Prostaglandin FP Receptors Do Not Contribute to 24-hour Intraocular Pressure Variation in Mice

Jonathan G. Crowston,¹ Christy A. Morris,² James D. Lindsey,² and Robert N. Weinreb²

PURPOSE. It is not known whether the prostaglandin FP receptor plays an important role in endogenous 24-hour regulation of intraocular pressure. The purpose of this study was to compare 24-hour intraocular pressure (IOP) in FP receptor-knockout mice with that of wild-type mice that have normal FP receptor expression.

METHODS. The 24-hour IOP profile was determined by rebound tonometry in FP-knockout and wild-type mice. Peak and trough IOP was then measured by microneedle cannulation of the anterior chamber in homozygous (FP−/−; n = 8), heterozygous (FP+/−; n = 14), and C57BL/6 background strain mice (FP+/+; n = 11). To confirm any differences in baseline IOP between genotypes, midaftemoon IOP was also measured in a larger, separate group of FP−/− mice (n = 20), FP+/− mice (n = 49), and FP+/+ (n = 23) wild-type littermates.

RESULTS. Trough IOPs were measured between 10 AM and 12 PM, peak IOPs were measured between 8 and 10 PM. For FP−/−, FP+/−, and FP+/+ mice trough IOP was 16.2, 15.3, and 15.1 mm Hg and peak IOP were 18.2, 18.4, and 17.7 mm Hg, respectively. There was no significant difference among genotypes for mean peak or mean trough IOP or for peak-trough difference in IOP among genotypes (P > 0.05, ANOVA). In addition, there was no significant difference in midaftemoon IOP between genotypes in a larger population (n = 92) of FP-knockout and wild-type mice.

CONCLUSIONS. An intact FP receptor does not appear to be critical for normal 24-hour IOP regulation in the mouse eye.


The 24-hour variation in intraocular pressure (IOP) is a consistent phenomenon in humans and other mammals,¹ but little is known of the molecular mechanisms that regulate this fluctuation. The recent development of accurate techniques for IOP measurement in wild-type and genetically modified mice permits evaluation of the role of individual genes in circadian IOP variation.¹²

The prostaglandin (PG) FP receptor is widely expressed in ocular tissues,³⁻⁵ but its function is poorly understood. PGF₂α and many of its analogues bind the FP receptor and lower IOP in humans,⁶ nonhuman primates,⁷ rabbits,⁸ and mice.⁹ IOP lowering is consistent over the entire 24-hour period,¹⁰⁻¹² and treatment is associated with a reduction in the 24-hour variation of IOP.¹¹ It has recently been shown that PGF₂α concentration in the aqueous humor of untreated rabbits exhibits circadian variation.¹³ Aqueous humor sampled during the light period, when IOP is low, has significantly higher PGF₂α levels than does aqueous taken during the dark phase, when IOP is higher. In contrast there is no significant variation in aqueous humor PGE₂ concentration over the same time period.¹³ These findings raise the possibility that FP receptor activation by aqueous PGF₂α has an important role in 24-hour IOP regulation.

We therefore hypothesized that mice lacking an intact FP receptor would have elevated IOP and increased 24-hour IOP variation compared with wild-type mice with intact FP receptors. The purpose of this study therefore was to compare IOP over the 24-hour circadian period in FP receptor knockout and wild-type mice.

METHODS

Animal Husbandry

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mice lacking the gene encoding the FP receptor were generated with a target vector (LacZ/Neo(r) that replaces the second exon of the FP gene (the generous gift of Shuh Narumiya Kyoto University, Japan). Homozygous knockout female mice fail to initiate parturition.¹⁴ For this reason, heterozygous (female) and homozygous (male) mating pairs were used to produce an F1 generation. C56BL/6 mice, which constitute the background strain, served as the control population. Mice were bred and housed in clean cages covered loosely with air filters containing white pine shavings for bedding. The environment was kept at 21°C. All mice were fed ad libitum. The animals’ ages ranged from 9 to 12 months.

Determination of Mouse Genotype

Mouse genotype was determined by polymerase chain reaction (PCR). DNA was extracted from 8-mm tail biopsy specimens of anesthetized adult mice (DNAnacsy tissue kit, cat. no. 69504H; Qiagen, Valencia, CA) according to the manufacturer’s guidelines. The oligonucleotide primers used to detect homologous translocation were: 5F (GCCCATCCT-TGGACACCGAGA), 6R (AGAGTCGGAAGCTGTGACTT) and NeoI (TGATATGCTGAAGCTTGG). 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 FP and 450 bp, corresponding to the LacZ/neo(r) cassette. DNA of the heterozygous FP−/− mice therefore produces two bands (700 and 450 bp), and that of homozygous FP−/− knockout mice produce a single band (450 bp).

IOP Measurement

Mice were exposed to a 12-hour light (6 AM to 6 PM) and 12-hour dark cycle for at least 2 weeks before the experiments. Trough IOP was measured between 10 AM and 12 PM, and peak IOPs were measured between 8 and 10 PM, with minimum red-light illumination, as described previously.¹
Cannulation IOP. Measurements were performed by cannulation of the anterior chamber, as described in detail previously (Fig. 1). Beveled microneedles were made of borosilicate glass with a tip diameter of 75 μm. The microneedle was mounted on a micromanipulator to enable accurate positioning and was connected to a pressure transducer (BLPR; World Precision Instruments, Sarasota, FL), which was calibrated against a manometer over the range of 0 to 30 mm Hg, as described previously. IOP was measured in both eyes within 7 minutes of the anesthetic administration. 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.

Induction-Impact (Rebound) Tonometry. Longitudinal IOP measurements over a single 24-hour period were performed with an induction impact tonometer described by Danias et al. (Fig. 1). This noninvasive tonometer permits IOP measurement in awake mice. The tonometer was calibrated in vivo, as described previously. A C57BL/6 mouse eye (background strain for FP receptor knockout mice) was cannulated close to the limbus with a needle connected to a fluid column and a pressure transducer. This setup permitted the eye pressure to be set by raising and lowering the fluid column. IOP measurements with the rebound tonometer were taken at intervals between 0 and 30 mm Hg. Longitudinal IOP measurement were made at 3-hour intervals in awake mice that were gently restrained to ensure no mechanical Valsalva effect that would elevate IOP. The tonometer probe was mounted on a micro-manipulator, and measurements were recorded from a distance of 3.0 ± 0.1 mm from the center of the cornea. A dissecting microscope was used to ensure good centration on the cornea.

RESULTS

Baseline IOP
IOP was measured at the same time (between 2 and 4 PM) in a larger population of homozygous and heterozygous FP receptor knockout mice, as well as in the wild-type background strain, by using the cannulation technique (FP+/−, n = 23; FP−/−, n = 49; FP−/−, n = 20). There was no statistically significant difference in IOP among the three genotypes (ANOVA; P > 0.05; Table 1).

Longitudinal IOP Measurement over 24 Hours
Calibration of the rebound tonometer in the C57BL/6 mouse provided a curvilinear calibration curve similar to that reported previously in Swiss White mice (Fig. 2). This noninvasive tonometer permitted multiple longitudinal IOP measurements in the same awake mice over a single 24-hour period. The experiment determined whether there was any obvious difference in the 24-IOP profiles or time of peak and trough IOPs.
among genotypes. The 24-hour IOP profile revealed no obvious phase-shift in the 24-hour IOP profile among genotypes (Fig. 2). For FP-knockout and wild-type mice, IOP was lower in the morning after onset of the light phase and increased after 6 PM with onset of the dark phase. No obvious difference was seen in the shape of the IOP profile between wild-type and homozygous FP-knockout mice.

Peak and Trough IOP
To determine the magnitude of IOP variation, peak and trough IOPs were measured between 10 AM and 12 PM and 8 and 10 PM by the cannulation method. The mean difference between trough and peak IOP was 1.8, 2.66, and 2.71 mm Hg in wild-type, heterozygous, and homozygous FP-knockout mice, respectively (Fig. 3). There was no significant difference ($P > 0.05$, ANOVA) in trough or peak IOP between genotypes. Similarly, there was no statistically significant difference in mean IOP change (peak - trough) between genotypes ($P > 0.05$, ANOVA).

Limits of Detection
The limit of detectable differences was calculated from the SD of mean IOP and the number of mice for right eye IOP measurements (StatMate; GraphPad Software Inc., San Diego, CA). For baseline IOP measurements, the study had an 80% power of detecting a minimum difference in IOP of 1.5 mm Hg between wild-type and homozygous FP-knockout mice and a minimum difference of 1.4 mm Hg between heterozygous and homozygous FP-knockout mice. For trough and peak measurements, the study had an 80% power to detect a minimum IOP difference between wild-type and homozygous mice of 2.3 mm Hg for trough IOP measurements and 2.8 mm Hg for peak IOP measurements.

DISCUSSION
These data demonstrate that there was no detectable difference in 24-hour IOP measurement between FP receptor-knockout and wild-type mice with intact FP receptors. Mice of both genotypes had lower IOPs in the early light phase and higher IOP in the early dark phase. These changes are similar to those reported previously in NIH Swiss White mice. The data indicate that an intact FP receptor is not critical for normal-appearing 24-hour IOP regulation in the mouse eye.

The human and mouse eye share several similarities with respect to aqueous humor dynamics. Both species have well-
defined trabecular and uveoscleral outflow pathways, and aqueous humor turnover is ~2.5% for both species. An important difference is the larger proportion of total outflow that passes via the uveoscleral outflow pathway in the mouse eye. Recent calculations based on the measurement of total outflow facility, episcleral venous pressure (EVP) and aqueous flow using the Goldmann equation indicated that ~80% of aqueous humor outflow passes through the uveoscleral pathway in the mouse. It is not known whether differences in the proportion of uveoscleral flow between mice and humans influences the relative contribution of the FP receptor to 24-hour IOP variation. We have previously demonstrated that prostaglandin FP-knockout mice have normal anterior segment anatomy and do not respond to topical PGF2α analogues. In comparison, wild-type mice responded to topical prostaglandins with a significant decline in IOP, indicating that FP receptor signaling lowers IOP in the mouse. We recently reported baseline (midafternoon) IOPs in a smaller cohort of FP-knockout mice. These data suggested a trend for increased IOP in FP-knockout mice. These data demonstrated previously that the IOP is minimally affected by prostaglandin FP receptor knockout mice. The magnitude of 24-hour IOP variation was greater, however, for both genotypes when IOP was measured with the rebound tonometer compared with IOP variation as measured by cannulation. This result is most likely a reflection of the reduced accuracy of our rebound tonometer for measuring higher IOPs, perhaps due to the curvilinear calibration curve obtained for both NIH Swiss White mice and the C57BL/6 wild-type mice used in this study. In consideration of this, we elected to measure peak and trough values by using the cannulation method. The disadvantage of this approach was that general anesthesia was required and longitudinal IOP measurement was not possible. A minimum of 1 week’s separation between consecutive IOP measurements was used to permit sealing of the corneal wound and resolution of any potential inflammation that may have occurred. It has been demonstrated previously that the IOP is minimally affected by ketamine-xylazine anesthesia in the first 8 minutes after intraperitoneal injection of anesthetic. All cannulation IOP measurements in this study were performed within 7 minutes of anesthetic administration. Another source of the greater variability of the rebound tonometer is that gentle restraint is required to perform rebound tonometry in the awake mouse. Therefore, it is possible that variable stress or a Valsalva-induced IOP increase occurred during IOP measurement. We believe that a Valsalva effect is unlikely, however, since the IOPs obtained by rebound tonometry were not consistently higher during the 24-hour period. In addition, evaluation of different restraint techniques performed before this study indicated that manual restraint induced less IOP elevation compared with a Decapicone (Braintree Scientific, Braintree, MA) or a custom-built restraint device. Despite the increase in IOP range, rebound tonometry performed longitudinally over a single 24-hour period demonstrated that there was no difference in the shape of the 24-hour IOP profile between wild-type and knockout mice.

In the present study, mice lacking an intact FP receptor did not have significantly different IOP or 24-hour IOP variation than did wild-type mice. An intact FP receptor therefore does not appear to be essential for a normal-appearing 24-hour IOP variation in the mouse eye. Further studies may clarify whether this reflects a lack of involvement of the FP receptor, or the presence of a compensatory mechanism that maintains IOP variation when the FP receptor is absent.

References