Diurnal and Nocturnal Variations in Aqueous Humor Dynamics of Patients With Ocular Hypertension Undergoing Medical Therapy

Vikas Gulati, MD; Shan Fan, MD; Min Zhao, MD; Matthew A. Maslonka, BS; Chiraag Gangahar, MD; Carol B. Toris, PhD

Objective: To evaluate the interaction of intraocular pressure (IOP)–lowering medications with physiologic day and night changes in aqueous humor dynamics in participants with ocular hypertension.

Methods: Thirty participants were enrolled in this double-masked, randomized, crossover study. Each participant underwent aqueous humor dynamics measurements at baseline and at 2 weeks of dosing in random order with latanoprost in the evening and placebo in the morning, timolol maleate twice daily, and dorzolamide hydrochloride twice daily. Measurements included central corneal thickness by ultrasound pachymetry, anterior chamber depth by A-scan, seated and habitual IOP by pneumatonometry, blood pressure by sphygmomanometry, episcleral venous pressure by venomanometry, and aqueous flow by fluorophotometry. Outflow facility was assessed by fluorophotometry and by tonography. Uveoscleral outflow was mathematically calculated using the Goldmann equation.

Results: Latanoprost use significantly decreased IOP during the day and night. It increased daytime uveoscleral outflow by a mean (SD) of 0.90 (1.46) µL/min (P = .048), but a nighttime increase of 0.26 (1.10) µL/min (P = .47) did not reach statistical significance. Timolol use decreased IOP during the day by reducing aqueous flow by 25%. Dorzolamide use lowered IOP only at the noon measurement and reduced daytime aqueous flow by 16%. Neither dorzolamide nor timolol use added to the physiologic 47% reduction in nighttime aqueous flow.

Conclusions: The daytime IOP-lowering effects of latanoprost are mediated by an increase in uveoscleral outflow, and those of timolol and dorzolamide are mediated by aqueous flow suppression. Nighttime physiologic changes in uveoscleral outflow limit the nighttime pharmacodynamic efficacy of latanoprost. Aqueous flow suppression with timolol and dorzolamide was ineffective in obtaining IOP lowering at night.

Trial Registration: clinicaltrials.gov Identifier: NCT00572936


Elevated intraocular pressure (IOP) is the primary risk factor for glaucomatous optic neuropathy and visual field damage. Lowering of IOP by medical or surgical means remains the only proven measure to slow the progression of glaucomatous damage. Most prescribed drugs lower IOP by decreasing aqueous flow (eg, β-blockers and carbonic anhydrase inhibitors), improving the outflow of aqueous humor (eg, prostaglandin analogues and cholinergic agents), or both (α2-adrenergic agonists).

The variables of aqueous humor dynamics undergo physiologic changes in a 24-hour period in people with ocular normotension and ocular hypertension. The nocturnal suppression of aqueous humor production has been well documented. Some evidence supports nighttime changes in outflow facility and uveoscleral outflow (Fu). The pharmacologic actions of drugs and the physiologic responses of the body may enhance or counteract each other, thereby affecting the pharmacodynamic efficacy of a drug throughout a 24-hour period. The daytime effects on IOP and aqueous humor dynamics of β-blockers, carboxic anhydrase inhibitors, and prostaglandin analogues have been carefully studied in volunteers with ocular normotension and hypertension, but nighttime effects have not been as extensively inves-
tigated. There are studies of nighttime drug effects on IOP and aqueous flow in control subjects, but there remains little information on the nighttime effects of these drugs on outflow in patients with elevated IOP. This study evaluated the effects of a prostaglandin analogue, a β-blocker, and a carbonic anhydrase inhibitor on daytime and nighttime IOP and variables of aqueous humor dynamics in individuals with ocular hypertension. Daytime and nighttime aqueous humor dynamics were measured in the same participants treated with each drug in a masked crossover manner, enabling a direct comparison while minimizing the variability due to sampling differences.

METHODS

This study of 30 participants with ocular hypertension had a prospective, double-masked, randomized, multiple-crossover design. The exclusion criteria included a history of ocular inflammation; glaucomatous visual field defects; a history of ocular trauma or infection within 6 months before enrollment; abnormalities that prevent reliable IOP or fluorophotometric readings; anterior chamber angles of less than a grade of 2 on gonioscopic examination; a cup-disc ratio greater than or equal to 0.8; a history of intolerance to topical fluorescein, timolol, latanoprost, or dorzolamide; a history of severe or unstable cardiovascular, pulmonary, hepatic, or renal disease; inability to discontinue contact lens wear during the study visits; and a history of ocular surgical procedures. Ocular hypertension was defined as documentation of IOPs of 21 mm Hg or greater in both eyes on 2 separate occasions at least 3 months apart with no glaucomatous optic nerve or visual field defects. Participants with ocular hypertension rather than glaucoma (with optic nerve or visual field damage) were sought for the study because of the ethical concern with the required prolonged (18 weeks total) periods without ocular medication use. This study was approved by the institutional review board of the University of Nebraska Medical Center. Informed consent was signed by all participants.

All 30 participants underwent a baseline daytime visit and a baseline nighttime visit 2 days later. The 2 days of rest between visits gave the participants’ eyes time to recover before initiating the numerous nighttime measurements. At the completion of the nighttime visit, participants started self-administering 1 of the masked randomly assigned study drugs, which included placebo in the morning and latanoprost, 0.004%, in the evening or timolol maleate, 0.5% (Falcon Pharmaceuticals), twice daily or dorzolamide hydrochloride, 2% (Falcon Pharmaceuticals), twice daily. The drops were administered at 9 AM and 9 PM each day. On the day of the aqueous humor dynamics measurements, the morning dose was administered after the 9 AM IOP measurement. Duration of treatment with each study drug was set at 2 weeks, which was considered adequate to obtain the full effect of the study drugs on aqueous humor dynamics. Two weeks later, participants underwent repeated daytime and nighttime study visits. At the end of the nighttime visit with the first drug, participants were instructed to discontinue using the study drug and to start the 6-week washout period, during which time IOP was monitored as before. After 6 weeks of washout, participants started taking the second randomly selected study drug and used it for 2 weeks before the next nighttime and daytime measurements. The same procedure was repeated for the third study drug.

The study procedures at each daytime and nighttime visit are summarized in Table 1. Between 10 PM and 4 AM on the night before each daytime visit, participants instilled 1 drop of 2% fluorescein into each eye at 5-minute intervals for a total of 6 to 8 drops. On the morning of the daytime visit, central corneal thickness and anterior chamber depth were measured by ultrasound pachymetry and A-scan (PacScan series 300; Sonomed, Inc), respectively. From these measurements, the anterior chamber volume was calculated for each eye. The IOP was measured using a pneumotonometer (Model 30 Classic; Reichert Technologies). Four sets of duplicate fluorophotometric scans of the cornea and anterior chamber were collected using a scanning ocular fluorophotometer (Fluorotron Master; OcuMetrics, Inc) (Table 1). These values were used to calculate baseline aqueous flow (the method is described in detail elsewhere). With the participant in the seated position, episcleral venous pressure (P_e) was measured using an episcleral venomanometer (Eyetech Ltd) during the morning of the daytime visit. Two measurements were made per eye and averaged. If the difference between the 2 measurements was more than 2 mm Hg, a third measurement was taken. Except for tonography, all the daytime visit measurements were made with the participant in the seated position.

Table 1. Schedule of study visits.

Visit 1: Screening
Visit 2: Daytime
Visit 3: Nighttime
Visit 4: Daytime
Visit 5: Nighttime
Visit 6: Daytime
Visit 7: Nighttime
Visit 8: Daytime
Visit 9: Nighttime
Table 1. Study Schedule and Procedures*

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Days 0-1, Daytime Visit</th>
<th>Day 3, Nighttime Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instill fluorescein</td>
<td>6-8 drops starting between 10 PM and 4 AM</td>
<td>3-4 drops starting at 5 PM</td>
</tr>
<tr>
<td>Pachymetry</td>
<td>9 AM</td>
<td>2 AM</td>
</tr>
<tr>
<td>IOP (multiple readings)</td>
<td>9 AM to 2 PM</td>
<td>10 AM to 5 AM</td>
</tr>
<tr>
<td>Seated</td>
<td>None</td>
<td>10 AM to 5 PM</td>
</tr>
<tr>
<td>Supine</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Episceral venous pressure</td>
<td>10 AM</td>
<td></td>
</tr>
<tr>
<td>Fluorophotometry</td>
<td>9 AM to 11:30 AM</td>
<td>10 AM to 2 AM</td>
</tr>
<tr>
<td>Aqueous flow</td>
<td>12:30 PM to 2 PM</td>
<td>None</td>
</tr>
<tr>
<td>Outflow facility</td>
<td>11:30 AM</td>
<td>2 AM</td>
</tr>
<tr>
<td>Sphygmonanometry</td>
<td>2 PM</td>
<td>5 AM</td>
</tr>
</tbody>
</table>

Abbreviation: IOP, intraocular pressure.

*a No correction was applied for participants’ individual daily schedules.

After the fourth set of scans, participants received oral acetazolamide (two 250-mg tablets). Because acetazolamide has been shown to reduce IOP by reducing aqueous flow,29,30,31 without affecting outflow variables,29,30 it was used to facilitate the calculation of fluorophotometric outflow facility as the ratio of the change in aqueous flow to the change in IOP induced by the aqueous flow suppressant.

After the administration of acetazolamide, fluorophotometric outflow facility was calculated 3 times at 45-minute intervals. Values were averaged and reported as fluorophotometric outflow facility. Seated systolic and diastolic blood pressures were measured by sphygmomanometer at approximately 11:30 AM.

At the end of the study visit, supine 2-minute tonography was performed using the tonography setting on the pneumotonometer. Data were imported into a spreadsheet program (Excel; Microsoft Corp), and a regression line of all points fitted to the data was used to obtain the tonographic outflow facility incorporated all data points recorded during the 2 minutes rather than just the initial and final recorded IOPs.

On the day of the nighttime visit, at 5 PM, 1 drop of proparacaine hydrochloride followed by 3 to 4 drops of fluorescein were instilled in each eye. The nighttime measurements were made in a hospital-based private hotel room. Fluorophotometric scans and IOP measurements were performed throughout the night at the times listed in Table 1. The IOPs were taken in dim lighting with the participant in the supine position followed by the seated position. Participants were instructed to return to sleep between measurements. Blood pressure and central corneal thickness were measured at 2 AM. Based on our past experience with aqueous humor suppressants failing to lower IOP at night, nighttime measurements of fluorophotometric outflow facility were not attempted.13 The last measurement was 2-minute tonography at 5 AM.

Nighttime P_{oc} was not measured but was calculated as 2 mm Hg less than the individual daytime value for the seated position and 1.5 mm Hg higher than the daytime value for the supine position. These values are based on 2 previous studies29,31 reporting the diurnal and positional variation of P_{oc}.

The P_{oc} was calculated using the following formula: F_{oc} = F_{a} - C (IOP - P_{ev}), where F_{a} indicates aqueous flow; and C, outflow facility. The daytime P_{oc} was calculated with P_{ev} measured during the daytime visit and C determined by either the acetazolamide method or tonography. Nighttime F_{oc} was calculated using fluorophotographic C, supine IOPs at 2 AM, and nighttime calculated supine P_{ev}.

STATISTICAL ANALYSIS

Mean values of each variable for the 2 eyes were used for the analysis. In cases in which measurements could be obtained for 1 eye only, that eye was used for the analysis. Normality of data distribution for each variable was checked using the Kolmogorov-Smirnov test. Parametric (analysis of variance [ANOVA]) with the Tukey post hoc test and nonparametric (Kruskal-Wallis test with Dunn post hoc comparisons) tests were used for comparison of means between different groups as appropriate. Measurements were compared between daytime and nighttime using paired tests (paired t test or Wilcoxon rank sum test). \( P \leq .05 \) was considered statistically significant. Unless otherwise indicated, values are reported as mean (SD).

RESULTS

Thirty-two patients with ocular hypertension were screened for this study. One patient who was taking latanoprost for ocular hypertension did not have an IOP rise greater than 20 mm Hg after discontinuing treatment for up to 12 weeks. A second patient who initially expressed interest chose to withdraw from the study. Of the 30 participants enrolled in the study, 17 were not taking topical drops, 7 were using a prostaglandin analogue in both eyes, 4 were using a prostaglandin analogue in 1 eye, and 2 were using timolol in both eyes. All 30 participants underwent baseline daytime and nighttime measurements. After the baseline visit, 1 participant was withdrawn from the study owing to a possible mild allergic response to acetazolamide, and a second participant opted out with reports of backache. One participant had a motor vehicle crash after completing the daytime and nighttime visits with the first drug and had to withdraw. Three participants had an IOP increase to unacceptable levels (as determined by the treating physician) during the washout period and were withdrawn from the study. One of these participants had an excessive rise in IOP between visits 5 and 6 (after completing visits with 1 study drug), and 2 had excessive increases in IOP between visits 7 and 8 (after completing visits with 2 study drugs). Overall, 24 participants successfully completed all the study visits. Participants completing daytime and nighttime measurements with individual drugs were 28 with latanoprost, 24 with timolol, and 26 with dorzolamide.

The mean (SD) age of the 30 study participants was 59.3 (11.0) years. There were 9 men and 21 women. Regarding race, 22 participants were white, 6 were African American, 1 was Hispanic, and 1 was American Indian.

INTRAOCULAR PRESSURE

Figure 2 shows the effects on IOP of the 3 study drugs. Latanoprost and timolol significantly lowered the mean (SD) IOP (17.6 [3.7] mm Hg and 16.4 [2.3] mm Hg, respectively) compared with the baseline value of 21.6 (5.0) mm Hg (\( P < .05 \), by ANOVA with the Tukey post hoc test). The mean (SD) 20.2 (4.8) mm Hg IOP with dorzolamide was not statistically significantly lower than
the baseline value at 9 AM (12 hours after administration of the last dose). The mean (SD) noon IOPs (17.0 [3.2] mm Hg with latanoprost, 17.1 [2.5] mm Hg with timolol, and 17.6 [2.4] mm Hg with dorzolamide) were significantly decreased compared with the baseline value (20.9 [4.2] mm Hg) (P < .05 for all). During the nighttime, latanoprost significantly lowered the seated (10 PM and midnight) and supine (10 PM and 2 AM) IOPs compared with baseline (P < .05 for all, by ANOVA with the Tukey post hoc test). The mean reduction in IOP at night (16% seated and 10% supine) with latanoprost was less than the reduction obtained during the day (19%). Neither timolol nor dorzolamide lowered the seated or supine IOP compared with baseline at any of the nighttime measurements.

**BIOMETRY AND BLOOD PRESSURE**

No study drug affected central corneal thickness during the day or night (P > .90 for all, by ANOVA). Values of central corneal thickness at night were higher compared with daytime values (P < .01 in all groups, by paired t test). Anterior chamber volume at 2 weeks of treatment with any of the study drugs was comparable with baseline (P = .84, by ANOVA). The P<sub>E</sub><sub>1</sub>, measured during the day only, showed no change with any of the study drugs (P = .84, by ANOVA). None of the study drugs had any effect on the daytime or nighttime seated systolic or diastolic blood pressure (P > .30 for all, by ANOVA). No day and night differences in blood pressure were found at baseline or with any study drug. These data are summarized in Table 2.

**AQUEOUS FLOW**

Aqueous flow data at all visits were found to be normally distributed (baseline mean [SD], 2.09 [0.71] µL/min) (Figure 3). Using ANOVA for comparison of all daytime values, P = .006. Post hoc group comparisons were performed using the Tukey test. Timolol achieved a significant 25% reduction in daytime aqueous flow (mean [SD], 1.57 [0.44] µL/min; P < .01). The 16% reduction in daytime aqueous flow caused by dorzolamide (mean [SD], 1.75 [0.54] µL/min) reached significance when using...
Glaucoma medications lower IOP by altering the variables of aqueous humor dynamics. These effects are confounded by the spontaneous 24-hour changes in normotonic outflow facility, which was not measured in this study. Therefore, future studies should focus on the effects of these drugs on the nocturnal outflow facility.

**Table 2. CCT, ACV, Pev, and BP at Baseline and After Treatment With 3 Study Medications**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline (n = 30)</th>
<th>Latanoprost (n = 28)</th>
<th>Timolol Maleate (n = 24)</th>
<th>Dorzolamide Hydrochloride (n = 26)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
<td>Night</td>
<td>Day</td>
<td>Night</td>
<td>Day</td>
</tr>
<tr>
<td>CCT, µm</td>
<td>570 (39)</td>
<td>585 (46)</td>
<td>564 (40)</td>
<td>585 (46)</td>
<td>.93</td>
</tr>
<tr>
<td>ACV, µL</td>
<td>198 (37)</td>
<td>ND</td>
<td>191 (45)</td>
<td>ND</td>
<td>.84</td>
</tr>
<tr>
<td>Pev, mm Hg</td>
<td>9.3 (1.1)</td>
<td>ND</td>
<td>9.4 (1.4)</td>
<td>ND</td>
<td>.84</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>140 (17)</td>
<td>142 (14)</td>
<td>136 (20)</td>
<td>143 (17)</td>
<td>.96</td>
</tr>
<tr>
<td>Diastolic</td>
<td>82 (10)</td>
<td>84 (12)</td>
<td>78 (12)</td>
<td>83 (11)</td>
<td>.96</td>
</tr>
</tbody>
</table>

Abbreviations: ACV, anterior chamber volume; BP, blood pressure; CCT, central corneal thickness; ND, not done; Pev, episcleral venous pressure.

**Figure 3.** Daytime and nighttime aqueous flow in participants with ocular hypertension at baseline and after 2 weeks of treatment with latanoprost, timolol maleate, or dorzolamide hydrochloride. Using analysis of variance, the difference between baseline and timolol treatment was statistically significant. Daytime changes in aqueous flow with dorzolamide were significant (P = .03 by paired t test). Error bars represent 1 SEM.

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Abbreviations: ACV, anterior chamber volume; BP, blood pressure; CCT, central corneal thickness; ND, not done; Pev, episcleral venous pressure.

**UVEOSCLERAL OUTFLOW**

Tonographic Fu data were normally distributed, whereas fluorophotometric data were not. These variables were treated as ordinal variables, and nonparametric tests were used for comparison of means. Mean (SD) baseline daytime F was calculated to be −0.09 (1.40) µL/min by fluorophotometry and −0.59 (1.67) µL/min by tonography. Detailed calculations of F were performed for each subject and drug, and a discussion of the results have been described in a recent article. Mathematically calculated F can vary significantly depending on the Pev used for calculation; however, the calculated changes in F were found to be robust and vary little irrespective of the value of Pev used in the calculations.

The drug-induced changes in tonographic F data are given in Table 4. At baseline, F by tonography showed a significant reduction at nighttime (by 0.61 µL/min) compared with at daytime. This difference was seen irrespective of the method used to calculate the nighttime F values (seated: P = .04, by Wilcoxon test; or supine: P = .03, by Wilcoxon test). Fluorophotometric calculation of F was not performed at nighttime. Uveoscleral outflow calculated using daytime tonography was significantly increased with latanoprost use (P = .048, by Kruskal-Wallis test with the Dunn post hoc test). The increase in fluorophotometrically calculated F approached significance (P = .07, by Kruskal-Wallis test). Timolol and dorzolamide did not have a significant effect on either fluorophotometric or tonographic F. The nighttime values for F were significantly lower than the daytime values in all the groups (P < .01, by Wilcoxon test).

**COMMENT**

Glaucoma medications lower IOP by altering the variables of aqueous humor dynamics. These effects are confounded by the spontaneous 24-hour changes in normotonic outflow facility, which was not measured in this study. Therefore, future studies should focus on the effects of these drugs on the nocturnal outflow facility.
The magnitude of the normal decrease in aqueous humor flow at night is about twice as much as that achieved by timolol during the daytime. This explains why nocturnal reduction in aqueous flow beyond the normal decrease at night could not be achieved and why nocturnal IOP was not affected by timolol. A previous crossover IOP study using timolol, latanoprost, and dorzolamide found significant nighttime IOP lowering with dorzolamide and latanoprost but not with timolol. A drug additivity study adding timolol or brinzolamide but not with timolol. Using timolol, brimonidine tartrate, latanoprost, and dorzolamide, one study found that all the medications lowered IOP at all measurement times. However, the IOP-lowering effect of dorzolamide seen in this study may be due to the limited power to detect a significant difference rather than due to evidence against efficacy in aqueous suppression.

The magnitude of the normal decrease in aqueous humor production that occurs naturally each night was twice as much as that achieved by timolol during the daytime. This explains why nocturnal reduction in aqueous flow beyond the normal decrease at night could not be achieved and why nocturnal IOP was not affected by timolol. A previous crossover study also found significant supine IOP lowering with latanoprost but not with timolol at night. In contrast, another crossover IOP study using timolol, latanoprost, and dorzolamide found significant nighttime IOP lowering with dorzolamide and latanoprost but not with timolol. A drug additivity study adding timolol or brinzolamide to latanoprost found additional nighttime IOP lowering with dorzolamide and latanoprost but not with timolol.

When analyzed by paired t test, the study confirms that the mechanism of action of timolol and dorzolamide is mediated through suppression of aqueous humor production. The magnitude of the aqueous flow decrease was greater with timolol than with dorzolamide. When evaluated by ANOVA and post hoc testing, the aqueous humor suppression achieved by dorzolamide did not reach statistical significance owing to the inherent correction applied for multiple comparisons. Hence, a lack of statistical significance for aqueous humor suppression achieved by dorzolamide seen in this study may be due to the limited power to detect a significant difference rather than due to evidence against efficacy in aqueous suppression.

The 2-week treatment duration in this study is likely to represent the full effect of the study medications. Lack of a difference between the aqueous humor dynamics measured at 1 and 6 weeks of treatment with timolol and latanoprost has been reported previously. To our knowledge, a formal direct comparison of aqueous humor suppression with dorzolamide over days or weeks has not been reported. However, a consistent unchanged IOP response from 1 to 28 days and from 4 to 52 weeks has been reported previously. This finding suggests indirectly that aqueous humor dynamics after 2 weeks of dorzolamide therapy likely represent aqueous humor dynamics at any time during dorzolamide therapy.

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Table 3. Outflow Facility Measured by Fluorophotometry (C_ν) and Tonography (C_{tuv}) at Baseline and With the 3 Study Medications

<table>
<thead>
<tr>
<th>Outflow Facility, µL/min/mm Hg</th>
<th>Baseline (n = 30)</th>
<th>Timolol Maleate (n = 26)</th>
<th>Dorzolamide Hydrochloride (n = 26)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime C_ν</td>
<td>0.20 (0.13)</td>
<td>0.23 (0.18)</td>
<td>0.23 (0.12)</td>
<td>.74</td>
</tr>
<tr>
<td>Daytime C_{tuv}</td>
<td>0.23 (0.11)</td>
<td>0.22 (0.10)</td>
<td>0.18 (0.08)</td>
<td>.38</td>
</tr>
<tr>
<td>Nighttime C_ν</td>
<td>0.20 (0.10)</td>
<td>0.21 (0.11)</td>
<td>0.17 (0.08)</td>
<td>.52</td>
</tr>
</tbody>
</table>

Table 4. Change in Uveoscleral Outflow Values When Adding 2, 4, and 6 mm Hg to Episcleral Venous Pressure in the Goldmann Equation

<table>
<thead>
<tr>
<th>Change In Episcleral Venous Pressure</th>
<th>Latanoprost (n = 28)</th>
<th>Timolol Maleate (n = 24)</th>
<th>Dorzolamide Hydrochloride (n = 26)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured P_{env} + 2 mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime F_{ufl}</td>
<td>0.43 (1.64)</td>
<td>-0.16 (1.13)</td>
<td>0.14 (1.21)</td>
<td>.07</td>
</tr>
<tr>
<td>Daytime F_{tuv}</td>
<td>0.90 (1.46)</td>
<td>0.70 (1.52)</td>
<td>0.58 (1.62)</td>
<td>.04</td>
</tr>
<tr>
<td>Nighttime F_{tuv}</td>
<td>0.26 (1.10)</td>
<td>0.50 (1.38)</td>
<td>0.15 (1.62)</td>
<td>.47</td>
</tr>
<tr>
<td>Measured P_{env} + 4 mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime F_{tuv}</td>
<td>0.71 (1.44)</td>
<td>0.12 (1.00)</td>
<td>0.36 (1.12)</td>
<td>.008</td>
</tr>
<tr>
<td>Daytime F_{tuv}</td>
<td>0.91 (1.34)</td>
<td>0.62 (1.35)</td>
<td>0.53 (1.51)</td>
<td>.02</td>
</tr>
<tr>
<td>Nighttime F_{tuv}</td>
<td>0.28 (0.98)</td>
<td>0.42 (1.19)</td>
<td>0.12 (1.45)</td>
<td>.41</td>
</tr>
<tr>
<td>Measured P_{env} + 6 mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daytime F_{tuv}</td>
<td>0.98 (1.31)</td>
<td>0.41 (0.94)</td>
<td>0.58 (1.07)</td>
<td>.006</td>
</tr>
<tr>
<td>Daytime F_{tuv}</td>
<td>0.92 (1.24)</td>
<td>0.54 (1.20)</td>
<td>0.48 (1.41)</td>
<td>.001</td>
</tr>
<tr>
<td>Nighttime F_{tuv}</td>
<td>0.31 (0.88)</td>
<td>0.35 (1.01)</td>
<td>0.10 (1.30)</td>
<td>.36</td>
</tr>
</tbody>
</table>

Abbreviations: B, baseline; F_{ufl}, fluorophotometric uveoscleral outflow; F_{tuv}, tonographic uveoscleral outflow; P_{env}, episcleral venous pressure.

Data are given as mean (SD).

Data are given as mean (SD) microliter per minute change in Fu from baseline.

By Kruskal-Wallis test.

Significant differences found with the Dunn test.

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timolol was found to be less during the night and that of dorzolamide was found to be less during the day. All the published studies showing a nighttime effect of topical carbonic anhydrase inhibitors used thrice-daily dosing compared with twice-daily dosing in the present study. The thrice-daily dosing of carbonic anhydrase inhibitors may lead to higher steady-state levels and nighttime IOP lowering that are not accomplished with twice-daily dosing. In clinical practice, topical carbonic anhydrase inhibitors are used at twice-daily and thrice-daily dosing. In the present study, twice-daily dosing was chosen to minimize the number of eye drops and maintain the double-masked nature of the study.

Some studies have reported nocturnal IOP lowering with timolol when used alone or as a fixed combination with either a carbonic anhydrase inhibitor or a prostaglandin analogue. Other studies, including the present one, have found a lack of an IOP-lowering effect with a β-blocker during the night.

Although no nighttime suppression of aqueous humor production was found with either timolol or dorzolamide in the present study, the drugs were different in terms of control of the 9 AM IOP, before administration of the next dose. Compared with baseline, timolol produced a significant lowering of 9 AM IOP, whereas dorzolamide did not. This likely is due to the longer half-life of timolol whereby the drug, although ineffective over-night, is available 12 hours after the last administration to have an effect on the morning IOP, likely by suppressing aqueous humor production as it starts to rise to daytime values. Such an effect was not seen with dorzolamide as it is likely eliminated before the morning surge in aqueous humor production.

Similar to the present findings, other studies have reported the nighttime IOP-lowering effect of prostaglandin analogues to be approximately half of the daytime effect. Based on the present study results, the IOP-lowering effect of latanoprost was mediated by its effects on F_u during the day and possibly during the night as well. Some previous studies also found effects of latanoprost on outflow facility. These differences may be related to the selection of participants (those with lower initial outflow facility values may show greater improvement), the duration of therapy, or both. A study of young Japanese adults given latanoprost or placebo twice daily for 3 days found daytime and nighttime increases in calculated F_u with latanoprost. However, unlike the present study, the Japanese study reported higher values of F_u at nighttime compared with daytime and a statistically significant increase in aqueous flow at night with latanoprost use compared with the contralateral control eye. The daytime and nighttime measurements were made in different participants in the Japanese study.

For the purpose of calculating F_u using the Goldmann equation, investigators have used measured, standard, and multiple values of assumed P_e. In addition to using the measured value, we provide offsets of 2, 4, and 6 mm Hg (Table 4). It was assumed that in all the participants, the true hydrostatic pressure downstream from the trabecular meshwork is offset from the measured P_e by a similar amount in all participants and is not altered by any of the study drugs. Participants taking latanoprost had a statistically significant increase in daytime F_u regardless of how it was calculated. Therefore, the daytime IOP-lowering effects of latanoprost can be explained by improvements in F_u. With tonographic outflow facility in the calculation, F_u increased by 0.9 µL/min. The spontaneous nighttime physiologic reduction in F_u (by tonography) was approximately 0.6 µL/min. The mean nighttime change in F_u (by tonography) with latanoprost was approximately 0.3 µL/min, which did not reach statistical significance. The numerical concordance of the previously mentioned data from this study may suggest that the potential maximal increase in F_u during the day may be partially negated by the spontaneous decrease in F_u at night. According to the Goldmann equation, a change in F_u of 0.3 µL/min adequately explains an IOP change of 1.5 mm Hg, assuming no change in any other variables of aqueous humor dynamics. The present data showed a mean nighttime lowering of habitual IOP by 2.2 mm Hg and that of seated IOP by 2.3 mm Hg. To fully explain a 2.2-mm Hg decrease in IOP, a 0.44-µL/min increase in F_u would be needed. The change in F_u at night in this study was slightly lower than this value. This study was not sufficiently powered to detect a statistically significant difference of this magnitude. A study designed to detect a difference of 0.3 µL/min with the baseline means and standard deviations seen in this study at a power of 80% would require a sample size greater than 200. Since neither the aqueous flow nor the outflow facility changed with latanoprost use at nighttime, an increase in F_u is the most likely explanation for the significant IOP decrease at night with latanoprost.

Nocturnal measurements made in this study required awakening the volunteers several times during the night to collect the data. This is likely a significant departure from the participants’ normal nocturnal schedule and has the potential to affect the measurements thus obtained. However, this disturbance was unavoidable owing to the limitations of the currently available techniques. Our understanding of aqueous humor dynamics at night will improve when new methods become available that can make all measurements under the closed eyelid of the sleeping individual.

In conclusion, significant nocturnal physiologic changes in aqueous humor dynamics affect the efficacy of commonly used glaucoma drugs. Newer drugs are still needed that can control IOP better at night. Drugs that could facilitate nocturnal aqueous humor suppression would reduce nocturnal IOP, but this mechanism of action is not desirable because aqueous humor and its flow serve important physiologic functions. Drugs that could improve outflow facility or negate the spontaneous reduction in F_u during the night can be potential candidates for IOP lowering at night. Future studies are necessary to better understand the mediating pathways for nocturnal changes and potentially use these for more efficient IOP lowering and control of glaucoma.

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REFERENCES


