Improved Corneal Penetration of Timolol by Prodrugs as a Means to Reduce Systemic Drug Load

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A major problem in the use of timolol in glaucoma therapy is its relatively high incidence of cardiovascular and respiratory side effects. This report presents preliminary evidence for the feasibility of a prodrug approach to minimize systemic absorption of timolol using a reduced topical dose made possible by improved corneal drug penetration. The results indicate that the O-acetyl, propionyl, butyryl, and pivalyl ester prodrugs of timolol were virtually completely hydrolyzed between 30 and 150 min when incubated with plasma, aqueous humor, and ocular tissue homogenates of pigmented rabbits. Moreover, the in vitro corneal penetration of all but the O-pivalyl prodrug was 2–3 times higher than timolol. This was accompanied by a four- to sixfold increase in aqueous humor concentration at 5 and 30 min post-installation of 15 mM solutions, although the plasma timolol concentration at the same time points was either unaltered or slightly reduced. Collectively, these preliminary findings suggest that at least a twofold reduction in the topical dose of timolol is possible by using prodrugs, thereby reducing timolol concentration in the systemic circulation and possibly the incidence of cardiovascular and respiratory side effects. Invest Ophthalmol Vis Sci 28:487–491, 1987

A major problem in the use of timolol in glaucoma therapy is its relatively high incidence of cardiovascular and respiratory side effects. This has prompted the screening of several other beta blockers, such as d-timolol,1 betaxolol,2 labetalol,3 and S-32-468,4 which are either expected or proven to be as effective as timolol, but with fewer side effects. This report presents preliminary evidence for the feasibility of a prodrug approach to minimize systemic absorption of timolol using a reduced topical dose made possible by improved corneal drug penetration. Specifically, the O-acetyl, propionyl, butyryl, and pivalyl esters of timolol were evaluated with respect to ocular hydrolysis, in vitro corneal transport, and ocular and systemic absorption from topical solution instillation in pigmented rabbits.

Materials and Methods

Materials

Male, Dutch belt pigmented rabbits, weighing 2.5–3.0 kg, were purchased from ABC Rabbitry (Pomona, CA). Timolol maleate and propranolol HCl were purchased from Sigma Chemicals (St. Louis, MO). O-Acetyl, propionyl, butyryl, and pivalyl timolol HCl salts were prepared as described elsewhere. O-Acetyl timolol, O-propionyl timolol, O-butyryl timolol, 9.2 min for O-pivalyl timolol, and 9.7 min for propranolol, the internal standard. The assay sensitivity was 0.5 nmol with respect to timolol and its prodrugs. The intra- and interrun variations were less than 5% and 7.5%, respectively.

In Vitro Ocular Hydrolysis of Timolol Prodrugs

This was studied by incubating, in triplicate, 50 μl each of a 0.2 mM timolol ester solution and of aqueous humor or an ocular tissue homogenate at 37°C for up...
to 360 min. Homogenates of freshly excised conjunctiva, corneal epithelium, corneal stroma-endothelium, and iris-ciliary body were prepared using a Potter-Elvehjem tissue homogenizer followed by centrifugation at 3,020 × g at 4°C for 10 min. The supernatants were adjusted to a protein concentration of about 1.2 mg/ml by adding 1.17% KCl solution.

The enzymatic reaction was terminated by adding 150 μl of acidified acetonitrile. Fifty μl of a 25 μg/ml propranolol solution was added to the supernatant, and 5–40 μl of the mixture was injected, in duplicate, into the HPLC. After correcting for chemical hydrolysis, the susceptibility of each prodrug to enzymatic hydrolysis in anterior segment tissue homogenates was expressed as nmol of prodrug hydrolyzed per min per mg protein.

In Vitro Corneal Permeation of Timolol Prodrugs

Pigmented rabbits were killed by an overdose of intravenous sodium pentobarbital solution. The eyes were enucleated with the conjunctivae and lids intact, and the corneas were excised and mounted in a lucite perfusion chamber exposing a surface area of 0.95 cm², as described by Hull et al.7 Two and one-half ml of glutathione bicarbonate Ringer's solution,7 pre-adjusted to pH 7.4, was added to the reservoir bathing the endothelial side. An equal volume of this solution containing 10 mM of timolol or its prodrug was immediately added to the epithelial side. Mixing in each chamber was achieved by bubbling a 95% O₂-5% CO₂ mixture at a rate of three to four bubbles per sec. The temperature of the bathing fluids was maintained at 34 ± 1°C by a circulating water bath.

At predetermined times for up to 4 hr, 100 μl of solution was sampled from the endothelial side and immediately replaced by an equal volume of bathing solution. After adding an equal volume of 0.1 N HCl containing 5 μg/ml propranolol to the sample collected, the mixture was immediately analyzed by HPLC.

At the end of each experiment, the cornea was trimmed of scleral and conjunctival tissues, weighed, dried overnight in an oven at 105°C, and reweighed. The hydration level of the corneas thus determined was 75 ± 2.5%, well within the physiological range on degree of hydration. At least six corneas were used per compound.

Ocular and Systemic Absorption of Topically Applied Timolol Prodrugs

Rabbits were placed in polycarbonate restraining boxes with no restriction in head or eye movement. Twenty-five μl of a 15 mM timolol or prodrug solution in isotonic 10 mM Tris buffer (pH 7.4) was instilled to the conjunctival sac of each rabbit as previously described.8 At 5 or 30 min post-dosing, the rabbit was killed by an overdose of pentobarbital solution administered via a marginal ear vein. The eyes were immediately rinsed with saline and blotted dry, and about 100–150 μl of aqueous humor was aspirated from the anterior chamber. One min prior to killing the animal, 5 ml of blood was collected from a precannulated ear artery and was immediately centrifuged at 4°C to yield plasma. Both aqueous humor and plasma samples were frozen immediately and stored at −20°C until assayed.

At the time of assay, an aqueous humor sample was mixed with an equal volume of methanol containing 6% perchloric acid and 0.5 μg/ml propranolol. After centrifugation, 10 to 20 μl of the supernatant was in-
Fig. 3. Percent of timolol prodrug in hydrolyzed form over the time course of corneal permeation in vitro. Error bars represent standard error of the mean for an average of six corneas. Plot A, O-acetyl timolol; plot B, O-propionyl timolol; plot C, O-butyryl timolol; plot D, O-pivalyl timolol.

Results

Figure 1 shows the initial hydrolytic rate of various timolol prodrugs in plasma, aqueous humor, and homogenates of anterior segment tissues of the pigmented rabbit. Except in the corneal stroma and aqueous humor, the butyryl ester was hydrolyzed most rapidly, followed by the propionyl, acetyl, and pivalyl esters. This rank order of susceptibility to hydrolysis in a given tissue was in agreement with that observed in l-naphthyl esters. However, the hydrolysis of O-pivalyl timolol in iris-ciliary body homogenate was three times slower than dipivalyl epinephrine. The latter is a phenolic ester and is therefore expected to be more reactive than the aliphatic timolol esters. Collectively, these prodrugs were somewhat more susceptible to hydrolysis in plasma than in ocular tissue homogenates. The O-butyryl prodrug was most susceptible to hydrolysis in the iris-ciliary body homogenate, followed by homogenates of the conjunctiva, corneal stroma, and corneal epithelium.

Figure 2 shows the total amount of timolol, ie, prodrug plus timolol formed, permeated across isolated corneas, and Figure 3 shows the percent of each prodrug in hydrolyzed form over the time course of corneal permeation. At 10 min, over 30% of each prodrug was in its hydrolyzed form, and by 120 min all but the O-pivalyl prodrug were totally hydrolyzed. Overall, the relative rates of timolol formation from the various prodrugs during corneal permeation, as shown in Figure 3, were consistent with the in vitro incubation results shown in Figure 1. From the plots in Figure 2, the apparent corneal permeability coefficient and corneal transport lag time of timolol and its prodrugs were determined. These parameters are summarized in Table 1 along with the n-octanol/pH 7.4 buffer distribution coefficients* of timolol and its prodrugs. Except

* The distribution coefficient was the ratio of timolol or prodrug concentration in n-octanol and buffer after shaking these two phases at 22°C for 5 min.
Figure 4. Total timolol concentration in aqueous humor and plasma of pigmented rabbits at 5 and 30 min post-instillation of 25 µl of 15 mM solutions of timolol and its prodrugs. At 5 min, the percent of timolol prodrug in hydrolyzed form in the aqueous humor was 74.3%, 85.4%, 88.3%, and 33.8% for the O-acetyl, propionyl, butyryl, and pivalyl esters, respectively. At 30 min, this increased to 98.2%, 100%, 100%, and 82.8% for the respective prodrugs. In the plasma, no intact prodrug was detected for any prodrug at either 5 or 30 min. Error bars represent standard error of the mean. Asterisks denote results which were significantly different from timolol administration ($P < 0.01$). At least six rabbits (12 eyes) were used per time point for each compound. Key: ○, timolol; ●, O-acetyl timolol; □, O-propionyl timolol; ▲, O-butyryl timolol; △, O-pivalyl timolol.

Figure 5 shows the total timolol concentration in the aqueous humor at 5 and 30 min following instillation of a 15 mM timolol solution was consistent with literature results.$^{11,12}$

Figure 5 shows that the timolol concentrations in the aqueous humor at 5 and 30 min post-instillation of 15 mM solutions of timolol or its prodrugs correlated well with the corneal permeability coefficients of these compounds determined in vitro.

Discussion

As a result of esterification, the lipophilicity of timolol was considerably increased (Table 1). This led to a four- to sixfold increase in the corneal absorption of timolol following topical prodrug administration (Fig. 4). Such a factor of increase was consistent with in vitro corneal transport results (Fig. 5). The systemic absorption of timolol was either unaffected or slightly reduced by moderate increases in lipophilicity, possibly because timolol already easily penetrated the conjunctival and nasal epithelia and, in turn, the underlying blood vessels. Consequently, further increases in lipophilicity did not appreciably enhance the systemic absorption of timolol.

The facile hydrolysis of all timolol prodrugs in ocular tissue homogenates indicates that timolol will be quantitatively regenerated upon ocular absorption (Fig. 1). Prodrug hydrolysis in the corneal epithelium has the additional effect of continuously lowering prodrug concentration in this tissue, thereby maximizing the driving force for prodrug absorption and possibly contributing to enhancement in the corneal absorption of timolol. The unexpectedly facile hydrolysis of timolol prodrugs in the aqueous humor, as well as in corneal stromal homogenate, was probably not mediated by esterases but possibly by solubilized mucopolysaccha-

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Table 1. Distribution coefficient, apparent corneal permeability coefficient, and corneal transport lag time of timolol and its prodrugs

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Distribution Coefficient</th>
<th>Corneal Permeability Coefficient (10^5 cm/s)*</th>
<th>Lag Time (min)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timolol</td>
<td>H</td>
<td>2.4</td>
<td>1.82 ± 0.32</td>
<td>40.8 ± 2.7</td>
</tr>
<tr>
<td>O-Acetyl Timolol</td>
<td>CH3CO</td>
<td>13.0</td>
<td>3.83 ± 0.42</td>
<td>15.4 ± 0.8</td>
</tr>
<tr>
<td>O-Propionyl Timolol</td>
<td>CH3(CH2)CO</td>
<td>42.0</td>
<td>4.89 ± 0.09</td>
<td>16.0 ± 0.2</td>
</tr>
<tr>
<td>O-Butyryl Timolol</td>
<td>CH3(CH2)2CO</td>
<td>120.0</td>
<td>5.30 ± 0.41</td>
<td>22.1 ± 1.2</td>
</tr>
<tr>
<td>O-Pivalyl Timolol</td>
<td>(CH3)3CCO</td>
<td>479.0</td>
<td>2.44 ± 0.57</td>
<td>66.0 ± 0.1</td>
</tr>
</tbody>
</table>

* Mean ± SD.

rives. Neither the aqueous humor nor corneal stroma is enriched with esterases.13

Overall, these preliminary findings suggest that, due to improved corneal absorption and unaltered or slightly reduced systemic absorption of timolol by prodrugs, the instilled dose of timolol can be reduced by at least two times, thereby reducing systemic drug load. This twofold dose reduction may be significant given the potency of timolol in causing cardiovascular and pulmonary side effects. Of the four prodrugs, the butyryl and pivalyl prodrugs warrant further investigation with respect to systemic absorption as well as ocular pharmacokinetics and pharmacodynamics. This is because these prodrugs represent extremes in corneal penetration and ocular hydrolytic rates. Further work is also necessary to determine the basis for the apparently unaltered or reduced systemic absorption of timolol following prodrug administration. Finally, since these prodrugs suffer from aqueous instability to the extent that aqueous solutions with a minimum shelf life of 2 yr are not feasible, work is in progress to determine the ocular and systemic bioavailability of timolol prodrugs from non-aqueous lipophilic vehicles, which are anticipated to confer enhanced chemical stability to these compounds.

Key words: timolol, prodrugs, corneal absorption, systemic absorption, ocular hydrolysis

References