Increase in Outflow Facility With Unoprostone Treatment in Ocular Hypertensive Patients

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Objective: To determine the mechanism by which 0.15% unoprostone isopropyl reduces intraocular pressure (IOP) by studying 33 patients with ocular hypertension or primary open-angle glaucoma.

Methods: At baseline, IOP was determined by pneumatonometry, aqueous flow and outflow facility by fluorophotometry, episcleral venous pressure by venomanometry, and uveoscleral outflow by mathematical calculation. Unoprostone was administered to one eye and placebo to the fellow eye of each patient twice daily in a randomized masked fashion. In patients who demonstrated an IOP reduction of 3 mm Hg or more in either eye on day 5±1 (n=29), determinations were repeated on that day and on day 28±2. Treated eyes were compared with control eyes, and treatment days were compared with baseline by paired t tests.

Results: Compared with baseline, unoprostone significantly (P<.001) reduced IOP by a mean±SEM of 5.6±0.4 mm Hg and 4.8±0.6 mm Hg on days 5 and 28, respectively. The change from baseline with unoprostone was significantly (P<.001) greater than with placebo by 2.8±0.4 mm Hg on day 5 and by 3.2±0.5 mm Hg on day 28. Compared with baseline, unoprostone significantly (P≤.001) increased outflow facility by 0.05±0.01 and 0.08±0.02 µL·min⁻¹·mm Hg⁻¹ on days 5 and 28, respectively. The baseline-adjusted between-treatment differences were significant (P=.04) on day 28 (0.06±0.02 µL·min⁻¹·mm Hg⁻¹). Other measures were not different from placebo.

Conclusion: In responsive patients, unoprostone decreased IOP by increasing outflow facility.

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INTRAOCULAR PRESSURE (IOP) IS maintained by the production of aqueous humor and its drainage through the anterior chamber angle. Current glaucoma therapies lower IOP by reducing aqueous humor production, increasing outflow through the uveoscleral pathway, or increasing the facility of trabecular outflow. Some medications, such as brimonidine tartrate,¹ have been shown to have multiple mechanisms of action. If target IOP is not reached after an appropriate period of monotherapy, combination treatments are used to achieve the desired IOP-lowering effect, especially combinations of drugs with differing modes of action.² An understanding of the IOP-lowering mechanism of action of each glaucoma medication would help predict additivity between drugs.

Unoprostone isopropyl is a structural analogue of prostaglandin (PG) F₂₅₅ and has been reported to be a docosanoid. It has been shown to be a safe and efficacious IOP-lowering drug.³⁻⁵ Unoprostone appears to lower IOP by increasing or facilitating outflow of aqueous humor. An increase in outflow facility⁶ and uveoscleral outflow⁷ has been reported after topical administration of unoprostone in rabbits. No effect on tonographic outflow facility was found in healthy humans⁸ or in patients with glaucoma,⁹ suggesting that a uveoscleral outflow effect accounted for the IOP decrease. Recently, Thieme and coworkers¹⁰ suggested that unoprostone lowers IOP by affecting aqueous outflow through the trabecular meshwork via inhibition of endothelin-dependent mechanisms.

This study was conducted to determine the effects of unoprostone on aqueous humor dynamics in patients with ocular hypertension (OHT) or primary open-angle glaucoma (POAG).

METHODS

This was a single-center, randomized, double-masked, placebo-controlled study in patients with OHT or POAG. The number of patients to enroll was determined before the start of the study by power estimates generated with nQuery Advisor Version 2.0 (Statistical Solu-
tions, Boston, Mass). A sample size of 30 subjects was needed to provide at least 75% power to detect a difference in aqueous flow of 15% between drug-treated and vehicle-treated eyes, assuming a standard deviation of 0.75 µL/min and a 2-sided significance level of .05. The study was approved by the University of Nebraska Medical Center Institutional Review Board, Omaha, and all patients provided written informed consent before initiation of any study-related assessments.

Patients were scheduled for 4 visits, consisting of screening, baseline, day 5±1 of treatment, and day 28±2 of treatment. The protocol is summarized in Table 1.

At visit 1 (screening), a medical history was collected from each patient and a complete ophthalmic examination was performed. Main inclusion criteria at visit 1 included diagnosis of open angle glaucoma (POAG) or OHT, before the baseline visit (visit 2), subjects who had been taking medication to treat the elevated IOP discontinued medication. The protocol is summarized in Table 1.

### Table 1. Schedule of Visits

<table>
<thead>
<tr>
<th>Visit 1 (Day 31 to −1) Screening</th>
<th>Visit 2 (Day 0) Baseline</th>
<th>Visit 3 (Day 5 ± 1)</th>
<th>Visit 4 (Day 28 ± 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous IOP therapy is discontinued. Washout period begins. Inclusion/exclusion criteria are evaluated. Complete ophthalmic examination is given. Urine pregnancy test is performed (if applicable).</td>
<td>IOP is checked at 8 AM ± 1 h. IOP should be 23-30 mm Hg in both eyes and ≤5 mm Hg difference between eyes. Baseline aqueous humor dynamic parameters are collected. After all measurements, 1 eye is randomly assigned to receive unoprostone isopropyl, while the other receives placebo twice daily</td>
<td>IOP is checked at 8 AM ± 1 h. IOP must be reduced by 3 mm Hg vs baseline in at least 1 eye and IOP should be ≤30 mm Hg in both eyes; if not, patient exits study. Masked study medication is instilled by investigator. Aqueous humor dynamic parameters are collected. Patient continues to self-administer unoprostone and placebo twice daily.</td>
<td>IOP is checked at 8 AM ± 1 h. Masked study medication is instilled by investigator. Aqueous humor dynamic parameters are collected. Complete ophthalmic examination is given. Urine pregnancy test is repeated (if applicable).</td>
</tr>
</tbody>
</table>

Abbreviation: IOP, intraocular pressure.

Between 9 PM and 4 AM the night before visit 2, patients in the acetazolamide or timolol; IOP at intervals x=1, 2, and 3 after acetazolamide-timolol; IOP before acetazolamide-timolol administration; IOP, average of IOP values taken at the beginning and end of intervals x=1, 2, and 3; and CFl, CFl at intervals x=1, 2, and 3. The 3 calculated CFl, measurements were averaged to obtain the reported CFl values.

Uveoscleral outflow (Fu) was calculated by means of the following formula:

\[
Fu = Fa - CFl(IOP - P_e)
\]

where \( Fa \) indicates aqueous flow rate before treatment with acetazolamide or timolol; \( F_{a_e} \), aqueous flow rate at intervals x=1, 2, and 3 after acetazolamide-timolol; IOP, the IOP just before acetazolamide-timolol administration; IOP, average of IOP values taken at the beginning and end of intervals x=1, 2, and 3; and CFl, CFl at intervals x=1, 2, and 3. The calculated CFl, measurements were averaged to obtain the reported CFl values.

Safety assessments were performed and eligible patients received 2 bottles identical in appearance and labeled only by the patient's identification number and the words right eye or left eye. One bottle contained 0.15% unoprostone isopropyl (Rescula; Novartis Ophthalmics, East Hanover, NJ) and the other contained vehicle (placebo). Patients were instructed to instill 1 drop in the appropriate eye twice daily (8 AM and 8 PM) for 4 to 6 days in a double-masked, randomized fashion. Patients were asked to record on a log sheet the time of each drug instillation and any omissions or errors in treatment. The night before visit 3, fluorescein was administered as before. At visit 3 (day 3 ± 1), only patients whose morning IOP was reduced by at least 3 mm Hg from baseline in at least one eye, and did not exceed 30 mm Hg, continued in the study. If the IOP criteria were not met, this visit served as an exit visit and further investigations were not performed. The morning dose of unoprostone or placebo was administered to the appropriate eye by the investigator in the clinic, and all measurements were repeated as at visit 2. That evening, patients continued treatment with 0.15% unoprostone twice daily in one eye and placebo twice daily in the fellow eye until visit 4. The night before visit 4, fluorescein was administered to each eye by the patient as before. At visit 4, the final dose of unoprostone or placebo was administered by the investigator immediately after the first IOP measurement. All measurements were repeated as at visit 2. The efficacy variables were the change from baseline in aqueous humor flow, fluorophotometric outflow facility, uveo-
Within each treatment group, tests were made to determine whether the mean change from baseline differed significantly from zero. The change-from-baseline values were used to determine whether the 2 treatment groups were different. These analyses were performed by means of a paired t test. As there was one primary time point (day 28) compared with baseline, there were no adjustments made for multiple comparisons. All statistical tests in this trial were 2 sided, and all tests with a corresponding $P \leq .05$ were considered statistically significant. All values are reported as mean ± SEM.

Changes from baseline in brachial artery blood pressure, radial pulse, and visual acuity were tested within each treatment group by a paired $t$ test. Changes from baseline in the slitlamp examination, ocular symptoms, and ophthalmoscopy were evaluated.

**RESULTS**

Thirty-three patients were enrolled in the double-masked treatment period of this study. Twenty-nine patients completed the study. Four patients were discontinued on day 5 (visit 3) because of insufficient IOP reduction in either eye.

The average age of the patients was $57.7 \pm 2.0$ years (range, 32–84 years). Thirteen (39%) of the patients were male and 25 (76%) were white. Fifteen patients (45%) had dark-colored (black or brown) irides and 18 patients (55%) had light-colored (hazel, green, blue, or gray) irides. Mean IOP was $15.6 \pm 0.4$ mm Hg at screening, ranging from 11 to 22 mm Hg before washout (intent-to-treat data set). All patients enrolled were diagnosed as having OHT except for one patient who was diagnosed as having POAG in the right eye and OHT in the left eye

In patients who completed the study, unoprostone significantly reduced IOP at days 5 and 28 compared with baseline and with placebo (Figure 1). Mean baseline IOP values were $25.5 \pm 0.6$ mm Hg and $25.7 \pm 0.7$ mm Hg in the unoprostone-treated eyes and placebo-treated eyes, respectively. Average reduction from baseline in eyes treated with unoprostone was $5.6 \pm 0.4$ mm Hg ($P < .001$) and $4.8 \pm 0.6$ mm Hg ($P < .001$) on days 5 and 28, respectively, whereas the average reduction in the placebo-treated eyes was $2.5 \pm 0.04$ mm Hg ($P < .001$) and $1.7 \pm 0.1$ mm Hg ($P = .008$; Figure 1), respectively. The baseline-adjusted between-treatment differences were statistically significant on day 5 ($2.8 \pm 0.4$ mm Hg; $P < .001$) and on day 28 ($3.2 \pm 0.5$ mm Hg; $P < .001$).

Compared with baseline values, both unoprostone and placebo significantly decreased aqueous humor flow on day 5 of treatment but not on day 28 (Table 2). The baseline-adjusted between-treatment differences were not statistically significant at either day 5 ($0.14 \pm 0.11 \mu L/\text{min}; P = .21$) or day 28 ($0.18 \pm 0.14 \mu L/\text{min}; P = .22$).

The average changes in outflow facility from baseline values in eyes treated with unoprostone were statistically significant ($P \leq .001$) on days 5 and 28, whereas the average changes in the placebo-treated eyes were not statistically significant (Table 2, Figure 2). The baseline-adjusted between-treatment differences were statistically significant on day 28 ($0.06 \pm 0.03 \mu L \cdot \text{min}^{-1} \cdot \text{mm Hg}^{-1}; P = .04$) but not day 5.

Unoprostone and placebo did not significantly alter episcleral venous pressure (Table 2). Both unoprostone and placebo reduced uveoscleral outflow on day 28 compared with baseline ($P \leq .04$; Table 2). However, the baseline-adjusted between-treatment differences were not statistically significant at day 5 or 28.

There were no serious adverse events and no clinical concerns detected during the comprehensive ophthalmic examinations. The incidence of burning, stinging, or conjunctival hyperemia on drug instillation was more frequent with unoprostone than with placebo treatment. Most reported adverse events were mild and ocular. No patients were discontinued from the trial because of adverse events.
Table 2. Aqueous Humor Dynamics Before and After Topical Application of Unoprostone or Placebo

<table>
<thead>
<tr>
<th></th>
<th>Baseline, Mean ± SEM</th>
<th>Day 5 Mean ± SEM</th>
<th>P Value†</th>
<th>Day 28 Mean ± SEM</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Outflow facility, µL·min⁻¹·mm Hg⁻¹</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoprostone eye</td>
<td>0.09 ± 0.01</td>
<td>0.15 ± 0.02</td>
<td>.001</td>
<td>0.18 ± 0.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Placebo eye</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>.55</td>
<td>0.14 ± 0.02</td>
<td>.24</td>
</tr>
<tr>
<td><strong>Uveoscleral outflow, µL/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoprostone eye</td>
<td>1.24 ± 0.09</td>
<td>1.02 ± 0.12</td>
<td>.14</td>
<td>1.00 ± 0.11</td>
<td>.02</td>
</tr>
<tr>
<td>Placebo eye</td>
<td>1.14 ± 0.11</td>
<td>0.99 ± 0.11</td>
<td>.57</td>
<td>0.88 ± 0.10</td>
<td>.04</td>
</tr>
<tr>
<td><strong>Episcleral venous pressure, mm Hg</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoprostone eye</td>
<td>10.6 ± 0.2</td>
<td>10.7 ± 0.3</td>
<td>.85</td>
<td>10.7 ± 0.2</td>
<td>.76</td>
</tr>
<tr>
<td>Placebo eye</td>
<td>10.4 ± 0.2</td>
<td>10.6 ± 0.2</td>
<td>.43</td>
<td>10.4 ± 0.2</td>
<td>.93</td>
</tr>
<tr>
<td><strong>Aqueous humor flow, µL/min</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unoprostone eye</td>
<td>2.37 ± 0.14</td>
<td>2.08 ± 0.11</td>
<td>.007</td>
<td>2.40 ± 0.11</td>
<td>.84</td>
</tr>
<tr>
<td>Placebo eye</td>
<td>2.50 ± 0.15</td>
<td>2.07 ± 0.11</td>
<td>.001</td>
<td>2.34 ± 0.12</td>
<td>.39</td>
</tr>
</tbody>
</table>

*Placebo or 0.15% unoprostone isopropyl was applied twice daily for 28 days in patients with ocular hypertension or glaucoma. Numbers of patients varied from 27 to 29 because some values were excluded for the following reasons: failure of the fluorophotometer preventing determination of aqueous flow; insufficient reduction in intraocular pressure or aqueous flow from timolol maleate resulting in inability to calculate outflow facility and uveoscleral outflow; and value of uveoscleral outflow calculated to be more than 2 SDs away from the mean (outlier).†Compared with baseline by means of a paired t test.‡Compared with contralateral placebo-treated eye by means of a paired t test.

bits treated with 1 drop of unoprostone. In vitro findings suggested that the IOP-lowering effect of unoprostone was likely due to its effect on calcium-gated potassium channels, intracellular calcium, and some degree of smooth-muscle relaxation in the trabecular meshwork. Taken together with data from the current study, these findings suggest that one possible mechanism of the IOP-lowering effect of unoprostone is modulation of the trabecular meshwork function.

Contrary to experiments in rabbits and the current clinical study, previous investigations of 0.12% unoprostone in ocular normotensive volunteers and patients with POAG did not find an effect on outflow facility when measured by tonography. These apparent discrepancies may be due to differences in study design, method of measurement, and concentration of drug. The current study enrolled only patients who responded to treatment with at least a 3-mm Hg decrease in IOP. Unoprostone appears to work well in some individuals and not well in others (unpublished pilot data, C.B.T., G.L.Z., and C.B.C., December 1998). Enrolling only responders increased the power of the study to find differences in the aqueous humor dynamic parameters under investigation and to identify the mechanism by which the IOP was decreased. The fluorophotometric method to assess outflow facility was used, which detects differences not always found by tonography. The 0.15% unoprostone in the current study was expected to provide a greater effect on IOP than the 0.12% used in some earlier studies. All of these differences in study design made it possible to detect an increase in outflow facility with unoprostone treatment.

The PGF₂α analogues increase outflow predominantly, or at least partially, through the uveoscleral pathway. Latanoprost in normotensive and hypertensive human eyes primarily affects uveoscleral outflow, though it and other PGF₂α analogues, including bimatoprost, have been found to increase outflow facility as well. The outflow facility increase with bimatoprost was insufficient to account for the entire IOP decrease, suggesting that uveoscleral outflow also was increased, similar to the effects of latanoprost. Travoprost increases uveoscleral outflow in monkeys without affecting other parameters of aqueous humor dynamics, but its effects in humans have not been reported.

A reduction of aqueous flow in unoprostone-treated eyes occurred after 5 days of treatment, but a similar decrease was also noted in placebo-treated eyes compared...
with baseline. The effect on aqueous flow disappeared after 28 days of dosing. This suggests a possible short-term, contralateral effect of unoprostone on aqueous flow, a finding that requires confirmation. At no point during the study was the aqueous flow difference between treated and contralateral control eyes statistically significant. Therefore, a unoprostone-induced effect on aqueous flow cannot account for the IOP reduction in the treated compared with contralateral control eyes.

Unlike most other PGF$_{2\alpha}$ analogues, unoprostone did not increase uveoscleral outflow in the present study. Instead, a reduction in uveoscleral outflow was observed compared with baseline measurements. Because a reduction in uveoscleral outflow also was observed in the contralateral placebo-treated eyes, with no difference between treated and the contralateral control eyes, the effect was not considered to be clinically important. The reduction of uveoscleral outflow is more likely the indirect effect of increased outflow facility than a direct effect on uveoscleral outflow. The balance in resistance factors between the trabecular meshwork and uveoscleral pathway may have shifted in favor of the trabecular meshwork, causing a redirection of some fluid from the uveoscleral pathway into the trabecular meshwork. In other words, the fluid took the path of least resistance.

The finding that unoprostone did not affect uveoscleral outflow is contrary to published studies in humans and rabbits. It should be noted, however, that the earlier clinical study concluded an effect on uveoscleral outflow only when an effect on aqueous flow and outflow facility was not detected. Our pilot study (unpublished data), which included some nonresponders and patients with relatively low baseline IOPs, also failed to find a significant effect on aqueous flow and outflow facility, and when calculated mathematically, uveoscleral outflow remained unchanged as well. It is possible that the power of the earlier studies was insufficient to detect changes in outflow facility. The increase in uveoscleral outflow with unoprostone treatment in rabbits not found in humans might be explained by species differences in the structures of the anterior chamber angle and the unique sensitivity of the rabbit blood-aqueous barrier, especially to topical PGs. Breakdown of the blood-aqueous barrier alone can increase uveoscleral outflow. Rabbit eyes do not respond well to topical latanoprost, yet this drug has become the gold standard for IOP reduction in humans. The rabbit is a poor model for the study of PGs and aqueous humor dynamics in humans.

It is always a concern in studies of this nature that patients may have instilled some of their drops in the incorrect eye at some time during the treatment period. These errors might account for apparent contralateral effects. Each patient was informed repeatedly of the need for accurate adherence to their regimen and the need to report any errors in drug administration. All patients filled out a daily log reporting the exact times of each drop application and any problems. Patients rarely reported omissions, delays in administration of drops, or administration of the wrong drop to an eye. The drops were administered by the investigator on each day of measurements to ensure that the treatment was correct while data were being collected. It is unlikely that sufficient numbers of patients administered the drops erroneously to account for the contralateral effects.

In clinical practice, IOP-lowering drugs often are combined to achieve target IOPs. Drugs that increase facility of outflow might be used in combination therapy with IOP-lowering drugs that inhibit inflow. Unoprostone and timolol (an aqueous flow suppressant) have been found to be additive in several clinical trials. On the other hand, combination therapy of unoprostone with drugs that increase aqueous humor outflow may or may not be effective. There is no apparent additivity of unoprostone with latanoprost.

In conclusion, unoprostone significantly reduced IOP in patients with ocular hypertension by increasing the facility of outflow through the trabecular meshwork. Unoprostone was safe and well tolerated, and may be a suitable adjunct drug to aqueous flow suppressants.

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