Effect of Latanoprost on Intraocular Pressure in Mice Lacking the Prostaglandin FP Receptor

Jonathan G. Crowston,1,2 James D. Lindsey,1,2 Makoto Aihara,3 and Robert N. Weinreb1,2

PURPOSE. To determine whether latanoprost lowers IOP in prostanlgandin FP receptor knockout mice.

METHODS. Mean IOP difference between treated and untreated fellow eyes was measured on three separate occasions, 2 hours after a 200-ng dose of latanoprost to the right eye of homozygous (n = 9) and heterozygous (n = 15) FP knockout mice. C57BL/6 (n = 10) and NIH Swiss white mice (n = 17), which have normal FP receptor expression, provided the control population. The investigator was masked to the genotype of the FP knockout mice at the time of IOP measurement.

RESULTS. Latanoprost had no effect on IOP in the homozygous FP knockout mouse, with an average IOP difference of −0.019 to +0.69. In contrast, latanoprost reduced IOP in the treated eye of the heterozygous FP knockout, C57BL/6, and Swiss white mice with mean differences and 95% confidence interval (CI) for the difference between means of −0.52 (−0.91 to −0.14), −1.38 (−2.1 to −0.70), and −1.29 (−1.78 to −0.79) mm Hg, respectively.

CONCLUSIONS. FP receptor signaling plays a crucial role in the early IOP response to latanoprost in the mouse eye. (Invest Ophtalmol Vis Sci. 2004;45:3555-3559) DOI:10.1167/iovs.04-03538

Topically administered prostaglandin (PG) F2α lowers intraocular pressure (IOP) in humans,1 nonhuman primates,2,3 and rodents.4,5 Although effective as an ocular hypotensive, (PG)F2α, has several ocular side effects that preclude its development for clinical use. In contrast, topical application of latanoprost (Prostaglanin [PhXA41]), a PGF2α analogue that is a selective agonist of the PG FP receptor, is well tolerated and has become the most commonly used drug to treat glaucoma and ocular hypertension.7 Although it is generally well accepted that latanoprost reduces IOP by increasing pressure-independent outflow, the exact molecular mechanisms responsible for this are not fully understood.

The FP receptor is a G-protein–coupled receptor that is expressed in the tissues of the uveoscleral outflow pathway including iris, ciliary body,6 and sclera,7 as well as in the trabecular meshwork.10 Pharmacologic studies indicate an important role for FP receptor activation in the IOP response to latanoprost.11-15 Despite the receptor selectivity demonstrated in vivo, prostaglandin analogues may act through mechanisms that do not involve FP receptor signaling in vivo. For example, studies that investigated the response to PGF2α and its analogues in the cat have suggested that IOP reduction and relaxation of the ciliary muscle were not mediated by FP receptor signaling.14,15 The role of the FP receptor in latanoprost-mediated lowering of IOP therefore needs further clarification.

The recent development of techniques that allow accurate measurement of IOP and aqueous humor dynamics in the mouse eye6,7 provides the opportunity for the investigation of knockout mice that lack the FP receptor. These mice were generated by homologous recombination with a target 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, whereas heterozygous knockouts transcribe reduced amounts.16 Both the homozygous and heterozygous knockout mice develop normally and have no gross abnormalities in behavior, macro- and microscopic anatomy, or biochemical or hematologic indices.17 Hence, the purpose of this study was to determine whether latanoprost lowers IOP in mice that do not express the PG FP receptor.

METHODS

Animal Husbandry

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. FP knockout mice were obtained as a gift from Shuh Narumiya (Kyoto University, Kyoto, Japan). Because homogygote knockout females fail to initiate parturition,17 heterozygous (female) and homozygous (male) mating pairs were used to generate an F1 generation. C57BL/6 mice, which constitute the background species of the FP knockout mice, were used as the wild-type control. NIH Swiss white mice were used as a further control population. Mice were bred and housed in clear cages covered loosely with air filters and containing white pine shavings for bedding. The environment was kept at 21°C with a 12-hour light (6 AM to 6 PM) and 12-hour dark cycle. All mice were fed ad libitum. Animal age ranged from 6 to 8 months. All measurements were performed between 2 and 6 PM, to minimize the influence of diurnal IOP rhythm and hence possible diurnal variation of mouse IOP.

Drug Application

Four μl 0.005% latanoprost solution (200 ng total; Pfizer, New York, NY) was applied to the right eye of each mouse. During the administration of treatment, animals were gently restrained in a tapered plastic film tube (Decapicone; Braintree Scientific Inc., Braintree, MA) to avoid stress. This 200-ng dose of latanoprost has been found to be the minimum needed to achieve a maximum IOP response in the Swiss white mouse.4

Anesthesia

The mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge,
FP knockout mice were generated with a target vector LacZ/neo(r), which replaces the second exon of the FP gene (A). PCR products showing single bands for homozygous knockout mice (FP−/−, *) and double bands for heterozygous FP+/− mice (B). Right lane: 100-bp molecular weight standard.

**Determination of Mouse Genotype by PCR**

DNA was extracted from 8-mm tail biopsy samples of anesthetized adult mice (DNeasy tissue kit, cat. no. 69504; Qiagen, Valencia, CA) according to the manufacturer’s guidelines. The oligonucleotide primers used to detect homologous translocation were: 5F (GCCCATCCT-TGGACACCGGAGA), 6R (AGAGTCGGAAGCTGACTC), and NeoII (TGATATTGCTGAAGACCTGG). Amplification was performed over 35 cycles of 94°C for 30 seconds, 65°C for 30 seconds, and 75°C for 10 minutes. Products were analyzed by electrophoresis in 1% agarose gels. The PCR product sizes were 700 bp for the FP receptor and 450 bp corresponding to the LacZ/neo(r) cassette. Thus, DNA from the heterozygous FP+/− mice produced two bands at 700 bp and at 450 bp, whereas DNA from the homozygous FP−/− knockout mice produced a single band at 450 bp (Fig. 1).

**Anterior Segment Histology**

Nine-month-old homozygous FP knockout and C57BL/6 mice were euthanatized by CO2 inhalation followed by intracardiac perfusion of approximately 40 mL of fixative containing paraformaldehyde (2%) and glutaraldehyde (2.5%) in 0.15 M sodium cacodylate buffer (pH 7.3). A full-thickness hole was cut in the posterior segment to allow free access of fixative and embedding medium. Eyes were enucleated, incubated overnight in fixative, and embedded in Epon. One- to 2-μm-thick sections were stained with toluidine blue, examined by light microscopy (Eclipse E800; Nikon, Melville, NY) and photographed with a cooled digital camera (SPOT Digital camera system; Diagnostic Instruments, Sterling Heights, MI).

**Measurement of IOP**

IOP measurements were performed by cannulation of the anterior chamber, as described previously.7 Microneedles were made of borosilicate glass with a tip diameter between 75 and 100 μm.16 The microneedle was mounted on a micromanipulator to enable accurate positioning. It was connected to a pressure transducer (Model BLPR; WPI, Sarasota, FL) which was calibrated against a manometer over the range of 0 to 30 mm Hg, as described previously.14 IOP was measured in both eyes within 7 minutes of the anesthetic injection, to minimize the effect of anesthesia.4,18 Left and right eyes were measured first in alternating rotation in successive mice. The second IOP was measured within 1 minute of the first eye recording. The investigator was masked to mouse genotype at the time of IOP measurement. The IOP response to latanoprost was measured three times in the FP knockout mice, because small IOP alterations could have been masked by physiological intermouse and intereye differences in IOP. As a larger reduction in IOP has been demonstrated previously in wild-type mice,4 the response to latanoprost in these mice was measured only once. A 1-week washout period was used between measurements.

**Statistical Analysis**

Paired Student’s t-tests were used to compare the IOP in treated and untreated fellow eyes of mice of the same genotype. The average reduction in IOP (mean IOP in treated eyes minus mean IOP in fellow untreated eyes) was expressed as the mean difference and 95% confidence interval (CI) for the difference between means. A significant reduction in IOP was defined by a 95% CI range that was less than zero. The Student t-test and analysis of variance (ANOVA) were used to compare the mean difference in IOP between genotypes. For comparison of two means, P < 0.05 was considered to be statistically significant. The Tukey-Kramer post hoc modification of ANOVA was performed to adjust for comparison between multiple groups.

**RESULTS**

**Baseline Characteristics in FP Knockout IOP**

The F1 generation of heterozygous female and homozygous male breeding pairs consisted of 15 heterozygous and 9 homozygous FP knockout mice. Two distinct bands were identified after electrophoresis of the PCR products: a 700-bp band corresponding to the FP gene and a 450-bp band corresponding to the lacZ/neo(r) cassette (Fig. 1).

Slit lamp biomicroscopy, gonioscopy, and fundoscopy revealed no anatomic difference between FP knockout and C57BL/6 mice. In addition, examination of toluidine-blue-stained plastic sections of eyes from adult FP homozygous knockout and C57BL/6 wild-type mice did not reveal any anatomic differences (Fig. 2). IOP in untreated eyes of the heterozygous FP knockout mice tended to be lower than in the homozygous FP knockout mice (Table 1). The mean difference and 95% CI for the difference in means was −1.9 mm Hg (−3.5 to +0.41) for comparisons of right eyes and −1.4 mm Hg (−3.6 to +0.02) for left eye comparison. These differences

![Figure 2. Anterior segment histology showing the angle structures in C57BL/6 (left) and homozygous FP knockout (right) mice. No significant anatomic differences were detected between the two genotypes.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932925/ on 11/21/2018)
were not statistically significant (P = 0.05 OD, P = 0.11 OS, nonpaired t-test assuming unequal variance). In addition, there was no significant difference in IOP between left and right eyes within either genotype. The baseline IOP in C57BL/6 mice was significantly lower in both eyes, compared with that in the FP homozygous mouse (P = 0.0013 OD, P = 0.0013 OS). There was a trend toward lower IOP in the C57BL/6 mice compared with heterozygous FP knockout mice (P = 0.066 OD, P = 0.046).

Effect of Latanoprost on IOP

Average IOP difference between the latanoprost-treated and untreated fellow eye in the homozygous FP knockout mouse was +0.25 mm Hg with a 95% CI of the mean IOP difference crossing zero (−0.19 to 0.69). This indicates that latanoprost did not lower IOP in the homozygous FP knockout. In contrast, IOP was reduced in the treated eye of the heterozygous FP knockout, C57BL/6, and Swiss white mice, as the 95% CI for the mean difference in IOP between treated and fellow eyes did not cross zero (Table 2, Fig. 3). ANOVA showed that the mean difference in IOP between treated and untreated fellow eyes of the homozygous FP knockout mouse was significantly smaller than the mean difference in IOP for the FP heterozygous (P = 0.03) C57BL/6 (P < 0.0001) and Swiss white mice (P = 0.006).

DISCUSSION

These data demonstrate that latanoprost does not reduce IOP in knockout mice that lack the FP receptor. This finding is consistent with the view that latanoprost-mediated lowering of IOP is a direct result of FP receptor signaling. Furthermore, the small reduction in IOP in the heterozygous mice that transcribe reduced quantities of FP mRNA17 and the larger reduction in IOP observed in the wild-type mice points to a possible dose relationship between drug response and FP receptor expression.

We examined the effect of latanoprost in the FP knockout mouse, as this is a selective agonist of the FP receptor. These data can provide a template for comparison with other PGs to determine whether their mechanism of action is independent of FP receptor signaling. Several potential limitations, however, should be considered. First, the IOP response was measured at a single time point in response to a single dose of latanoprost. The measurement time and dosage strategy chosen in this study were based on previous work that showed that latanoprost induces maximum lowering of IOP in the mouse eye 2 hours after application of a 200-ng dose.4 Although it is possible that maximum lowering of IOP may be altered by the genetic background or by gene elimination, it is unlikely that such a shift would completely account for the elimination of IOP reduction. This is supported by the significant reduction in IOP in the treated eyes of heterozygous FP knockout mice at 2 hours. Second, IOP lowering was presented in terms of mean IOP reduction in the treated eye compared with the untreated fellow eye. It is possible that a contralateral response in the untreated eye would minimize the difference observed between the two eyes. This approach was used to minimize the variability in IOP among mice of the same species that could mask small changes in IOP induced by latanoprost. Despite these potential limitations, these results provide strong evidence that the mechanism of early reduction of IOP by latanoprost in the mouse is critically dependent on FP receptor expression.

Activation of the FP receptor initiates several downstream events, including an increase in inositol phosphate, protein kinase C activation, calcium release, and myosin light chain phosphorylation.10–19 How these events in turn lead to the lowering of IOP is not completely understood, but several different mechanisms may play a role. There is evidence that an increase in uveoscleral outflow after multiple applications is a consequence of extracellular matrix remodeling.22 These changes may result from drug-induced upregulation of matrix metalloproteinases, a family of neutral proteinases, which specifically degrade extracellular matrix.23 Latanoprost acid, the active metabolite of latanoprost, increases the expression of matrix metalloproteinases at the protein24 and RNA levels25 in cultured human ciliary smooth muscle cells and in sclera.25 In addition, repeat treatment with latanoprost leads to a reduction in collagen I, III, and IV; fibronectin; laminin; and hyaluronan in the ciliary muscles of monkeys, which was associated with increased expression of MMP-2 and -3.26 However, it is unlikely that extracellular matrix remodeling occurs within the 2-hour time-point examined here, as experimental studies indicate this time is insufficient for the induction of MMP gene transcription.28 Whether the FP receptor is critical for the IOP reduction after repeat applications of latanoprost remains to be investigated. Further studies to determine the effect of treatment on MMP expression and extracellular matrix remodeling

### Table 1. Baseline IOP for FP Knockout Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Mean IOP OD (mm Hg, SE)</th>
<th>Mean IOP OS (mm Hg, SE)</th>
<th>Weight (g, SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>9</td>
<td>14.6 (0.73)</td>
<td>13.9 (0.77)</td>
<td>34.1 (1.54)</td>
</tr>
<tr>
<td>Heterozygote FP&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>15</td>
<td>16.4 (0.55)</td>
<td>16.1 (0.55)</td>
<td>29.4 (1.19)</td>
</tr>
<tr>
<td>Homozygote FP&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9</td>
<td>18.3 (0.64)</td>
<td>17.5 (0.69)</td>
<td>30.4 (1.53)</td>
</tr>
</tbody>
</table>

### Table 2. Mean Difference in IOP between Latanoprost-Treated and Fellow Untreated Eyes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number</th>
<th>Mean Difference in IOP&lt;sup&gt;−&lt;/sup&gt; (mm Hg)</th>
<th>95% CI for Difference in Means</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>9</td>
<td>−1.38</td>
<td>−2.1 to −0.70</td>
</tr>
<tr>
<td>Swiss white</td>
<td>17</td>
<td>−1.29</td>
<td>−1.78 to −0.79</td>
</tr>
<tr>
<td>Heterozygote FP&lt;sup&gt;+/−&lt;/sup&gt;</td>
<td>15</td>
<td>−0.52</td>
<td>−0.91 to −0.14</td>
</tr>
<tr>
<td>Homozygote FP&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9</td>
<td>+0.25</td>
<td>+0.19 to +0.69</td>
</tr>
</tbody>
</table>

<sup>−</sup> Treated minus untreated eyes.
in the FP knockout and wild-type mice would further clarify the role of the FP receptor in prostaglandin-mediated alterations of the aqueous outflow pathways.

This study is the first to report the use of genetically modified mice to investigate the mechanism of action of an ocular hypotensive agent. The observation that mice that lack the FP receptor do not respond to latanoprost provides definitive evidence that the FP receptor is critical for the early IOP response in the mouse. Further studies with genetically modified mice should help clarify the molecular mechanism of PG-mediated IOP lowering.

References