Aqueous Humor Pilocarpine and Timolol Levels After Instillation of the Single Drug or in Combination

Philip P. Ellis, Ping-Yuan Wu, and Maris Riegel

Aqueous humor levels of pilocarpine and timolol in rabbits after administration of either 2% pilocarpine or 0.5% timolol in single-drug solutions were compared with the concentrations found after instillation of a fixed combination of 0.5% timolol and 2% pilocarpine drops. Time intervals considered were 15 min, 30 min, 1 hr, 4 hr, and 8 hr after application. Drug concentrations were analyzed in individual aqueous samples by high-performance liquid chromatography. No statistically significant differences in either pilocarpine or timolol concentrations in aqueous humor were found at any time tested between the single-drug preparations and the combination. Invest Ophthalmol Vis Sci 32:520-522, 1991

Recent studies suggest that administration of a mixture of timolol and pilocarpine provides additive ocular hypotensive effects.1-7 Obvious advantages of this combination are a single application and improved patient compliance. We undertook this study to establish whether comparable levels of the drugs were obtained in the aqueous humor of the rabbit eye after instillation of the combination drops compared with the application of single-drug preparations.

Materials and Methods

Commercial preparations of 2% pilocarpine (Isoprotocarpine; Alcon, Ft. Worth, TX) and 0.5% timolol (Timoptic; Merck, Sharp & Dohme, West Point, PA) were used. The combination drops containing 2% pilocarpine and 0.5% timolol (TP2) were furnished by Merck, Sharp & Dohme.

The 0.5% timolol formulation contained timolol maleate in a pH 7 phosphate buffer; the pH of the 2% pilocarpine preparation was approximately 4. Both formulations contained benzalkonium chloride as a preservative. In addition, the 2% pilocarpine contained hydroxypropyl methylcellulose 2910. The mixture (TP2) was formulated with timolol maleate, pilocarpine hydrochloride, and benzalkonium chloride (0.1 mg/ml) at a pH of approximately 3.5. A mixture of phosphate buffer (pH 7.8-9.2) and benzalkonium chloride (0.1 mg/ml) was separated from the drug mixture by an internal plug which was dislodged just before use. On mixing the two solutions a pH of 6.8 was reached. The final TP2 product contained a buffered, isotonic, 0.5% timolol maleate and 2% pilocarpine hydrochloride solution which was stable for 28 days. Unlike the 2% pilocarpine, TP2 contained no vehicle.

The drug preparations were assayed in duplicate before use. The solvents for chromatography were distilled-in-glass grade; all other chemicals were reagent grade.

New Zealand white rabbits weighing 2-3 kg were used throughout the study. Paracentesis was done on each eye twice with 7-day recoveries between operations.8 Both eyes of the rabbit were used; however, instillation of the drug was staggered so that the two aqueous humor samples from one animal would not contribute to the same time point. At paracentesis, each aqueous humor sample in a group (n = 7-11) was obtained from a different rabbit. The experimental design was predicated on the assumption that the administration of the two drugs together in a mixture would not change the kinetics of absorption of either drug.

Fifty microliters of pilocarpine, timolol, or TP2 were instilled into the rabbit eye. Before paracentesis, the rabbits were sedated and anesthetized with xylazine 10 mg/kg of body weight and ketamine hydrochloride 50 mg/kg of body weight. The aqueous humor was aspirated through the limbus with a 26-gauge needle attached to a 1-ml tuberculin syringe and frozen at -20°C for subsequent assay. The rabbits were used in accordance with National Institute
Table 1. Aqueous humor pilocarpine levels following combination drops (TP2) or 2% pilocarpine

<table>
<thead>
<tr>
<th>Time</th>
<th>Pilocarpine</th>
<th>Number</th>
<th>TP2 combination</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>0.71 ± 0.11</td>
<td>7</td>
<td>0.65 ± 0.14</td>
<td>7</td>
</tr>
<tr>
<td>30 min</td>
<td>3.72 ± 0.43</td>
<td>11</td>
<td>3.18 ± 0.51</td>
<td>7</td>
</tr>
<tr>
<td>1 hr</td>
<td>2.53 ± 0.25</td>
<td>11</td>
<td>2.14 ± 0.18</td>
<td>11</td>
</tr>
<tr>
<td>4 hr</td>
<td>0.42 ± 0.17</td>
<td>7</td>
<td>0.50 ± 0.21</td>
<td>7</td>
</tr>
<tr>
<td>8 hr</td>
<td>0.21 ± 0.09</td>
<td>7</td>
<td>0.35 ± 0.09</td>
<td>7</td>
</tr>
</tbody>
</table>

Concentrations in μg/ml ± standard error of the mean following instillation of a 50 μl dose.

Table 2. Aqueous humor timolol concentrations following combination drops (TP2) or 0.5% timolol

<table>
<thead>
<tr>
<th>Time</th>
<th>Timolol</th>
<th>Number</th>
<th>TP2 combination</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>1.11 ± 0.11</td>
<td>7</td>
<td>1.11 ± 0.17</td>
<td>7</td>
</tr>
<tr>
<td>30 min</td>
<td>1.98 ± 0.22</td>
<td>11</td>
<td>1.39 ± 0.11</td>
<td>7</td>
</tr>
<tr>
<td>1 hr</td>
<td>1.66 ± 0.17</td>
<td>11</td>
<td>1.22 ± 0.28</td>
<td>11</td>
</tr>
<tr>
<td>4 hr</td>
<td>0.20 ± 0.06</td>
<td>7</td>
<td>0.19 ± 0.03</td>
<td>7</td>
</tr>
<tr>
<td>8 hr</td>
<td>0.04 ± 0.03</td>
<td>7</td>
<td>0.03 ± 0.03</td>
<td>7</td>
</tr>
</tbody>
</table>

Concentrations in μg/ml ± standard error of the mean following instillation of a 50 μl dose.

Results

The pilocarpine concentration assayed in the commercial preparation was 1.9% (0.95 mg per dose); that in the mixture was 1.8% (0.9 mg per dose). The commercial preparation contained 0.5% timolol (0.25 mg per dose); the timolol concentration in the mixture was 0.54% (0.27 mg per dose).

The drug concentrations assayed in the aqueous humor of rabbits are summarized in Tables 1 and 2. In Figures 1 and 2, the logarithm of the respective drug concentration was plotted against time to depict the first-order kinetics during decay. Standard errors are included in the tables; however, they are omitted on the logarithmic graphs for clarity.

Pilocarpine concentrations in the aqueous humor were similar (Table 1) whether the drug was instilled as the single component or as the mixture. Likewise, the concentrations of timolol were comparable whether the drug was instilled as a single medication or as the mixture (Table 2). At none of the times tested could a statistically significant difference be found between the drug concentrations when the medications were administered individually or in...
combination, although slightly higher levels were observed at 30 min and 1 hr after administration of the single-drug preparations (Tables 1, 2). When the data were adjusted for differences in dose, the difference in pilocarpine concentrations at 15 min, 30 min, and 1 hr was reduced. Differences in the timolol concentrations were somewhat greater. In no case was the difference statistically significant.

Discussion

Timolol, a β-adrenergic blocker, and pilocarpine, a cholinergic agent, are both commonly prescribed drugs for reducing intraocular pressure. Aqueous humor production is reduced by timolol and the facility of outflow is increased with pilocarpine. A combination of these drugs could be expected to show at least partially additive effects. This has been demonstrated in clinical studies.3-7

Tear secretion is an important factor in regulating ocular drug availability and absorption. Although unchanged by timolol, pilocarpine has a muscarinic action stimulating the lacrimal gland.12,13 Nonphysiologic pH also can stimulate lacrimation and reduce drug available for absorption.14 In our study, the pilocarpine concentrations reached in the aqueous humor were statistically the same after administration of either the TP2 (pH 6.8) or of the single solution of pilocarpine (pH 4).

Our study demonstrated that a single instillation of the TP2 produces aqueous humor concentrations of the two drugs in the rabbit eye that were similar to those found after administration of the individual medication preparations. Even when the data were adjusted for the differences in dose, concentration differences were not significant. Although the results of animal studies cannot be applied rigorously to the human eye, it is reasonable to assume similar pharmacokinetics would exist. The combination medication would appear to be of clinical value in reducing the frequency of drug applications and thereby improving patient compliance.

Key words: timolol, pilocarpine, aqueous humor, combination drops, concentrations

References

6. Maclure GM, Vogel R, Sturm A, and Binkowitz B: Effect on the 24-hour diurnal curve of intraocular pressure of a fixed ratio combination of timolol 0.5% and pilocarpine 2% in patients with COAG not controlled on timolol 0.5%. Br J Ophthalmol 73:827, 1989.