The Effects of Prostaglandin Analogues on IOP in Prostanoid FP-Receptor–Deficient Mice

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PURPOSE. This study was designed to clarify the involvement of the prostanoid FP receptor in the intraocular pressure (IOP)-lowering effects of latanoprost, travoprost, bimatoprost, and unoprostone with the use of FP-receptor-deficient (FPKO) mice.

METHODS. FPKO and wild-type (WT) mice were bred and acclimatized under a 12-hour light-dark cycle. IOP was measured under general anesthesia by a microneedle method. To evaluate the effects of each drug, a single drop (3 µL) of each drug solution was topically applied in a masked manner to a randomly selected eye. IOP reduction was evaluated by the difference in IOP between the treated eye and the untreated contralateral eye in the same mouse. First, the diurnal variation and baseline IOP in WT and FPKO mice were measured. Then, to determine the window feasible for demonstrating the most marked ocular hypotensive effect, 0.005% latanoprost was applied to WT mice during the day or at night. The time when the ocular hypotensive effect was larger was selected for further studies to evaluate the effects of latanoprost (0.005%), travoprost (0.004%), bimatoprost (0.05%), and unoprostone (0.12%). In addition, bunazosin (0.1%) was also applied to demonstrate functional uveoscleral outflow in FPKO mice. All experiments were conducted under a masked study design.

RESULTS. The baseline IOP (mean ± SEM) in WT and FPKO mice was 15.0 ± 0.2 and 15.0 ± 0.3 mm Hg, respectively, during the day, and 18.9 ± 0.4 and 19.2 ± 0.4 mm Hg, respectively, at night. In WT mice, latanoprost significantly lowered IOP both during the day and at night, at 2 to 6 hours and 1 to 6 hours after application, respectively. Maximal IOP reduction was observed at 3 hours after drug instillation both during the day (10.9 ± 1.8%) and at night (23.2 ± 1.1%). At 3 hours after instillation, latanoprost (10.9 ± 1.8% and 23.2 ± 1.1%, daytime and nighttime, respectively), travoprost (15.9 ± 1.4% and 26.1 ± 1.2%), and bimatoprost (8.8 ± 2.0 and 19.8 ± 1.5%) significantly lowered IOP in WT mice both during the day and at night; isopropyl unoprostone significantly lowered IOP at night (15.7 ± 1.9%) but not during the day (5.3 ± 3.2%). In FPKO mice, latanoprost, travoprost, bimatoprost, and unoprostone showed no significant IOP-lowering effect. Bunazosin significantly lowered IOP in both WT (22.1 ± 1.6%) and FPKO mice (22.2 ± 2.1%).

CONCLUSIONS. A single application of latanoprost, travoprost, bimatoprost, or unoprostone had no effect on IOP in FPKO mice with presumed functional uveoscleral outflow pathways. The prostanoid FP receptor plays a crucial role in the mechanism of early IOP lowering of all commercially available prostaglandin analogues. (Invest Ophthalmol Vis Sci. 2005;46: 4159–4163) DOI:10.1167/iovs.05-0494

Prostaglandin (PG) analogues have been widely used as ocular hypotensive drugs for the treatment of glaucoma and ocular hypertension, because of both a greater effect in lowering intraocular pressure (IOP) and fewer systemic side effects than β-blockers. Currently, four different types of PG analogues, latanoprost, travoprost, bimatoprost, and isopropyl unoprostone (unoprostone), are used for the treatment of glaucoma. Latanoprost and travoprost are thought to lower IOP mainly via the FP receptor because of their high degree of specificity to this receptor. 1 This is supported by a recent report that latanoprost showed no IOP-lowering effect in FP-receptor-deficient (FPKO) mice. 2 On the other hand, the IOP-lowering mechanisms of bimatoprost and unoprostone are not fully understood.

Bimatoprost, which has the structure of a prostamide, was originally reported not to act as a PG analogue because of not binding to all prostanoid receptors. 3 So far, no specific receptor for bimatoprost has been determined. However, its metabolite, bimatoprost free acid, binds to the FP receptor with a potency similar to that of latanoprost. 4–11 Recently, bimatoprost has been shown to be readily hydrolyzed to the free acid in human eyes 12 (Dahlin DC. IOVS 2004;45:ARVO E-Abstract 2096). In addition, bimatoprost itself has a weak affinity to FP, leading to increased intracellular calcium concentration. 2 Thus, bimatoprost may exert FP agonistic activity.

Unoprostone shows low affinities for all prostanoid receptors. 1 However, there are several hypotheses to explain unoprostone-induced IOP reduction. Unoprostone free acid and further metabolites have been reported to stimulate PGE 2 release, which may play a role in IOP reduction by unoprostone. 13–14 Unoprostone free acid activates maxi-K channels to inhibit trabecular meshwork contraction, which can lead to increases in aqueous outflow. 15 Finally, unoprostone can induce cellular responses similar to those induced by other FP agonists in cultured ciliary muscle cells and trabecular meshwork cells, probably by acting as a weak FP agonist. 16–18

The discrepancies in many reports may be due to the limitations of the results generated by pharmacological in vitro experiments. For this reason, the transgenic mouse is a powerful tool for investigating single-gene-related function in vivo. In an attempt to shed light on the IOP-lowering mechanism of bimatoprost and unoprostone and to confirm the FP-receptor-mediated action of latanoprost and travoprost, we examined the effects of four commercially available PG analogues, latanoprost, travoprost, bimatoprost, and unoprostone, on IOP in prostanoid FP-receptor-deficient mice. In addition, the response to bunazosin HCl, which has been reported to decrease IOP by increasing uveoscleral outflow, 16,17 was measured to establish the presence of functional uveoscleral outflow pathways in FPKO mice.

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**Materials and Methods**

**Animals**

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. FPKO mice were generated by homologous recombination with a targeting vector that replaces the second exon of the FP gene with the β-galactosidase- and neomycin-resistant gene. Homozygous knockout mice do not transcribe FP-receptor mRNA. Both homozygous and heterozygous knockout mice develop normally, and have no gross abnormalities in behavior, macro- or microscopic anatomy, or biochemical or hematologic indices. Because homozgyote knockout females fail to initiate parturition, heterozygous (female) and homozygous (male) mating pairs were used to generate an F1 generation. Murine genotype was determined by PCR and FP-receptor homozygous knockout mice were used. C57BL/6 mice, which constitute the background species of the FPKO mice, were used as the wild-type (WT) control. Mice were bred and housed in clear cages, loosely covered with air filters, and white chips provided bedding. The environment was kept at 21°C with 50% humidity and a 12-hour light:12-hour dark cycle. All mice were fed ad libitum and acclimatized to the environment for at least 2 weeks before the experiments. We used mice older than 8 weeks of age in our study.

**Preparation and Instillation of Ophthalmic Solution**

Latanoprost and bimatoprost were purchased from Cayman Chemical Co. (Ann Arbor, MI). Latanoprost (0.005%) was dissolved in its vehicle solution as reported previously. Bimatoprost (0.03%) was dissolved in a phosphate-buffered saline (PBS). Travoprost (0.004%), unoprostone (0.12%), and bunazosin HCl (0.1%) ophthalmic solutions, and the vehicle solution as reported previously. Bimatoprost (0.03%), was dissolved in phosphate-buffered saline (PBS). Travoprost (0.004%), unoprostone (0.12%), and bunazosin HCl (0.1%) were provided by Alcon Inc. (Fort Worth, TX), R-Tech Ueno Ltd. (Hyoego, Japan), and Santen Pharmaceutical (Osaka, Japan), respectively. With a micropipette, 3 μL of each drug solution was topically applied in a masked manner to a randomly selected eye, and the contralateral, untreated eye served as the control.

**IOP Measurement**

IOP was measured by a microneedle method in mice anesthetized with ketamine and xylazine, as described previously. Briefly, a borosilicate glass microneedle (100-μm tip diameter and 1.0-mm outer diameter; World Precision Instruments [WPI], Sarasota, FL) was connected to a pressure transducer (Model BLPR, WPI). The system pressure detected by the transducer was recorded by a data acquisition and analysis system (PowerLab; ADInstruments, Colorado Springs, CO). The microneedle was placed in the anterior chamber, and the conducted pressure was recorded in both eyes during a 4- to 7-minute time window after anesthesia. Until the mouse was placed on the table for IOP measurement, room lighting was maintained similarly to that in the vivaria. For dark-phase measurements, all procedures were performed under red light illumination to eliminate the effect of lighting on IOP. The effect of each drug was calculated as the ratio of IOP (mm Hg) measured under red light illumination to eliminate the effect of lighting. The time point at which the ocular hypotensive effect was greatest was used for further studies to evaluate the effects of PG analogues.

**Effect of Vehicle Solutions on IOP in WT and FPKO Mice**

To confirm that the vehicle solutions did not affect IOP, 3 μL vehicle solution for each PG analogue and bunazosin was administrated topically to one randomly chosen eye in both WT and FPKO mice at 6 AM or 6 PM. Three investigators instilled eye drops under masked test protocols. A fourth investigator, also masked to the treatment, measured IOP 3 hours after drug instillation, as described above. Thus, all measurements were performed under masked conditions.

**IOP-Lowering Effects of PG Analogues on WT and FPKO Mice during the Day and at Night**

Three microliters of elanoprost (0.005%), travoprost (0.004%), bimatoprost (0.03%), or isopropyl unoprostone (0.12%) was administrated topically to one randomly chosen eye in both WT and FPKO mice at 6 AM or 6 PM. Three investigators instilled eye drops under masked test protocols. A fourth investigator, also masked to the treatment, measured IOP 3 hours after drug instillation, as described above. Thus, all measurements were performed under masked conditions.

**Statistical Analysis**

The Mann-Whitney U test was used for comparison of baseline IOP between daytime and nighttime values in each strain and also for comparison of IOP reduction between WT and FPKO mice. The Wilcoxon signed-ranks test was used for comparison of IOP between the treated eye and the contralateral eye. The Kruskal-Wallis test was used for multiple comparisons of IOP. Values of P < 0.05 were considered significant. All data are presented as means ± SEM.

**Results**

**Diurnal Variation of Baseline IOP in WT and FPKO Mice**

Mean IOP during the day (9 AM) and at night (9 PM) was 15.0 ± 0.2 and 18.9 ± 0.4 mm Hg, respectively, in WT mice, and 15.0 ± 0.3 and 19.2 ± 0.4 mm Hg, respectively, in FPKO mice.

**Table 1. Baseline IOP in WT and FPKO Mice during the Day and at Night**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>WT (mm Hg)</th>
<th>FPKO (mm Hg)</th>
</tr>
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<tbody>
<tr>
<td>Day (9 AM)</td>
<td>15.0 ± 0.2 (11)</td>
<td>15.0 ± 0.3 (11)</td>
</tr>
<tr>
<td>Night (9 PM)</td>
<td>18.9 ± 0.4* (12)</td>
<td>19.2 ± 0.4* (10)</td>
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</tbody>
</table>

IOP was measured by a microneedle method under general anesthesia. Data are expressed as means ± SEM (n). There was no significant difference between WT and FPKO mice at either time point.

* Different from daytime (Mann-Whitney U test), P < 0.0001.
were made at 3 hour after drug administration, both during the day and at night.

**Effect of Vehicle Solutions on IOP in WT and FPKO Mice**

At 3 hours after treatment with each vehicle solution, the mean IOP reductions during the day and at night ranged from −2.0% to 1.6% and from −2.2% to 2.1%, respectively, in WT mice and from −1.9% to 2.1% and from −1.2% to 2.5%, respectively, in FPKO mice. None of the vehicle solutions showed a significant effect on IOP.

**Effect of PG Analogues on IOP in WT and FPKO Mice during the Day and at Night**

During the day, latanoprost (10.9 ± 1.8%, P = 0.0117), travoprost (15.9 ± 1.4%, P = 0.0180), and bimatoprost (8.7 ± 2.0%, P = 0.0180) significantly lowered IOP in WT mice (Fig. 2A), whereas these four drugs had no effect on IOP in FPKO mice (Fig. 3A). At night, latanoprost (23.2 ± 1.1%, P = 0.0033), travoprost (26.1 ± 1.2%, P = 0.0051), bimatoprost (19.8 ± 1.5%, P = 0.0051), and unoprostone (13.7 ± 1.9%, P = 0.0051) significantly lowered IOP in WT mice (Fig. 2B), whereas they had no significant effect on IOP in FPKO mice (Fig. 3B). In WT mice, the IOP-lowering effect of each PG analogue was greater at night than during the day (Figs. 2A and B); conversely, in FPKO mice, the IOP-lowering effects of PG analogues did not differ significantly between daytime and nighttime administration (Figs. 3A and B).

**Effect of Bunazosin HCl on IOP in WT and FPKO Mice at Night**

Bunazosin significantly lowered IOP at 3 hours after instillation in both WT (22.1 ± 1.6%, P = 0.0053) and FPKO mice (22.2 ± 2.1%, P = 0.0051) (Fig. 4). However, there was no significant difference in the magnitude of IOP reduction between WT and FPKO mice (P = 0.7782).

**DISCUSSION**

In this study, we examined the effects of PG analogues on IOP in FPKO mice. In the absence of the prostanoid FP receptor, the IOP-lowering effects of a single instillation of PG analogues including latanoprost, travoprost, bimatoprost, and unoprostone were completely eliminated. On the other hand, bunazosin, which lowers IOP by improving uveoscleral outflow,16,17 lowered IOP in FPKO mice. Thus, FPKO mice do not likely have functional abnormalities in uveoscleral outflow. These results suggest that the prostanoid FP receptor plays a crucial role in the early IOP-lowering mechanism of all commercially available PG analogues. However, the intracellular signaling pathway and cellular response to FP-receptor binding have not been fully elucidated.
been fully clarified. Especially with regard to the short-term effect, there must be some cellular responses different from the induction of matrix metalloproteinase and the subsequent remodeling of extracellular matrices, which occur over a long time span.22,23

Our results confirm those of a previous study in which latanoprost failed to induce an IOP-lowering effect in FPKO and FP-receptor heterogeneous knockout mice; these mice had no obvious anatomic differences compared to WT mice.5 In the previous study, however, IOP was measured during the day (between 2 and 6 PM), when IOP in the WT mice was not high (13.9–14.6 mm Hg), likely due to the 24-hour variation in IOP. Thus, the measured IOP reduction induced by latanoprost in the WT mice was small (−1.4 mm Hg) and not necessarily adequate for comparison with that in the FPKO mice. Moreover, only the structural similarity in the uveoscleral outflow pathway between FPKO and WT mice was histologically examined. In light of these previous results, the baseline IOP and the response to latanoprost were measured both during the day and at night, and the nighttime measurement was also adopted to detect the IOP response in FPKO mice. In addition, the functional integrity of the uveoscleral outflow pathway in FPKO was suggested by bunazosin.

Interestingly, bimatoprost and unoprostone completely failed to lower IOP in FPKO mice. Thus, bimatoprost and unoprostone may lower IOP in mice via their converted acid forms, which are bound to the FP receptor; this is supported by reports of previous in vitro studies.1,4,11 Recent reports also indicate that bimatoprost can stimulate human FP receptors.1,5,7,8 However, it is possible that FP agonists produced by bimatoprost and unoprostone act on FP receptors via unknown signal pathways. To clarify this issue, determination of PG analogues in the aqueous after instillation of bimatoprost and unoprostone in FPKO mice will be required.

The mechanism of IOP reduction by bimatoprost has been thought to involve improvements in conventional and uveoscleral outflow pathways in monkeys and humans.24,25 Thus, bimatoprost may also mediate IOP reduction via receptors other than FP receptors, which mainly affect the uveoscleral outflow pathway. Bimatoprost free acid can also bind to the EP1 receptor.1 However, the absence of IOP reduction in FPKO mice suggests that the contributions of other receptors are limited, as far as the early IOP-lowering mechanism of bimatoprost is concerned.

Unoprostone may act as a weak FP agonist, despite having low affinities for all prostanoid receptors.1 This is also supported by the cellular responses seen in cultured ciliary muscle cells and trabecular meshwork cells.1,5,7,8 Stimulation of PGE2 release by unoprostone free acid and further metabolites13,14 may be unrelated to the IOP-lowering effect, at least in mice.

Travoprost significantly lowered IOP in WT mice both during the day and at night in the present study. These results are comparable with those of our previous study using ddY mice.21 The absence of a travoprost-induced IOP-lowering effect in FPKO mice is an expected outcome considering its higher selectivity, potency, and affinity for the FP receptor compared to latanoprost.5,7

It must be noted that the present results involve the ocular hypotensive effect after a single instillation of a PG analogue in mice, which implies that direct extrapolation of the present results to humans or glaucoma patients may be difficult. Clinically it is well known that IOP reduction after a single instillation of latanoprost is not so remarkable as after once-daily long-term instillation. The mechanism of action after a single instillation may be different from that after long-term use. Ocular hypotensive effects by various PG analogues obtained in mice do not seem to parallel those in humans. In fact, the ocular hypotensive effect of latanoprost tended to be a little greater than that of bimatoprost in mice (Fig. 2), while previous studies suggested that the ocular hypotensive effect of bimatoprost tended to be a little greater than that of latanoprost in human patients.26–29 In the present study, we demonstrated that IOP reduction after a single application of PG analogues in mice was FP-receptor dependent, suggesting that FP receptors are at least partly involved in the ocular hypotensive effect of latanoprost, travoprost, bimatoprost, or unoprostone in humans as well. Effects of bimatoprost or unoprostone in humans cannot be explained only by FP receptors. Therefore, further studies are required to clarify the involvement of...
the FP receptor in IOP reduction with long-term administration of FP agonists.

Because IOP lowering is primarily mediated via FP receptors, it is an interesting question whether ocular side effects induced by PG analogues are also observed in FPKO mice. Since FPKO mice have similar IOP patterns and seemingly normal functional uveoscleral outflow pathways (both functionally and structurally), FPKO mice may be useful in developing new PG-analogue drugs with greater ocular hypotensive potential and lesser ocular side effects.

In conclusion, the finding that travoprost, bimatoprost, and isopropyl unoprostone, as well as latanoprost, failed to lower IOP in FPKO mice provides evidence that the FP receptor plays a critical role in the IOP-lowering effect of these PG analogues, at least as far as short-term effects are concerned.

References


