with the contralateral control eyes. The pupil size was smaller (P<.01) in the treated eyes by as much as 1.0 ± 0.2 mm for 4 hours. In 8 glaucomatous monkey eyes, the application of 0.05% and 0.1% 8-iso PGE<sub>2</sub> reduced the IOP (P<.01) for as long as 2 and 5 hours, respectively. The maximum reduction in the IOP was 4.6 ± 0.8 mm Hg (0.05%) and 6.0 ± 0.8 mm Hg (0.1%) compared with baseline measurements. The magnitude and duration of the ocular hypotensive effect were enhanced with twice-a-day administration for 5 consecutive days. Outflow facility in normal monkey eyes was increased (P<.05) by 48% in the treated eyes, and aqueous humor flow was unchanged (P>.10), compared with vehicle-treated contralateral control eyes. Mild eyelid edema, conjunctival edema, hyperemia, and discharge appeared in some eyes treated with the 0.1% drug concentration.

Conclusions: The use of 8-iso PGE<sub>2</sub> reduces the IOP in both normal and glaucomatous monkey eyes. An increase in outflow facility appears to account for most of the IOP reduction in normal monkeys.

Clinical Relevance: The application of 8-iso PGE<sub>2</sub> may have potential for the treatment of glaucoma as an outflow facility–increasing drug.


Several prostaglandin (PG) F<sub>2α</sub> analogues and their prodrugs are potent ocular hypotensive agents in monkey eyes with laser-induced glaucoma<sup>4-6</sup>and are effective and well tolerated in patients with ocular hypertension or glaucoma.<sup>4-5</sup>The mechanism by which these drugs lower the intraocular pressure (IOP) differentiates them from other agents used to treat glaucoma. Prostaglandin F<sub>2α</sub> derivatives reduce the IOP primarily by increasing uveoscleral outflow, with minimal or no increase in trabecular outflow facility and without altering the aqueous humor flow rate.<sup>6</sup> Prostaglandins E<sub>1</sub> and E<sub>2</sub> and some of their derivatives reduce the IOP in rabbits and in normotensive human volunteers following topical application.<sup>7-8</sup> However, the isoprostanes are unique prostanooids formed by peroxidation of arachidonic acid.<sup>9</sup> This formation is not inhibited by cyclooxygenase inhibitors. This study evaluates the effects of the isoprostane 8-iso PGE<sub>2</sub> on IOP following single- and multiple-dose applications in normal and glaucomatous monkey eyes and the mechanism by which 8-iso PGE<sub>2</sub> alters IOP in normal monkeys.

RESULTS

The unilateral topical application of 0.1% 8-iso PGE<sub>2</sub> to the eyes of 6 normal monkeys reduced (P<.01) the IOP for 4 hours in the treated eyes. The maximum difference in IOP between treated eyes and contralateral control eyes was 3.2 ± 0.2 mm Hg (Figure 1). The pupil size was smaller (P<.01) in the treated eyes than in the contralateral control eyes by as much as 1.0 ± 0.2 mm for 4 hours following treatment (Figure 2). Mild eyelid edema, conjunctival edema, and discharge occurred in 1 of 6 treated eyes, but aqueous flare and cells were not observed.

In 8 monkeys with unilateral glaucoma, administration to the glaucomatous eye of a single dose of 0.05%, 0.1%, and 0.2% 8-iso PGE<sub>2</sub> reduced (P<.01) the IOP for as long as 2, 5, and 3 hours, respectively. The maximum reduction in IOP occurred 2 hours after dosing with each of the 3 concentrations and was 4.6 ± 0.9 mm Hg (0.05%), 6.0 ± 0.8 mm Hg (0.1%) and 5.8 ± 0.5 mm Hg (0.2%).
MATERIALS AND METHODS

Fourteen female cynomolgus monkeys, each weighing 3 to 5 kg, were used in this study. Six of the animals had IOPs in the normal range. In 8 of the animals, glaucoma had been induced unilaterally by repeated argon laser (65-120 spots; power, 1.1-1.5 W; size, 50 µm; duration, 0.5 second) or diode (60-120 spots; power, 1.1-1.2 W; size, 75 µm; duration, 0.5 second) photocoagulation of the midtrabecular meshwork for 360°.

On each day of the study, the IOP was measured with a calibrated pneumatonometer (Model 30 classic, Mentor Inc, Norwell, Mass) before drug administration (baseline), at 0.5 hour, and then hourly until 6 hours after drug administration. Five minutes before tonometry, 0.5% proparacaine hydrochloride, 1 drop, was applied topically, and ketamine hydrochloride, 1 to 5 mg/kg of body weight, was administered intramuscularly for adequate sedation. The pupil diameter was measured in normal monkeys with a millimeter ruler under standard illumination immediately before and after each IOP measurement. Slitlamp examination for the detection of aqueous humor flare and cells was performed in a dark room before drug treatment and at 1, 3, and 5 hours after treatment.

8-Isoprostaglandin E2 (prosta-5,13-dien-1-oxic acid, 11,15-dihydroxy-9-oxo-[5Z,8b]-13,15S), Cayman Chemical Co Inc, Ann Arbor, Mich) was freshly prepared by dissolving in dimethyl sulfoxide (100 g/L). This stock solution was further diluted with 0.9% sodium chloride to 0.05%, 0.1%, and 0.2% concentrations. Single-dose testing was performed in 6 normal monkeys with the 0.1% concentration and in 8 glaucomatous monkey eyes with 0.05%, 0.1%, and 0.2% concentrations. In normal monkeys, one 25-µL drop of 8-iso PGE2 was randomly applied to 1 eye, and an equal volume of isotonic sodium chloride solution (the vehicle) was applied to the contralateral control eye. In the monkeys with glaucoma, the first day was the baseline day, and one 25-µL drop of isotonic sodium chloride was administered to the glaucomatous eye at 9:30 AM. On the second day, 25 µL of 8-iso PGE2 was applied to the glaucomatous eye at 9:30 AM. Following 1 baseline day (neither vehicle nor drug was administered) and 1 vehicle-treated day (vehicle to the glaucomatous eye at 9:30 AM and 3:30 PM), a multiple-dose study was carried out in 8 monkeys with unilateral glaucoma, with 0.1% 8-iso PGE2 applied to the glaucomatous eye twice a day (at 9:30 AM and 3:30 PM) for 5 consecutive days.

Outflow facility was measured with an electronic indentation tonograph (EDT-103, Alcon Laboratories, Inc, Ft Worth, Tex) 4 hours before dosing and was remeasured 2 hours after unilateral dosing with one 25-µL drop of 0.1% 8-iso PGE2 in 6 normal monkeys. Aqueous humor flow was measured with a scanning computerized fluorophotometer (Coherent Fluorotron, Coherent Corp, Palo Alto, Calif) in 6 normal monkeys. (Breakdown of the blood-aqueous barrier in glaucomatous monkey eyes makes them poor models to use for aqueous flow measurements.) Iontophoresis was performed in the central corneas of both eyes of each monkey for 7 minutes using 10% fluorescein in 2% agar gel at 9 PM on the day before aqueous flow measurements. Baseline aqueous humor flow rates were measured hourly for 4 hours beginning at 9:30 AM. The following day, one 25-µL drop of 0.1% 8-iso PGE2 was applied to 1 eye of each monkey, and the same volume of vehicle was instilled in the contralateral eye at 8:30 AM. Flow rates were measured at the same times as on the baseline day beginning 1 hour after drug administration. The washout period between each test on the same animal was at least 1 week.

The 2-tailed paired t test was used for statistical analysis before and after single-dose treatment, and the Bonferroni t test was used for the analysis of the multiple-dose study. The Pearson product moment correlation coefficient was used to analyze the relationship between IOP and outflow facility. All experimental studies complied with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research and were approved by the Mount Sinai School of Medicine, New York, NY, Institutional Animal Care and Utilization Committee.

Data are given as mean ± SEM.

The magnitude and duration of the ocular hypotensive effect were enhanced with twice-a-day administration of 8-iso PGE2 in normal monkey eyes. The IOP was significantly (P < .05) reduced for 4 hours after the first dose and for 18 hours after the sixth dose compared with values obtained on the day of vehicle treatment. The IOP was maximally reduced 2 hours after dosing and was 5.0 ± 0.3 mm Hg on day 1 and 9.6 ± 0.6 mm Hg on day 3, comparing the eyes with glaucoma on the drug- and vehicle-treated days (Figure 4). Intracocular pressures on the baseline and vehicle-treated days were similar (P > .40). Mild superficial punctate keratopathy appeared in 1 of the eyes after the fourth dose and persisted thereafter. Mild conjunctival hyperemia and mucous discharge appeared in 2 of the eyes.

Two hours after the unilateral application of 25 µL of 0.1% 8-iso PGE2 to 6 normal monkeys, outflow facility was increased (P < .05) by 48% in the drug-treated eyes compared with the vehicle-treated control eyes and by 43% compared with baseline measurements. The IOP was reduced (P < .05) at 2 hours in the drug-treated eyes when measured tonographically (Table). A significant correlation was found between the IOP reduction and the increase of outflow facility (r = 0.81, P < .05). The coefficient of determination (r² = 0.66) indicated that the increase in outflow facility accounted for most of the IOP reduction in these normal monkey eyes.

For 4 hours following the administration of a single dose of 0.1% 8-iso PGE2 to the treated eyes of 6 normal monkeys, aqueous humor flow rates were unchanged (P > .10) compared with baseline values or values obtained in the contralateral vehicle-treated eyes (Table).

Single-dose administration of PGE1 and PGE2 to rabbits and PGE2 to normotensive volunteers ultimately reduced the IOP after an initial rise.26 In contrast to PGE1 and PGE2, several PGE derivatives—RS-61565 and RS-20216—specific for the EP3 prostanoid receptor, produced greater reductions of the IOP without an initial ocular hypertensive response and less ocular irritation. In the present study, initial ocular hypertension was not observed following single doses of 8-iso PGE2 in normal and in glaucomatous mon-
A dose-dependent ocular hypotensive effect was observed in the glaucomatous monkey eyes, with 0.1% 8-iso PGE2 producing a greater magnitude and a longer duration of IOP reduction than the 0.05% concentration. Increasing the concentration to 0.2% did not increase the magnitude of the IOP reduction. The 0.1% 8-iso PGE2 dosage produced measurements that appeared to be near the top of the dose-response curve and, with twice-a-day administration, produced a sustained reduction of IOP for 5 days in glaucomatous monkey eyes. The prostanoid-receptor profile of 8-iso PGE2 has not been reported.

The effect of PGF2α on outflow facility measured in cynomolgus monkeys has varied from increases of 25% to no increases at all.10,11 Clinical trials, however, demonstrated only small increases in tonographically measured outflow facility in patients with ocular hypertension or primary open angle glaucoma following the topical administration of PGF2α-isopropyl ester12 or PhXA34.13 Compared with PGF2α, the administration of 8-iso PGE2 gave a consistent increase in tonographic outflow facility of 50% in monkeys (Table), with little effect on aqueous humor flow. Analysis of linear regression in the present study shows an apparent close relationship ($r = 0.81$, $P < .05$) between IOP reduction and increased pressure-dependent outflow facility. If several assumptions are made, eg, episcleral venous pressure is unchanged, the coefficient determination ($r^2 = 0.66$) indicates that more than two thirds of the reduction of IOP induced by 8-iso PGE2 may be explained by the increase in outflow facility measured tonographically. Previous studies14-16 have demonstrated that PGF2α congeners can enlarge the spaces between the ciliary muscle bundles, reduce their connective tissue content, and increase uveoscleral outflow, which under normal circumstances is predominantly pressure independent. This is the
major mechanism of action of PGF$_{2\alpha}$ congeners, with little effect on trabecular facility. Furthermore, in isolated human anterior-segment preparations that measure only outflow facility, PGE$_2$, but not PGF$_{2\alpha}$, increases facility. Based on the comparison with PGF$_{2\alpha}$, we interpret the tonographic response to 8-iso PGE$_2$ to be the result of an increase in trabecular outflow facility rather than any alternative explanation. However, iso-prostaglandins could cause uveoscleral outflow to become more pressure dependent, and this mechanism may contribute to the measured tonographic facility.

In this study, a few adverse ocular effects are noted, including mild eyelid edema, conjunctival edema and discharge, mild conjunctival hyperemia, and superficial punctate keratopathy, the last observed in 1 glaucomatous monkey eye during the multiple-dose study. These findings may be related to the frequent tonometry and instillation of local anesthetic drugs or to the relatively high dose of 8-iso PGE$_2$. Changes in the formulation may reduce or eliminate these adverse effects if caused by the drug itself.

The application of 8-iso PGE$_2$, which is structurally different from PGF$_{2\alpha}$ and PGE$_2$, reduces the IOP in both normal and glaucomatous monkey eyes with few adverse effects. A high correlation exists between the reduction of the IOP and the increase of tonographic outflow facility; the latter appears to account for most of the reduction of IOP in normal monkey eyes, in contrast to the predominant ocular hypotensive mechanism of drugs related to PGF$_{2\alpha}$, such as latanoprost. These findings have possible clinical relevance because they show that prostaglandins with unusual stereochemical or geometric configurations can affect aqueous humor dynamics by different mechanisms than those with the normal stereochemistry and geometric configuration that are in current clinical use.