The Effect of Pilocarpine on Ocular Levobunolol Absorption from Ophthalmic Solutions

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ABSTRACT

Studies in vitro and in vivo were conducted to investigate the effect of pilocarpine on ocular absorption of levobunolol when both drugs were formulated in one solution dosage. The ocular absorption of levobunolol is pH-dependent. Due to the large buffering capacity of pilocarpine at pH 5.5, the ocular absorption of levobunolol from pilocarpine-containing solutions was reduced by approximately four-fold as compared to a non-pilocarpine-containing formulation at pH 7.2. The ocular absorption of levobunolol in the presence of pilocarpine at acidic pH was enhanced by the use of sulfosuccinates, specifically Schercopol CMS.

INTRODUCTION

In many patients, the disease of glaucoma is manifested by elevated intraocular pressure. The present treatment of glaucoma focuses on lowering the IOP to levels that might not produce further damages to the optic nerve head and deterioration of the visual field. Efforts in lowering the IOP involve the use of pharmacological agents that reduce the aqueous humor formation (e.g., β-adrenergic antagonists) or enhance aqueous humor outflow (e.g., muscarinic agonists). Many patients require the concomitant use of drugs from more than one pharmacologic class in order to achieve the desired decrease in IOP.

Levobunolol, a β-adrenergic antagonist, and pilocarpine, a muscarinic agonist, when used individually have been efficacious in lowering the IOP in glaucomatous patients. They are also prescribed as concomitant therapy in an attempt to lower the IOP to a greater extent in some patients. To address such a need, efforts were expended to evaluate potential interactions between
levobunolol and pilocarpine when both drugs were formulated into one ophthalmic solution dosage. A single ophthalmic dosage form will improve patient compliance while achieving the therapeutic endpoint, pressure control.

Both ocular levobunolol and pilocarpine are rapidly absorbed when administered individually. After an eyedrop instillation, levobunolol concentration in the aqueous humor rose quickly and reached the maximum within 30 minutes post-dosing (1-3). The formation of its major metabolite dihydrobunolol in the cornea was pH-dependent and capacity-limited (3). However, the effect of formulation pH on the ocular absorption of levobunolol has not been reported. The ocular pharmacokinetics of pilocarpine has been well documented (4-7). Similar to levobunolol, pilocarpine was rapidly absorbed into the eye, reaching maximum aqueous humor concentration within 30 minutes post-dosing in both rabbit and man (4,7). Because of the rapid ocular absorption of levobunolol and pilocarpine, aqueous humor samples were collected within 30 minutes post ocular drug instillation to rabbits to detect the effect of concomitant drug administration on the ocular bioavailability of levobunolol and pilocarpine.

Ion-pairing agents have been used to improve ocular absorption of cromoglycate and several cationic drugs (8,9). Due to the reduced ocular bioavailability of levobunolol at low pH, anionic sulfosuccinates of low ocular irritation and toxicity were utilized in order to improve ocular absorption of ionized levobunolol.

METHODS AND MATERIALS

Schercopol-CMS (SP-CMS, disodium cocamido MEA sulfosuccinate) and Varsulf SBL 203 (V-SBL, disodium lauramido MEA sulfosuccinate) were obtained from Scher Chemicals (Clifton, NJ) and Sherex Chemical Co. (Dublin, OH), respectively. All other chemicals and reagents were of the highest grade available unless noted otherwise.

The 0.25% and 0.5% (w/v) levobunolol HCl ophthalmic solutions at pH 7.2 were commercial preparations (Betagan™, Allergan, Inc., Irvine, CA). Naive female New Zealand albino rabbits, weighing 2 to 3.5 Kg, were used.

Partitioning Studies

The distribution coefficients of levobunolol (LBUN) and pilocarpine (PILO) were measured by mixing equal volumes of n-octanol and the formulation of 0.5% LBUN/4% PILO at pH 5.5 containing sulfosuccinates at different concentrations. The two phases were allowed to separate and equilibrate overnight. Concentrations of levobunolol and pilocarpine in both phases were measured by HPLC.

Ocular Absorption of Levobunolol and Pilocarpine In Vitro:

Four ophthalmic solutions were used to investigate the effect of pH and pilocarpine on levobunolol absorption:
A 100 μl aliquot of each formulation was introduced into the anterior chamber of a corneal perfusion system. The flow through the anterior chamber, facing the corneal epithelium, was approximately 0.055 ml/min, simulating the tear turnover in vivo. A detailed description of this perfusion system was reported previously (10). The effluent from the anterior chamber and aliquots from the posterior chamber were collected periodically for three hours post-dosing and the concentrations of levobunolol, its metabolite (dihydrobunolol, DHB), and pilocarpine were assayed by HPLC. At the end of experiment, the cornea was removed, weighed, and extracted with ethanol for assay of drug contents.

The ocular absorption of levobunolol and pilocarpine from additional formulations, LP5.5 fortified with 0.2%, 0.5%, and 1.5% (w/v) Schercopol-CMS, were also evaluated.

Ocular Absorption of Levobunolol In Vivo:

Rabbits were randomly divided into groups of fifteen. All animals received one eyedrop (50 μl) instillation in the left eye. The ophthalmic solutions used were 0.5% levobunolol HCl/2% pilocarpine HCl at pH 5.5 or 0.25% levobunolol HCl at pH 7.2. The sacrifice times were 10 and 30 minutes post-dosing. After animal euthanasia with an intravenous dose of T-61 (American Hoechst Corp., Somerville, NJ), aqueous humor and cornea were collected and assayed for levobunolol and dihydrobunolol.

Two additional groups of eight rabbits were administered one 50 μl dose of the 0.5 % LBUN/4% PILO formulations at pH 5.5 with or without 1.5% Schercopol-CMS. At 15 minutes post-dosing, rabbits were euthanized and cornea and aqueous humor samples collected for drug assay.

HPLC Analysis

Pilocarpine and levobunolol were separately quantified. Levobunolol and dihydrobunolol concentrations in ocular tissues and fluids were measured by an HPLC method with a dual UV/fluorescence detection mode (11). Pilocarpine was quantitated separately using a previously published HPLC method (12). Both methods have well-documented accuracy, precision, and selectivity (11,12).

DATA ANALYSIS

The distribution coefficient (DC) was the ratio of the drug concentrations in the n-octanol phase and in the aqueous phase. The Setschenow constant (13) was calculated using the following equation:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Code</th>
<th>Levobunolol</th>
<th>Pilocarpine</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>LP5.5</td>
<td>0.5%</td>
<td>4%</td>
<td>5.5</td>
</tr>
<tr>
<td>II</td>
<td>L5.5</td>
<td>0.5%</td>
<td>-</td>
<td>5.5</td>
</tr>
<tr>
<td>III</td>
<td>P5.5</td>
<td>-</td>
<td>4%</td>
<td>5.5</td>
</tr>
<tr>
<td>IV</td>
<td>LP7.2</td>
<td>0.5%</td>
<td>4%</td>
<td>7.2</td>
</tr>
</tbody>
</table>
$$K_S = \frac{\log(\frac{DC}{DC_0})}{C}$$

where DC and DC$_0$ were the distribution coefficients measured in the presence and in the absence of sulfosuccinate in the dosage, respectively, and C was the molar sulfosuccinate concentration in the solution dosage. This number is an indicator of whether a salting-out effect could account for the shift in DC.

The buffering capacity was calculated by titration of the solutions, levobunolol with or without pilocarpine, with sodium hydroxide solutions of known molarity. The difference in buffering capacity in the stated pH region was the difference in the amount of sodium hydroxide needed to titrate the solution upward one pH unit.

In vitro ocular bioavailability was determined as the percent of the dose recovered as the drug and metabolite from the posterior chamber. Mean absorption time of the drug (MAT) was $AUC_{X_{max}-X_t}$ divided by $X_{max}$, where $X_{max}$ was the amount of drug in the posterior chamber when the absorption was complete and $AUC_{X_{max}-X_t}$ was the trapezoidal area under the $X_{max}-X_t$ and time curve during the absorption period (14).

RESULTS

Levobunolol, dihydrobunolol, and pilocarpine were quantitatively recovered in the in vitro absorption experiments. Approximately 0.5 to 0.75% of the pilocarpine dose penetrated the cornea after a bolus dose into the anterior chamber (Table I). The absorption of pilocarpine increased by 50% when the pH in the formulation increased from pH 5.5 to 7.2. The ocular absorption of levobunolol ranged between 0.6 to 1.9% of the dose and was significantly increased when the formulation pH increased from 5.5 to 7.2 and when 4% pilocarpine was removed from the dosage (Table I). The ocular absorption of levobunolol was lowest among the three solution dosages when the dose contained 4% pilocarpine at pH 5.5. Approximately 40% of the levobunolol dose that was absorbed into the posterior chamber in vitro was

Table I. In vitro Ocular Bioavailability of Pilocarpine and Levobunolol$^1$.

<table>
<thead>
<tr>
<th></th>
<th>Pilocarpine (%) Dose Penetrated</th>
<th>LBUN + DHB (%) Dose Penetrated</th>
<th>% Absorbed As DHB</th>
<th>MAT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% LBUN &amp; 4% PILO at pH 5.5 (LP5.5)</td>
<td>0.471 (0.148)$^*$</td>
<td>0.552 (0.129)$^+$</td>
<td>46.4 (6.1)</td>
<td>27.8 (4.0)</td>
</tr>
<tr>
<td>0.5% LBUN at pH 5.5 (LP5.5)</td>
<td>NA</td>
<td>1.88 (0.54)$^+$</td>
<td>38.9 (8.2)</td>
<td>24.2 (5.0)</td>
</tr>
<tr>
<td>4% PILO at pH 5.5 (LP5.5)</td>
<td>0.641 (0.163)</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>0.5% LBUN &amp; 4% PILO at pH 7.2 (LP7.2)</td>
<td>0.745 (0.154)$^*$</td>
<td>1.15 (0.23)$^+$</td>
<td>45.3 (3.9)</td>
<td>22.5 (5.0)</td>
</tr>
</tbody>
</table>

$^1$ Mean (SD), n=4 to 7.
$^* +$ Statistically significantly different, p<0.05.
the metabolite, dihydrobunolol, formed in the transcorneal penetration process. The metabolism and mean absorption time across the cornea of levobunolol were not affected by pilocarpine.

In the *in vivo* study, there were no significant differences in corneal concentrations of levobunolol and dihydrobunolol between the 0.5% LBUN/2% PILO pH 5.5 and 0.25% LBUN pH 7.2 formulations at both time points (Figure 1). However, the aqueous humor concentrations of levobunolol and dihydrobunolol after dosing with the 0.5% LBUN/2% PILO pH 5.5 formulation were approximately half of those observed after dosing of the 0.25% LBUN pH 7.2 formulation, indicative of a four-fold reduction in
Table II. The Enhancement Effect of Schercopol-CMS on the Ocular Bioavailability of Pilocarpine and Levobunolol from a 0.5% Levobunolol and 4% Pilocarpine Ophthalmic Solution at pH 5.5 in vitro.1

<table>
<thead>
<tr>
<th>Concentration of Schercopol-CMS</th>
<th>Pilocarpine (% Dose Penetrated)</th>
<th>Levobunolol (% Dose Penetrated)</th>
<th>% Absorbed As DHB</th>
<th>MAT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>0.471 (0.148)</td>
<td>0.552 (0.129)†</td>
<td>46.4 (6.1)†</td>
<td>27.8</td>
</tr>
<tr>
<td>0.2%</td>
<td>0.649 (0.178)</td>
<td>0.917 (0.185)</td>
<td>30.5 (5.2)</td>
<td>22.8</td>
</tr>
<tr>
<td>0.5%</td>
<td>0.750 (0.240)</td>
<td>0.952 (0.321)</td>
<td>26.1 (9.5)</td>
<td>23.1</td>
</tr>
<tr>
<td>1.5%</td>
<td>0.735 (0.234)</td>
<td>1.03 (0.28)</td>
<td>23.6 (3.5)</td>
<td>23.6</td>
</tr>
</tbody>
</table>

1 Mean (SD), n=4 to 7.
† Statistically significantly different from all Schercopol-CMS formulations, p<0.05.

intraocular absorption of levobunolol in the presence of 2% pilocarpine at pH 5.5.

The effect of both sulfosuccinates (SP-CMS and V-SBL) on the distribution coefficient of levobunolol and pilocarpine are shown in Figure 2. As the sulfosuccinate concentration in the dosage increased to 0.75% and 1.25% for V-SBL and SP-CMS, respectively, the partitioning of both levobunolol and pilocarpine into n-octanol was enhanced by an order of magnitude. The Setschenow constant was greater than 28 and 39 at all sulfosuccinate concentrations tested for pilocarpine and levobunolol, respectively. Since a molar proportionality constant between 0.1 and 1 is expected for salting-out phenomena (13,15), the large KS values in our experiment suggest micelle formation or ion-pairing rather than salting-out.

When corneal penetration of levobunolol and pilocarpine was examined in vitro, SP-CMS significantly enhanced levobunolol absorption without changing the MAT (Table II). SP-CMS significantly reduced corneal metabolism of levobunolol to dihydrobunolol. The corneal penetration of pilocarpine was not affected by SP-CMS. When ocular absorption of levobunolol was evaluated in vivo, SP-CMS significantly improved the ocular absorption of levobunolol in the cornea and aqueous humor at pH 5.5 (Figure 3).

DISCUSSION

Although drug concentrations in both the cornea and aqueous humor were measured from the in vivo study, aqueous humor data had less variability and were more reliable in predicting ocular absorption of levobunolol. Because the sampling time points were close to the dosing time, it was likely that the corneal surface contained variable amounts of residual drug from the dosages. Therefore, the aqueous humor concentration was a more sensitive indicator of ocular bioavailability than the corneal drug concentrations.
Levobunolol is a base with a pKa of 9.3 and has relatively little buffering capacity in the pH range of 6 to 7. The commercial ophthalmic solution was weakly buffered at pH 7.2 and produced good ocular bioavailability. At pH values lower than neutrality, levobunolol is more than 99% ionized, resulting in reduced corneal absorption and metabolism (6). However, if the ophthalmic solution of levobunolol was weakly buffered at acidic pH (e.g., the 0.5% LBUN formulation at pH 5.5), the ocular absorption and metabolism of levobunolol would not be significantly affected because the tears would titrate the instilled drug solution up to the physiologic pH.
Concentrations of levobunolol (LBUN) and dihydrobunolol (DHB) in cornea and aqueous humor 15 minutes after one eyedrop instillation of a 0.5% LBUN solution at pH 7.2, a 0.5% LBUN/4% pilocarpine (PILO) solution at pH 5.5, or a 0.5% LBUN/4% PILO/1.5% Schercopol CMS solution at pH 5.5 to rabbit eyes. *Signifies statistically significant differences between the latter two formulations.

range within a short time. The effects of pH and buffering on the absorption of ionizable compounds have been discussed in the literature (16-19).

Pilocarpine has a pKa of 6.85. Its ocular absorption is also dependent upon pH (Table I) (19). The molar concentration of a 4% pilocarpine solution is 164 mM. Adding 4% pilocarpine to solution causes a 90-fold increase in buffer capacity at pH 6. Since pilocarpine is stable only within a narrow pH range (i.e., 4 to 6), it presents a challenge to formulate a combination solution dosage providing both good pilocarpine stability as well as good levobunolol bioavailability. At pH 5.5, the combination dosages containing 2% or 4% pilocarpine have high buffer capacity and are very resistant to pH changes towards the basic direction. Our study results demonstrate that the ocular
absorption of levobunolol was significantly reduced by formulating levobunolol and pilocarpine together in solution at pH 5.5 so that desirable shelf-life for pilocarpine can be achieved.

In this study, we have chosen sulfosuccinates with low ocular adverse effects as ion-pairing agents. The critical micellar concentration of SP-CMS in pH 7.4 0.01 M phosphate buffer is 0.06%, as measured by tensiometry (20), which is lower than the concentrations used in this study. Therefore, some micelle or complex coacervate formation and single ion-pairing might have occurred. The larger complexes could act as reservoirs for ion-pair formation upon instillation at the corneal surface. SP-CMS improved the ocular bioavailability of levobunolol which was reduced in the presence of high concentrations of pilocarpine (2 to 4%) at pH 5.5. Acute toxicological studies indicated that a concentration of 1.5% SP-CMS produced corneal toxicity but not at 0.2%. Therefore, SP-CMS could potentially be used to formulate a safe and comfortable ophthalmic dosage.

The distribution coefficient data suggested the sulfosuccinates formed ion-pairs with levobunolol and pilocarpine. The levobunolol-sulfosuccinate ion-pairs had approximately two orders of magnitude higher log DC values, in a range favorable for corneal penetration, than the pilocarpine-sulfosuccinate ion-pairs. This explains the penetration enhancement effect on levobunolol, but not on pilocarpine, of Schercopol CMS in this study. It is of interest to note that the ion-pair formation with sulfosuccinate did not significantly alter the mean absorption time of levobunolol across the cornea. This suggests that the main chemical species penetrating the cornea was the free form of levobunolol and that the physical environment within the cornea was not altered by the sulfosuccinate. It is possible that the environment in the corneal epithelium was not conducive to ion-pair formation since ion-pairing is a solvent-medicated phenomenon. Thus, ion-pairing would primarily occur at the tear-cornea interface, the effect sufficient to enhance the extent of drug absorption into the cornea.

Our study results demonstrated a four-fold decrease in ocular bioavailability of levobunolol due to the presence of pilocarpine in the same ophthalmic solution. Many combination dosages were developed to improve patient compliance and convenience. In the case of developing a combination dosage for β-adrenergic antagonist (pka ~8 and 9) and pilocarpine, the clinical implications of reduced but not additive therapeutic efficacy should be considered.

REFERENCES


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