Aqueous Humor Flow in Normal Human Eyes Treated With Brimonidine and Timolol, Alone and in Combination

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Objective: To compare the effect on aqueous humor flow and intraocular pressure (IOP) of topically applied 0.2% brimonidine tartrate with topically administered 0.5% timolol maleate, alone and in combination.

Design: A randomized, double-masked, placebo-controlled study of 20 human subjects was carried out. The topical drugs were instilled twice daily the day before and again on the morning of the day of the measurements. Aqueous humor flow was measured by clearance of topically applied fluorescein with a fluorophotometer, and IOP was measured with an applation tonometer.

Results: Brimonidine reduced the aqueous humor flow by 33.1%; timolol, by 49.9%; and the combination of brimonidine and timolol, by 58.9%. Brimonidine reduced the IOP by 20.3%; timolol, by 22.9%; and the combination of brimonidine and timolol, by 34.7%.

Conclusions: Brimonidine suppressed aqueous humor flow, but not as effectively as timolol. However, the effects on the IOP of both drugs separately were comparable. The short-term effect of brimonidine was partly additive to timolol, and the combination treatment caused a further reduction in aqueous humor flow and IOP. The IOP reduction by timolol could be explained solely by aqueous humor flow reduction. Much of the IOP reduction caused by brimonidine, but not all, could be explained by suppression of the aqueous humor flow, suggesting an additional mechanism for the ocular hypotensive effect of brimonidine.

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The adrenergic agonists apraclonidine hydrochloride1-6 and brimonidine tartrate7-12 are powerful ocular hypotensive agents when applied topically. They are α-adrenergic receptor agonists, but brimonidine has a notably higher affinity for the α2-receptor.13 This selectivity and the chemical structure of the drug may provide an advantage for brimonidine over apraclonidine. The long-term use of apraclonidine has been limited by allergy and tachyphylaxis,14-18 but no major drift of reduction in the intraocular pressure (IOP) was found in patients with glaucoma or ocular hypertension treated for 1 year with brimonidine.18

Studies19-22 of apraclonidine have shown that the IOP-lowering effect can be attributed to suppression of aqueous humor flow. The ocular hypotensive effect of brimonidine has been shown to be caused primarily by reduction in aqueous humor production,13,22-25 but increased uveoscleral outflow has also been reported in rabbits26 and humans.27 Brimonidine added to therapy with β-receptor antagonists significantly lowered the IOP during a 3-month study period.27 Since β-blockers are known to reduce the aqueous humor flow,28 the question arises whether a combination of brimonidine and β-blockers can cause even a further reduction in aqueous humor production, or if the decrease in IOP can be attributed to another mechanism, such as increased uveoscleral outflow, as previously suggested.25,26 The present study measures aqueous humor flow and IOP after topical administration of brimonidine, alone and in combination with timolol maleate, to determine the efficacy of the 2 different drugs.

RESULTS

The rates of aqueous humor flow are given in Table 1. Brimonidine reduced aqueous humor flow statistically significantly compared with placebo, but less efficiently than timolol (P<.001). When timolol and brimonidine were applied in combination, aqueous humor flow was reduced...
SUBJECTS AND METHODS

Twenty healthy volunteers were enrolled in the study. There were 12 women and 8 men (mean age, 30.0 years; age range, 19-58 years). All subjects underwent a screening, including a medical and an ophthalmological history, a visual acuity measurement, a slitlamp examination,planation tomometry, and optical coherence tomography. Exclusion criteria were ocular disease, systemic disease requiring long-term medical treatment, pregnancy or lactation, inability to comply with tonometry or fluorophotometry, an IOP difference between the 2 eyes greater than 3 mm Hg, and known drug hypersensitivity. The research protocol followed the tenets of the Declaration of Helsinki and was approved by the Ethical Committee of Uppsala University, Uppsala, Sweden. Informed consent was obtained from all participants.

The study was performed in 2 parts. In part 1, the effect of 0.2% brimonidine vs placebo on eyes was studied. In part 2, 0.5% timolol was topicaly added to both eyes. Two distinct treatment regimens could thus be compared, with 20 eyes in each treatment group: (1) placebo-treated eyes, (2) brimonidine-treated eyes, (3) timolol-treated eyes, and (4) brimonidine and timolol-treated eyes. There was a washout period of at least 14 days between the parts of the study to ensure complete elimination of the drugs.

The study was randomized, double masked, and placebo controlled. The brimonidine, timolol, and placebo eyedrops were given by random assignment and were administered from identical-appearing dropper bottles labeled by subject number, sequence, and right and left eye. These sterile dropper bottles containing 0.2% brimonidine tartrate (Alphagan; Allergan, Inc, Irvine, Calif), 0.5% timolol maleate (Blocadren; Merck Sharp and Dohme Inc, Whitehouse Station, NY), or placebo (Isopto-Plain; Alcon Laboratories, Inc, Fort Worth, Tex) were prepared by the staff of the Uppsala University Hospital Pharmacy. Each part of the study was performed on 2 consecutive days. The subjects reported to the test area at 8 AM on the day before aqueous humor flow was measured. They were given approximately 20 µL of 0.2% brimonidine in one eye and approximately 20 µL of placebo in the other eye. The procedure was repeated at 5 PM. The next day, when aqueous humor flow was measured, eyedrops were reintilled at 8 AM. In part 2, brimonidine and placebo eyedrops were administered according to the same schedule as in part 1, but on every point for eyedrop instillation, approximately 20 µL of 0.5% timolol was also administered to both eyes 5 minutes after the other eyedrops, ie, timolol was also administered twice daily. Because of the risk of error with eyedrop self-administration, research personnel administered all eyedrops, except fluorescein.

At 2 AM on the day of the flow measurements, each subject instilled approximately 20 µL of 2% fluorescein into each eye 3 to 5 times, according to age, at 5-minute intervals, and then returned to sleep. The subjects reported to the research area at 8 AM and underwent measurements of the fluorescense of the cornea and the anterior chamber with a fluorophotometer (Fluorotron Master; Coherent Radiation, Palo Alto, Calif). The procedure was repeated every other hour until 4 PM. After the last fluorophotometric reading at 4 PM, the IOP was measured with a Goldmann tonometer. Tonometry was started in the right eye, then alternated between the eyes for a total of 3 readings per eye. The IOP was then recorded as the mean of the 3 measurements.

Aqueous humor flow was calculated from the clearance of fluorescein on each 2-hour interval by using the following equation: Clearance = ΔM/(C × Δt), where ΔM is the loss of mass of fluorescein in the combined cornea and anterior chamber during an interval of time (Δt) and C is the average concentration in the anterior chamber during the interval, estimated from the initial and final fluorescein and assuming a single exponential decay. Aqueous humor flow was determined from the rate of clearance of fluorescein after subtracting the presumed rate of diffusional clearance (0.23 µL/min).29 The variable apparent resistance to outflow calculated for each eye from the following relation: apparent resistance = IOP/aqueous humor flow.30

Differences in IOP, and apparent resistance between groups were analyzed using a 2-sided t test for paired samples. P<.05 was considered statistically significant. The coefficient of variation of measurements of aqueous humor flow under similar conditions as used in this experiment is approximately 23%.31 The mean±SD aqueous humor flow in daytime has been reported as 2.75±0.63 µL/min.31 A sample size of 20 in each group would provide a power of 95% for detecting a true difference of 20% between the eyes.32

The results of this study indicate that brimonidine suppresses aqueous humor formation, but not as efficiently as timolol. However, the effects on the IOP of both drugs separately were comparable. When timolol and brimonidine were applied in combination, a further reduction of aqueous humor flow and IOP was seen. The effect on the IOP was greater than expected from the reduction of flow. Calculations based on outflow pressure made the discrepancy smaller, but an added effect of brimonidine on outflow, such as uveoscleral outflow, was not excluded. Toris and coworkers32 studied patients with ocular hypertension (with baseline IOPs of 21 mm Hg) who were...
in 1972 by Bárány and colleagues,33 and later discussed by Reiss and Brubaker,34 Brubaker,35 and Lee and coworkers.35 As defined earlier in this article, apparent resistance is calculated as IOP divided by the aqueous humor flow rate. It can be useful when studying a drug and its site of action for the ocular hypotensive effect; a reduction of aqueous humor flow will yield an increase in apparent resistance, while an increased outflow of aqueous humor results in a decreased apparent resistance. In the present study, the apparent resistance was increased for brimonidine and timolol, suggesting that they both act as aqueous suppressants. However, the increase in apparent resistance for brimonidine compared with placebo was only 21.6%, while the corresponding figure for timolol was 52.0%. This difference in apparent resistance was statistically significant (P<.001), supporting the suggestion that brimonidine has some additional hypotensive effect that is not mediated through inhibition of aqueous formation but rather through enhancing aqueous outflow.25,26

In a study by Maus and colleagues,36 the effect of short-term administration of brimonidine, apraclonidine, and latanoprost on timolol-treated eyes was studied. Apraclonidine and brimonidine reduced the IOP in the timolol-treated eyes by further suppressing aqueous humor flow, while latanoprost reduced the IOP without changing the aqueous production. Calculated apparent resistance did not change statistically significantly after the addition of apraclonidine or brimonidine, but increased after the addition of latanoprost, indicating that the additional IOP reduction of brimonidine could be attributed to decreased aqueous humor production. The present study also examined the short-term administration of drugs in healthy volunteers, but we were unable to explain brimonidine’s effect on IOP exclusively by a reduction in aqueous humor since the difference in apparent resistance suggested an additional effect by increased aqueous outflow. The reduction in IOP by timolol could, however, be explained solely by suppression of aqueous humor flow.

Schadlu and coworkers22 measured aqueous humor flow after short-term administration of apraclonidine and brimonidine in healthy volunteers. They found that both drugs suppressed aqueous humor formation sufficiently to account for the respective drug’s ability to lower the IOP. They also found a consensual effect on aqueous humor flow in the fellow eye: 16% for apraclonidine and 17% for brimonidine. The total reduction by

![Table 1. Aqueous Humor Flow From 8 AM to 4 PM*](image1)

<table>
<thead>
<tr>
<th>Drug Applied</th>
<th>Aqueous Humor Flow, µL/min†</th>
<th>Reduction in Aqueous Humor Flow (vs Placebo), %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>3.02 ± 0.53</td>
<td>. . .</td>
<td>.</td>
</tr>
<tr>
<td>Brimonidine tartrate</td>
<td>2.02 ± 0.41</td>
<td>33.1 &lt;.001</td>
<td>.</td>
</tr>
<tr>
<td>Timolol maleate</td>
<td>1.51 ± 0.22</td>
<td>49.9 &lt;.001</td>
<td>.</td>
</tr>
<tr>
<td>Brimonidine and timolol</td>
<td>1.24 ± 0.17</td>
<td>58.9 &lt;.001</td>
<td>.</td>
</tr>
</tbody>
</table>

*There were 20 eyes in each treatment group. Ellipses indicate data not applicable.
†Data are given as mean ± SD.

![Table 2. Intraocular Pressure at 4 PM*](image2)

<table>
<thead>
<tr>
<th>Drug Applied</th>
<th>Intraocular Pressure, mm Hg†</th>
<th>Reduction in Intraocular Pressure (vs Placebo), %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>11.8 ± 1.9</td>
<td>. . .</td>
<td>.</td>
</tr>
<tr>
<td>Brimonidine tartrate</td>
<td>9.4 ± 1.8</td>
<td>20.3 &lt;.001</td>
<td>.</td>
</tr>
<tr>
<td>Timolol maleate</td>
<td>9.1 ± 1.8</td>
<td>22.9 &lt;.001</td>
<td>.</td>
</tr>
<tr>
<td>Brimonidine and timolol</td>
<td>7.7 ± 1.8</td>
<td>34.7 &lt;.001</td>
<td>.</td>
</tr>
</tbody>
</table>

*There were 20 eyes in each treatment group. Ellipses indicate data not applicable.
†Data are given as mean ± SD.

treated with brimonidine for 8 days. They found a suppression of aqueous humor flow by 20% and an increase of the uveoscleral outflow by 5 times. In the present study, a larger part of the effect on the IOP could be explained by brimonidine’s effect on aqueous humor flow, but there was a discrepancy between the effect on flow and the reduction of IOP. The 2 studies are not directly comparable since they differed in design on several points. Toris and colleagues studied patients with ocular hypertension and used a drug exposure of 8 days, whereas we studied healthy volunteers and used a drug exposure of 1 day. Since the present study comprised only healthy volunteers with a rather low baseline mean±SD IOP of 11.8±1.9 mm Hg, the difference in IOP reduction not explained by reduced aqueous humor production could be an error in the pressure measurements. A deviation of 1 to 2 mm Hg from the true IOP lies within the technique. However, if we assume that the IOP measurements are completely correct, there must be another mechanism apart from the decreased aqueous humor flow that accounts for the decrease in IOP.

In the present study, the IOP measurements were taken at 4 PM, ie, 8 hours after the last dose of drug was administered. For brimonidine, this would correspond to a trough effect on IOP, whereas this would not be as pronounced for timolol since it has a longer duration of action. The IOP measurement would, therefore, be advantageous for timolol concerning a reduction. Thus, a reduction in IOP could have been underestimated for the brimonidine-treated and the brimonidine and timolol-treated eyes.

The concept of apparent resistance to outflow to study the nonlinearity of outflow resistance was first introduced
brimonidine in the aqueous humor flow was 44% to 48% in their study. Taking into account the consensus effect, the reduction of 33.1% by brimonidine alone that was found in the present study would correspond well with their findings. However, a greater reduction of the IOP was found in our study. Also, the consensual effect of brimonidine could affect the second part of the present study, when the combination of brimonidine and timolol was administered to one eye and timolol was instilled in the other eye. The aqueous humor flow measured in the timolol-treated eye could thus in part reflect a crossover effect of brimonidine.

Timolol exerts its ocular hypotensive effect by reduction of aqueous humor. This effect is consistent, and ranges from 47% to 32% in studies on dogs. In another study, it was between 30% and 39% in other studies. In the present study, the flow reduction was 50%, which is somewhat more pronounced compared with the previously mentioned studies, but still in the same range.

In conclusion, the effect of short-term administration of brimonidine was partly additive to timolol, and the combination treatment caused a further reduction of aqueous humor flow and IOP. Most of the reduction of the IOP by brimonidine could be explained by a suppression of aqueous humor flow, but calculation of apparent resistance to outflow supports the theory that brimonidine has some additional ocular hypnotensive effect through enhancement of aqueous outflow.

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