Pharmacokinetic Basis for Nonadditivity of Intraocular Pressure Lowering in Timolol Combinations

Vincent H. L. Lee, Amy M. Luo, Shaoyong Li, Samir K. Podder, James Shih-Chieh Chang, Shigehiro Ohdo, and George M. Gross

The authors determined whether the ocular absorption of topically applied timolol in the pigmented rabbit was affected significantly by coadministration with either pilocarpine or epinephrine in the same drop to explain the nonadditivity in intraocular pressure lowering (IOP) seen clinically. They instilled 25 μl of 0.65% timolol maleate solution (equivalent to 0.5% timolol), both in the presence and absence of 2.6% pilocarpine nitrate or 1% epinephrine bitartrate, into pigmented rabbit eyes. The time course of timolol concentration in the conjunctiva, anterior sclera, corneal epithelium, corneal stroma, aqueous humor, iris-ciliary body, and lens was monitored for 360 min by using reversed-phase high-performance liquid chromatography. The area under the timolol concentration-time curve in all but one of the anterior segment tissues was reduced by 20-50% (mean, 40%) when timolol was coadministered with pilocarpine and by 20-70% (mean, 42%) when timolol was coadministered with epinephrine. Such an effect was not a result of alterations in corneal permeability or aqueous humor turnover rate, nor was it related to the extent of systemic absorption caused by pilocarpine and epinephrine. Rather, the reduction in ocular timolol absorption may have been caused by the accelerated washout of timolol by tears stimulated by the coadministered drugs and, to a lesser extent, by the loss of timolol through binding to the increased amount of tear proteins induced by the coadministered drugs. Thus, the nonadditivity in IOP lowering from timolol–pilocarpine and timolol–epinephrine combinations is probably caused by changes in precorneal timolol clearance. Invest Ophthalmol Vis Sci 32:2948–2957, 1991

Combination or multiple drug therapy often is prescribed during the advanced stages of glaucoma when single-drug therapy is incapable of maintaining intraocular pressure (IOP) adequately. Typically, such combinations are created to provide an additive, cooperative, or synergistic lowering of the IOP by using two drugs that together affect both inflow and outflow mechanisms. Thus, timolol, which reduces aqueous humor inflow,1 was used in combination with drugs that enhance outflow, such as pilocarpine,2–4 epinephrine,5–6 dipivefrin,27 and others.3–5 A similar strategy recently was reported for betaxolol11–13 and levobunolol.14

The long-term effectiveness of multiple drug therapy is drug dependent. Thus, the IOP-lowering effect of timolol is enhanced consistently by pilocarpine15–20 but not by epinephrine and dipivefrin, its prodrug.6,15,21,22 Moreover, wherever there is an additive effect, it is often less than the arithmetic sum of the IOP-lowering effects of the individual drugs.3–5,15,23–25 This phenomenon may be attributed to the nature of the disease state, to the order of and the time interval between the instillation of drops, or to drug interactions at the absorption site.26 However, none of these factors has been studied carefully to our knowledge. Most of the attention on drug interactions in ophthalmology has been directed toward those that affect the receptors involved in the regulation of aqueous humor dynamics.6,24,27,28 There are, however, few reports of drug interactions mediated at the level of tear flow, protein binding, loss of drug to the systemic circulation, corneal drug penetration, or intraocular drug distribution. Any of these factors may alter the drug concentration at its target site and may be significant, particularly when the alteration affects the portion of the dose–response curve where small changes in drug concentration may cause large changes in the drug response.

We wished to determine whether the ocular absorption of timolol from a 0.65% timolol maleate solution (equivalent to a 0.5% timolol solution) from the eyes of the pigmented rabbit was affected significantly by the coadministration with either 2.6% pilocarpine ni-
trate or 1% epinephrine bitartrate in the same drop, thus explaining the nonadditivity in IOP lowering seen clinically.15-22 We also were interested in the mechanisms by which possible changes in ocular timolol absorption were mediated.

Materials and Methods

Materials

Timolol maleate, epinephrine bitartrate, pilocarpine nitrate, histamine HCl, and rabbit serum albumin were purchased from Sigma (St. Louis, MO). Propranolol HCl and triethylamine HCl were purchased from Aldrich (Milwaukee, WI). Male Dutch-belted pigmented rabbits, weighing 2.5-3.0 kg, were housed in standard laboratory rabbit cages and were fed a regular diet with no restrictions on the amount of food or water consumed. All experiments conformed to the ARVO Resolution on the Use of Animals in Research.

Preparation of Drug Solutions

Drug solutions for ocular and systemic absorption experiments were prepared in 10 mM Tris buffer, adjusted to pH 7.4 with 10 N NaOH, and rendered isotonic by adding NaCl. Solutions for corneal penetration experiments were prepared in glutathione bicarbonate Ringer’s (GBR) solution.30 In this instance, 0.2% sodium bisulfite was added to the solutions containing epinephrine to protect the drug from being oxidized during the experiment. All solutions were prepared immediately before each experiment. Solutions containing rabbit serum albumin were allowed to equilibrate at 37°C for at least 30 min before use.

High-Performance Liquid Chromatographic Assay

Timolol was quantified using reversed-phase high-performance liquid chromatography on a Beckman Ultrasphere ODS column (column size, 25 cm × 4.6 mm; particle size, 5 μm) fitted with a OD-GU RP18 5-μm precolumn (1.5 cm) (West Coast Scientific, Hayward, CA), as previously described.30 The system consisted of a SCL-6A system controller, two LC-6A pumps, a SIL-6A autoinjector, a SPD-6A spectrophotometric detector, and a CR-3A integrator (Shimadzu, Baltimore, MD). The mobile phase consisted of varying proportions of 10% acetonitrile in methanol and 0.2% triethylamine HCl in 5% acetonitrile at pH 3. The proportion of organic phase was kept at 40% for 8 min, changed from 40% to 50% for the next 7 min, kept at 50% for another 2 min, decreased from 50% to 40% for the next 2 min, and kept at 40% for the final 4 min. The flow rate was 1 ml min⁻¹. Timolol was monitored at 294 nm. The retention time was 6.0 ± 0.5 min for timolol and 15.0 ± 0.5 min for propranolol, the internal standard. Under these chromatographic conditions, neither pilocarpine nor epinephrine appeared in the chromatogram. The sensitivity of the assay was better than 5 nmol with respect to timolol in a 2-ml plasma sample. The intra- and interrun variations were 5% and 7.5%, respectively.

At the time of assay, an aqueous humor sample (about 80 μl) was mixed with 40 μl of 25 μg ml⁻¹ propranolol HCl and 80 μl of 0.1 N HCl in acetonitrile. After centrifugation, 80-120 μl of the supernatant was injected into the chromatograph. Excised tissues were soaked in 1 ml of 0.6% HClO₄ at 8°C for 12 hr. Thereafter, the sample was mixed with 0.1 ml of 10 μg ml⁻¹ of propranolol HCl solution and 0.5 ml of 1 M ammonium acetate buffer (pH 9), extracted with 8 ml of diethyl ether by vortexing for 3 min, and then centrifuged at 1500 g for 10 min. The upper organic layer was transferred to a 15-ml screw-capped conical centrifuge tube containing 200 μl of 0.2 N HCl, vortexed for 3 min, and centrifuged at 1500 g for 10 min. The organic phase was discarded, and 100-120 μl of the aqueous phase, containing timolol and propranolol, was injected into the chromatograph. The same procedure was used to extract timolol from 2 ml of plasma. The extraction efficiency was better than 85% for both ocular tissue and plasma samples.

Ocular Absorption of Topically Applied Timolol

Unanesthetized rabbits were placed in polycarbonate restraining boxes with no restriction in head or eye movement. We instilled 25 μl of the test solution directly onto the cornea of both eyes of each rabbit, collecting in the cul-de-sac. At 5, 15, 30, 60, 90, 150, 180, 240, or 360 min postdosing, the rabbit was killed with an overdose of sodium pentobarbital (Eutha-6; Western Medical Supply, Arcadia, CA) administered through a marginal ear vein. After thoroughly rinsing the corneal and conjunctival surfaces with 1.17% KCl solution and blotting them dry, the corneal epithelium was scraped with a no. 11 scalpel. Approximately 150-200 μl of aqueous humor was aspirated from the anterior chamber using a 1-ml tuberculin syringe fitted with a 27-gauge needle. The corneal stroma was excised by cutting at the corneolimbal margin. The iris and ciliary body were difficult to separate from each other, and they were removed as one piece. The lens was separated from the vitreous, which was discarded. The anterior sclera was dissected and trimmed to remove other tissue fragments. Finally, 8-10-mm tangential sections of conjunctiva were dissected from the upper and lower eyelids with a scalpel. Dissection of both eyes was completed...
within 10 min. All excised tissues were rinsed with ice-cold KCl solution, blotted dry, transferred to preweighed microcentrifuge tubes containing 200 μl of 0.6% HClO₄, and stored at −70°C. At least four eyes were used per time point.

**Systemic Absorption of Topically Applied Timolol**

The dosing procedure was as described in the ocular absorption experiments. Fifteen minutes before solution instillation, the rabbits were cannulated in a central ear artery using polyethylene tubing (PE-50; Intramedic) and heparinized with 1000 units of sodium heparin (Western Medical Supply). Thereafter, 25 μl of a dosing solution was instilled into each eye of a given rabbit. At 0, 3, 6, 10, 15, 30, 45, 60, 90, and 120 min postdosing, 4-ml blood samples were collected into heparinized tubes. The volume of blood aspirated was replenished with 50 ml of lactated Ringer’s solution (Travenol, Deerfield, IL) through an intravenous drip during the experiment. Preliminary experiments revealed that the plasma timolol concentration fell below the detection limit after 120 min; therefore, blood was collected until then. The blood was centrifuged at 1500 g for 10 min to yield 2 ml of plasma, quickly frozen, and stored at −20°C until assayed. At least four rabbits were used.

**Influence of Epinephrine on the Systemic Absorption of Timolol From the Conjunctival and Nasal Mucosae**

We wished to determine whether the effect of epinephrine on the systemic absorption of timolol was mediated at the conjunctival or the nasal mucosa. Absorption from the conjunctival mucosa was determined by instilling 25 μl of a 0.65% timolol maleate solution with and without 1% epinephrine bitartrate in rabbits whose conjunctival sac was isolated from the nasolacrimal duct by a punctum plug. This plug was fashioned from a 5-mm segment of a polyethylene tubing (PE 50; outer diameter, 1 mm) preinserted 15–20 mm into the lacrimal duct by a punctum plug. This plug was fashioned from a 5-mm segment of a polyethylene tubing (PE 50; outer diameter, 1 mm) that had been heat sealed at one end and beveled at the other. 31 Absorption from the nasal mucosa was determined by instilling 25 μl of a 0.65% timolol maleate solution nasally through a polyethylene catheter (PE 50; outer diameter, 1 mm) preinserted 15–20 mm into the lacrimal sac and nasolacrimal duct of the same group of rabbits after a 7-day washout period. In each case, serial blood samples were collected from each rabbit until 120 min had elapsed and assayed for timolol as described earlier. Immediately after the last sampling, the rabbits in the conjunctival-administration experiment were killed, and their anterior segment tissues were excised and assayed for timolol as described earlier. At least four rabbits were used per condition.

**Influence of Pilocarpine and Epinephrine on Systemic Timolol Pharmacokinetics**

In this experiment, we tried to determine whether changes in the plasma timolol concentration–time profiles seen in timolol–pilocarpine combinations and timolol–epinephrine combinations, compared with timolol instillation, were caused by changes in the plasma clearance of timolol. We injected 50 μl of a 0.65% timolol maleate solution with and without 2.6% pilocarpine nitrate or 1% epinephrine bitartrate into the marginal ear vein of pigmented rabbits. The sampling schedule and sample processing procedure were the same as in the topical solution-instillation experiment described previously. At least four rabbits were used per dosing time.

**Influence of Histamine and Rabbit Serum Albumin on the Ocular and Systemic Absorption of Topically Applied Timolol**

We wished to determine whether the ocular and systemic absorption of topically applied timolol was affected by the coadministration of 0.5% histamine HCl and 7% rabbit serum albumin. The dosing, sampling, and sample processing procedures were as described earlier for the ocular and systemic absorption experiments, except that ocular absorption was evaluated only at 30 min postdosing.

**Influence of Pilocarpine and Epinephrine on the Corneal Penetration of Timolol**

In this experiment, we tried to determine whether changes in the ocular absorption of topically applied timolol in the presence of either pilocarpine or epinephrine were caused by changes in corneal permeability. The rabbits were killed by a rapid injection of sodium pentobarbital (Eutha-6) into the marginal ear vein. The eyes were enucleated immediately with the conjunctiva and lids intact. The corneas were excised and mounted in a Lucite-block perfusion chamber. 29 We added 2.5 ml of GBR solution to the endothelial side, and an equal volume of the same solution containing drug was added immediately to the epithelial side. Mixing in each chamber was achieved by bubbling a mixture of 95% O₂ and 5% CO₂. The chambers’ temperatures were kept at 37°C by a circulating water bath. At predetermined times up until 240 min, 50-μl aliquots were sampled from the endothelial side and immediately replaced by an equal volume of GBR solution. The aliquots were assayed for timolol by reversed-phase high-performance liquid chromatography. After correcting for the corneal surface area (1.075 cm²) and initial timolol concentra-
tion was the conjunctiva, in which the Cmax was two-  
fold higher and the AUC was sixfold higher when ti-  
molol was coadministered with epinephrine. The in-  
crease in timolol concentration in the conjunctiva did  
not, however, lead to a corresponding increase in the  
plasma AUCs. The AH-plasma ratio was 266 in the  
intravenous experiment differently than it did in the  
topical administration experiment. The plasma timo-  
lol concentration was highest in the corneal epithe-  
ilium, followed by the iris-ciliary body, corneal  
stroma, conjunctiva, aqueous humor, sclera, and lens  
in that order (Figs. 1A–G, Table 1).

Under all experimental conditions, timolol concen-  
tration reached its peak in the conjunctiva, corne-  
al epithelium, and corneal stroma relatively early (5  
min) compared with the aqueous humor (15 min),  
and iris-ciliary body (240 min) (Figs. 1A–G, Table 1).  
Surprisingly, the peak time of timolol concentration  
in the sclera (30–60 min) was much longer than that in  
the overlying conjunctiva (5 min) from which the sclera derived its drug. As can be  
seen in Table 1, the long peak time in the sclera was  
probably a result of its slower rate of elimination (ka)  
in the sclera than that in the conjunctiva.

Pilocarpine did not affect the peak time of timolol  
concentration in any anterior segment tissues except  
the sclera, which showed a decrease. Epinephrine pro-  
longed the peak time in the conjunctiva, corneal epipi-  
thelium, and corneal stroma and shortened it in the  
sclera, iris-ciliary body, and lens. It did not affect it  
in the aqueous humor (Table 1). Prolongation of the  
peak time in the conjunctiva was probably a result of  
the vasoconstrictive effect of epinephrine.

The MRT of timolol in most anterior segment tis-  
ues was not altered by coadministration with either  
pilocarpine or epinephrine. This is consistent with the  
general lack of change in the magnitude of ka and  
k (Table 1), which are related to the MRT through the  
relationship: MRT = 1/ka + 1/k.

Although both pilocarpine and epinephrine re-  
duced the AUCs of timolol in the anterior segment  
tissues, they differed on how they affected the rate and  
extent of timolol absorption in plasma (Fig. 1H). Spe-  
cifically, pilocarpine affected none of the pharmaco-  
kINETIC parameters of timolol in plasma except ka (Table  
1), whereas epinephrine reduced the Cmax and AUC  
of timolol by 72% and 45%, respectively, and increased  
its MRT 2.2 times (Table 1). The increase in MRT by  
epinephrine was caused by reduction in ka rather than  
k; the MRT of timolol in the direct intravenous ad-  
ministration experiment was unaffected by coadmi-  
istration with epinephrine (Fig. 2, Table 2). Reduction  
of systemic timolol absorption by epinephrine was  
related to its vasoconstricting effect at both the  
nasal and conjunctival mucosa (Fig. 3, Table 3). In  
the presence of epinephrine, the systemic absorption  
of timolol from the nasal and the conjunctival mu-  
cosa was 72% and 50% of control based on the AUC  
and 40% and 33% based on Cmax. The timolol concen-  
tration in the anterior segment tissues at 120 min after  
conjunctival administration, however, was 1.6–7.1-  
fold higher in the presence of epinephrine than in its  
absence (Table 4).

Pilocarpine affected the plasma timolol AUC in the  
intravenous experiment differently than it did in the  
topical administration experiment. The plasma timo-  
lol AUC was reduced by intravenously administered  
pilocarpine (Table 2) but was unaffected by topically  
applied pilocarpine (Table 1).

The net effect of coadministration of timolol with  
pilocarpine or epinephrine was a 50% reduction or no  
change in the therapeutic index of timolol, respecti-  
vely. Therapeutic index is defined as the ratio of  
aqueous humor–plasma AUCs or iris-ciliary body–  
plasma AUCs. The AH–plasma ratio was 266 in the
Fig. 1. Concentration-time profiles of timolol in the conjunctiva (plot A), anterior sclera (plot B), corneal epithelium (plot C), corneal stroma (plot D), aqueous humor (plot E), iris-ciliary body (plot F), lens (plot G), and plasma (plot H) of the pigmented rabbit following the topical instillation of 25 μl of 0.65% timolol maleate solutions alone (O) or in combination with 2.6% pilocarpine nitrate (●) or 1% epinephrine bitartrate (□). Error bars represent standard error of the mean for n = 4–8 for each experimental condition.
Table 1. Influence of 2.6% pilocarpine nitrate and 1% epinephrine bitartrate on the pharmacokinetic parameters of timolol in plasma and various anterior segment tissues following topical instillation of 25 μl of 0.65% timolol maleate solutions to each eye.

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Additive</th>
<th>Plasma</th>
<th>Conj†</th>
<th>Sclera</th>
<th>CE</th>
<th>CS</th>
<th>AH</th>
<th>ICB</th>
<th>Lens</th>
</tr>
</thead>
<tbody>
<tr>
<td>ka (min⁻¹)</td>
<td>None</td>
<td>2.21</td>
<td>—§</td>
<td>0.077</td>
<td>0.50</td>
<td>0.62</td>
<td>0.14</td>
<td>0.0062</td>
<td>0.0088</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>0.82</td>
<td>0.21</td>
<td>0.12</td>
<td>0.72</td>
<td>3.40</td>
<td>0.21</td>
<td>0.0080</td>
<td>0.0296</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>0.15</td>
<td>0.18</td>
<td>0.13</td>
<td>0.95</td>
<td>0.31</td>
<td>0.72</td>
<td>0.0061</td>
<td>0.0159</td>
</tr>
<tr>
<td>ke (10² min⁻¹)</td>
<td>None</td>
<td>2.28</td>
<td>—§</td>
<td>0.40</td>
<td>1.34</td>
<td>2.21</td>
<td>0.88</td>
<td>0.36</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>2.07</td>
<td>4.42</td>
<td>0.47</td>
<td>1.42</td>
<td>1.61</td>
<td>0.80</td>
<td>0.42</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>0.63</td>
<td>1.51</td>
<td>0.51</td>
<td>1.14</td>
<td>1.16</td>
<td>0.56</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>tmax (min)</td>
<td>None</td>
<td>4.67</td>
<td>5</td>
<td>60</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>240</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>13.00</td>
<td>5</td>
<td>45</td>
<td>5</td>
<td>5</td>
<td>15</td>
<td>240</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>44.00</td>
<td>15</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>150</td>
<td>90</td>
</tr>
<tr>
<td>Cmax (µg/ml or µg/g)</td>
<td>None</td>
<td>0.051</td>
<td>0.62</td>
<td>0.26</td>
<td>92.29</td>
<td>18.03</td>
<td>1.76</td>
<td>37.82</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>0.050</td>
<td>1.19</td>
<td>0.11</td>
<td>7.75</td>
<td>3.75</td>
<td>0.57</td>
<td>(11.3)</td>
<td>(0.11)</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>0.065</td>
<td>0.75</td>
<td>0.06</td>
<td>23.76</td>
<td>1.43</td>
<td>0.23</td>
<td>(3.02)</td>
<td>(0.09)</td>
</tr>
<tr>
<td>AUC (ng X min/ml or µg X min/g)</td>
<td>None</td>
<td>1.59</td>
<td>271.1</td>
<td>100.1</td>
<td>14227</td>
<td>1892</td>
<td>4342</td>
<td>16686</td>
<td>136.0</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>1.77</td>
<td>359.8</td>
<td>51.1</td>
<td>7400</td>
<td>1200</td>
<td>235.3</td>
<td>10100</td>
<td>82.9</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>0.87</td>
<td>1699.9</td>
<td>68.4</td>
<td>11587</td>
<td>1317</td>
<td>231.3</td>
<td>8319</td>
<td>38.2</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>None</td>
<td>44.16</td>
<td>86.4</td>
<td>225.4</td>
<td>88.4</td>
<td>124.8</td>
<td>177.9</td>
<td>206.7</td>
<td>469.3</td>
</tr>
<tr>
<td></td>
<td>Pilocarpine</td>
<td>48.40</td>
<td>68.2</td>
<td>224.8</td>
<td>79.1</td>
<td>74.8</td>
<td>164.8</td>
<td>197.3</td>
<td>459.5</td>
</tr>
<tr>
<td></td>
<td>Epinephrine</td>
<td>142.04</td>
<td>78.9</td>
<td>214.3</td>
<td>103.6</td>
<td>92.6</td>
<td>169.2</td>
<td>199.0</td>
<td>438.8</td>
</tr>
</tbody>
</table>

* See Methods for explanation of abbreviations.† Conj = conjunctiva, CE = corneal epithelium, CS = corneal stroma, AH = aqueous humor, ICB = iris-ciliary body.‡ Figures in parentheses represent standard error of the mean, n = 4.§ Cannot be estimated from data.

As shown in Table 5, neither pilocarpine nor epinephrine affected the corneal penetration of timolol in vitro. By contrast, the antioxidant sodium bisulfite reduced the corneal permeability coefficient of timolol 1.5-fold.

Table 2. Influence of 2.6% pilocarpine nitrate and 1% epinephrine bitartrate on the pharmacokinetic parameters of timolol in plasma following intravenous bolus administration of 50 μl of 0.65% timolol maleate solutions

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Timolol alone</th>
<th>With pilocarpine</th>
<th>With epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>k12 (10² min⁻¹)</td>
<td>5.79 ± 2.56$§</td>
<td>7.25 ± 0.95$§</td>
<td>7.58 ± 1.49$§</td>
</tr>
<tr>
<td>K21 (10² min⁻¹)</td>
<td>5.20 ± 1.34$</td>
<td>4.33 ± 1.30$</td>
<td>3.61 ± 0.60$</td>
</tr>
<tr>
<td>k10 (10³ min⁻¹)</td>
<td>5.30 ± 0.89$</td>
<td>8.30 ± 1.76$</td>
<td>11.12 ± 1.27$</td>
</tr>
<tr>
<td>Vc (L)</td>
<td>1.54 ± 0.56</td>
<td>2.29 ± 0.76</td>
<td>1.04 ± 0.30$</td>
</tr>
<tr>
<td>Vdss (L)</td>
<td>3.48 ± 1.79</td>
<td>6.03 ± 0.63</td>
<td>3.32 ± 1.16$</td>
</tr>
<tr>
<td>AUC (µg X min/ml)</td>
<td>3.40 ± 0.48$</td>
<td>1.66 ± 0.32$</td>
<td>2.47 ± 0.33$</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>31.9 ± 2.6$</td>
<td>31.5 ± 4.9$</td>
<td>24.7 ± 4.0$</td>
</tr>
</tbody>
</table>

* See Methods for explanation of abbreviations.† Mean ± SEM, n = 4.$ Significantly different from timolol alone at P < 0.035 by one-way ANOVA.§ Not significantly different at P = 0.2 by one-way ANOVA.
Fig. 2. Effect of 2.6% pilocarpine nitrate and 1% epinephrine bitartrate on the concentration-time profiles of timolol in the plasma of the pigmented rabbit following the intravenous administration of 50 μl of 0.65% timolol maleate solutions. Error bars represent standard deviation for n = 4 for each experimental condition. ● = timolol alone; ○ = timolol plus pilocarpine; △ = timolol plus epinephrine.

Both 0.5% histamine and 7% rabbit serum albumin reduced timolol concentrations in the corneal stroma and iris–ciliary body at 30 min postdosing by approximately 44% and 16%, respectively (Table 6). The reduction in timolol concentrations in the conjunctiva, sclera, and lens was even more extensive (Table 6). Only 0.5% histamine reduced the timolol concentrations in the corneal epithelium and aqueous humor (Table 6). One-way analysis of variance revealed that neither treatment affected the systemic bioavailability of topically applied timolol (P = 0.05). The AUC was 1.67 ± 0.31 μg·min/ml in the presence of 0.5% histamine and 1.53 ± 0.31 μg·min/ml in the presence of 7% rabbit serum albumin, compared with 1.59 ± 0.35 μg·min/ml for the control.

Table 3. Influence of 1% epinephrine bitartrate on plasma timolol pharmacokinetics following the instillation of 0.65% timolol maleate solutions ocularily, conjunctivally, and nasally in the pigmented rabbit

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Epinephrine†</th>
<th>Ocular</th>
<th>Conjunctival</th>
<th>Nasal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (μg/ml)</td>
<td>–</td>
<td>0.051 ± 0.006†</td>
<td>0.015 ± 0.001</td>
<td>0.065 ± 0.008</td>
</tr>
<tr>
<td>tmax (min)</td>
<td>+</td>
<td>0.014 ± 0.002</td>
<td>0.005 ± 0.001</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td>ka (10² min⁻¹)</td>
<td>–</td>
<td>4.67 ± 1.44</td>
<td>6.00 ± 0</td>
<td>6.33 ± 2.03</td>
</tr>
<tr>
<td>ke (10² min⁻¹)</td>
<td>+</td>
<td>44.00 ± 16.52</td>
<td>48.75 ± 3.75</td>
<td>24.00 ± 6.00</td>
</tr>
<tr>
<td>AUC (μg × min/ml)</td>
<td>–</td>
<td>221 ± 24</td>
<td>57.7 ± 14.3</td>
<td>53.6 ± 22.7</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>+</td>
<td>15.0 ± 1.5</td>
<td>2.3 ± 0.5</td>
<td>59.0 ± 0.5</td>
</tr>
<tr>
<td>ke (10² min⁻¹)</td>
<td>–</td>
<td>2.28 ± 0.08</td>
<td>1.7 ± 0.08</td>
<td>4.7 ± 0.01</td>
</tr>
<tr>
<td>ke (10² min⁻¹)</td>
<td>+</td>
<td>0.63 ± 0.02</td>
<td>1.1 ± 0.5</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>AUC (μg × min/ml)</td>
<td>–</td>
<td>1.59 ± 0.18</td>
<td>0.72 ± 0.06</td>
<td>1.82 ± 0.20</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>+</td>
<td>0.87 ± 0.12</td>
<td>0.36 ± 0.08</td>
<td>1.31 ± 0.10</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>–</td>
<td>0.16 ± 0.23</td>
<td>40.23 ± 2.15</td>
<td>27.21 ± 0.74</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>+</td>
<td>142.04 ± 25.38</td>
<td>67.75 ± 3.47</td>
<td>46.33 ± 2.75</td>
</tr>
</tbody>
</table>

* See Methods for explanation of abbreviations.
† – = without epinephrine; + = with epinephrine.
‡ Mean ± SEM, n = 4.

Discussion

A significant finding in our study was that the coadministration of 0.65% timolol maleate with either 2.6% pilocarpine nitrate or 1% epinephrine bitartrate resulted in a 50% reduction in its extent of absorption into the eye (Figs. 1A–G, Table 1). It thus follows that unless the ocular absorption of pilocarpine or epinephrine is increased by timolol, an unlikely situation, the nonadditivity in intraocular pressure lowering seen in timolol combinations with pilocarpine or epinephrine is caused, at least in part, by a reduction in timolol absorption by the coadministered drug. The reduction in systemic absorption of timolol by epi-

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933382/ on 11/01/2018
though, for assay-sensitivity reasons, the precorneal ble 1) supports the notion of accelerated precorneal changes in precorneal clearance of timolol caused by pilocarpine and epinephrine (Table 4).

Table 4. Effect of 1% epinephrine bitartrate on timolol concentrations in anterior segment tissues at 120 min post-dosing following conjunctival application

<table>
<thead>
<tr>
<th>Tissue/fluid</th>
<th>Without epinephrine</th>
<th>With epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>1.75 ± 0.19</td>
<td>12.46 ± 2.09† (7.1)‡</td>
</tr>
<tr>
<td>Sclera</td>
<td>0.44 ± 0.08</td>
<td>2.32 ± 0.28§ (5.2)‡</td>
</tr>
<tr>
<td>Corneal epithelium</td>
<td>159.2 ± 15.8</td>
<td>461.4 ± 64.5 (2.9)‡</td>
</tr>
<tr>
<td>Corneal stroma</td>
<td>16.1 ± 1.85</td>
<td>50.15 ± 7.58† (3.1)‡</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>2.22 ± 0.21</td>
<td>4.14 ± 0.48† (1.9)‡</td>
</tr>
<tr>
<td>Iris-ciliary body</td>
<td>77.61 ± 13.28</td>
<td>126.35 ± 12.91† (1.6)‡</td>
</tr>
<tr>
<td>Lens</td>
<td>0.15 ± 0.03</td>
<td>0.48 ± 0.09† (3.3)‡</td>
</tr>
</tbody>
</table>

* Mean ± SEM, n = 8.
† Statistically different from control at P < 0.02 by Student's t-test.
‡ Factor of increase over control.

epinephrine (Fig. 1H) did not lead to an increase in the extent of ocular absorption (Figs. 1A–G). This finding was similar to that of others, who pretreated the rabbit eye with 50 μl of 2% epinephrine 5 min before instilling timolol solution, and consistent with the larger role played by the nasal than the conjunctival mucosa in systemic timolol absorption.31 Only when access of the administered dose to the nasal cavity was denied was there an increase (about twofold) in the ocular tissue concentrations by the coadministered epinephrine (Table 4).

The reduction in timolol concentrations in the anterior segment tissues caused by pilocarpine and epinephrine may be attributed to changes in precorneal drug loss, corneal drug penetration, or drug elimination from the target tissue or fluid. The last possibility can be excluded because no changes are seen in the k of timolol when coadministered with pilocarpine or epinephrine, even though both drugs decrease outflow resistance and, hence, aqueous humor turnover rate34 (Table 1). The second possibility—changes in corneal drug penetration—can also be excluded because the in vitro corneal permeability experiments revealed no change in the corneal permeability coefficient of timolol by pilocarpine or epinephrine (Table 5). Therefore, the most probable explanation for the reduction in ocular timolol concentrations was changes in precorneal clearance of timolol caused by pilocarpine and epinephrine, a major component of which is solution drainage.35

The approximately equal extent of reduction in timolol concentrations in all anterior segment tissues in the presence of either pilocarpine or epinephrine (Table 1) supports the notion of accelerated precorneal clearance of timolol by the coadministered drugs. Although, for assay-sensitivity reasons, the precorneal clearance of timolol was not measured, there is evidence that both pilocarpine and epinephrine can stimulate tear production,36–38 thus indirectly increasing the solution drainage rate.39 Moreover, both cholinergic and adrenergic stimulation are known to increase tear protein secretion.40–42 thereby potentially increasing loss of timolol through binding to the increased amount of tear proteins. Others showed that timolol at 0.1 μg ml⁻¹ is 60% bound to serum proteins.43 Nevertheless, under our experimental conditions, loss of timolol to protein binding probably played only a modest role in reducing ocular timolol absorption. As shown in Table 6, the extent of ocular timolol absorption from a solution containing 7% rabbit serum albumin was as high as 85% of the control on average. A recent finding of the lack of statistically significant difference in the aqueous humor timolol and pilocarpine concentrations between the single drug preparations and their fixed-ratio combination in anesthetized albino rabbits44 lends credence to this hypothesis of accelerated precorneal clearance of timolol by pilocarpine- and epinephrine-induced lacrimation. Because tearing is inhibited by anesthesia,45 pilocarpine would not be able to express its lacrimation effect in this previous study.44

In addition to increasing the tear flow and protein secretion rates, pilocarpine can reduce timolol concentration in the conjunctival sac through its vasodilation effect. Such an effect increased the clearance of glycerin from the conjunctival sac of anesthetized rabbits by approximately threefold.35 The precorneal clearance of timolol may be affected similarly in light of the 40% reduction in the extent of ocular timolol absorption by 0.5% histamine, a potent vasodilator (Table 6). By contrast, the systemic absorption of timolol was not affected in either instance or when timolol was coadministered with pilocarpine. This is not surprising because timolol is 80% absorbed sys-

Table 5. Influence of 2.6% pilocarpine nitrate, 0.2% sodium bisulfite (SBS), and 1% epinephrine bitartrate on the corneal permeability coefficient (Papp) of 0.65% timolol maleate in the pigmented rabbit

<table>
<thead>
<tr>
<th>Additive</th>
<th>Papp of timolol (10⁻⁶ cm/sec)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>12.27 ± 0.84</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>10.56 ± 0.67†</td>
</tr>
<tr>
<td>Sodium bisulfite</td>
<td>4.93 ± 0.31</td>
</tr>
<tr>
<td>Epinephrine/sodium bisulfite</td>
<td>4.82 ± 0.33‡</td>
</tr>
</tbody>
</table>

* Mean ± SD, n = 4.
† Not statistically significant from timolol alone by Student’s t-test (P > 0.05).
‡ Not statistically significant from timolol plus sodium bisulfite by Student’s t-test (P > 0.05).
Moreover, there is the possibility that timolol was diluted and washed out of the nasal cavity more rapidly by the increased lacrimation rate.

Apparently, the vasoconstrictive effect of epinephrine, which theoretically can increase the amount of drug in the conjunctival sac for ocular absorption, is more than offset by its lacrimation effect. This is indicated by the 50% reduction in ocular absorption of timolol when coadministered with epinephrine (Figs. 1A–G, Table 1). Because of the rapid washout of timolol into the nasal cavity, reduction in the systemic absorption of timolol by epinephrine must be a result more of its vasoconstrictive effect on the nasal mucosa than on the conjunctival mucosa, even though epinephrine was somewhat less effective in reducing systemic timolol absorption when applied directly to the nasal mucosa (Fig. 3, Table 3). A minor contributing factor to the reduction in the plasma timolol AUC by epinephrine was an increase in the elimination rate constant (k_{10}) of timolol from plasma by epinephrine, as shown in the intravenous administration experiment (Table 2). In this instance, the plasma timolol AUC in the presence of epinephrine was only 73% of the control.

In summary, the possibility that the ocular absorption of one drug may be affected by a coadministered drug must be considered in evaluating ophthalmic drug candidates for use in combinations, particularly when the precorneal clearance, corneal permeability, and intraocular distribution of one drug are likely to be affected by the other drug. This is the case when timolol is coadministered with either pilocarpine or epinephrine. There is, therefore, a pharmacokinetic basis for the nonadditivity in intraocular pressure lowering seen clinically when these drugs are used in combination to treat advanced glaucoma.

Key words: ocular drug interactions, ocular drug pharmacokinetics, precorneal drug clearance, timolol–epinephrine combinations, timolol–pilocarpine combinations

### Table 6. Influence of 7% rabbit serum albumin and 0.5% histamine on timolol concentrations in ocular tissues at 30 min following the topical instillation of 25 μl of 0.65% timolol maleate solutions in the pigmented rabbit

<table>
<thead>
<tr>
<th>Tissue/fluid</th>
<th>Control</th>
<th>7% Albumin</th>
<th>0.5% Histamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctiva</td>
<td>3.66 ± 1.18</td>
<td>1.36 ± 0.33(37%)††</td>
<td>1.31 ± 0.59 (36%)‡‡</td>
</tr>
<tr>
<td>Anterior sclera</td>
<td>0.39 ± 0.08</td>
<td>0.04 ± 0.04 (9%)‡</td>
<td>0.12 ± 0.04 (30%)‡‡</td>
</tr>
<tr>
<td>Corneal epithelium</td>
<td>116.37 ± 25.0</td>
<td>115.21 ± 16.22 (99%)</td>
<td>57.83 ± 1.28 (50%)‡‡</td>
</tr>
<tr>
<td>Corneal stroma</td>
<td>12.64 ± 2.03</td>
<td>9.30 ± 1.91 (74%)‡</td>
<td>5.92 ± 0.95 (47%)‡‡</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>2.42 ± 0.35</td>
<td>2.22 ± 0.15 (92%)</td>
<td>1.83 ± 0.36 (76%)‡‡</td>
</tr>
<tr>
<td>Iris-ciliary body</td>
<td>16.27 ± 4.85</td>
<td>12.07 ± 1.96 (74%)‡</td>
<td>8.72 ± 0.50 (54%)‡‡</td>
</tr>
<tr>
<td>Lens</td>
<td>0.11 ± 0.05</td>
<td>0 (0%)§</td>
<td>0.008 ± 0.008 (7%)§§</td>
</tr>
</tbody>
</table>

* Mean ± SEM, n = 3-6. † Percent of control. ‡ Significantly different from control at P < 0.05 by Student’s t-test.

### References

2. O’Connor MA and Mooney DJ: The additional pressure-lowering effect in patients with glaucoma of pilocarpine 2%, adrenaline 1%, or guanethidine 3% with adrenaline 0.5% and timolol 0.25%. A double-blind cross over study. Trans Ophthalmol Soc UK 103:588, 1983.


24. Schenker HI, Yablonski ME, Podos SM, and Linder L: Fluoro-


26. Chrai SS, Makoid MC, Eriksen SP, and Robinson JR: Drop

27. Boas RS, Messenger MJ, Mittag TW, and Podos SM: The ef-

28. Crawford K and Kaufman PL: Pilocarpine antagonizes prosta-

29. O'Brien WJ and Edelhauser HF: The corneal penetration of


34. Kaufman PL and Barany EH: Loss of acute pilocarpine effect on outflow facility following surgical disinsertion and retrodis-


36. de Haas EBH: Lacrimal gland response to parasympathomime


41. Friedman Z, Lowe M, and Selinger Z: Beta adrenergic recep-


43. Belpaire FM, Bogaert MC, and Rosseneu M: Binding of β-

44. Ellis PP, Wu PY, and Riegel M: Aqueous humor pilocarpine and timolol levels after instillation of the single drug or in com-