Low Dose O-Butyryl Timolol Improves the Therapeutic Index of Timolol in the Pigmented Rabbit

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The degree of enhancement in the ocular absorption of timolol afforded by its O-butyryl prodrug and the possibility of reducing its systemic absorption through a proportional reduction in its instilled dose were investigated in the pigmented rabbit. A reversed phase HPLC procedure was used to assay for timolol and O-butyryl timolol in the aqueous humor and plasma over 120 min post-instillation of 25 μl of drug solutions. We found that the ocular absorption of the O-butyryl prodrg of timolol from a 15 mM solution was 5.5 times greater than that of the parent drug while the systemic absorption was comparable. When a lower concentration of the prodrug (3.75 mM) was used the therapeutic index as assessed by the ratio of aqueous humor to plasma concentrations seemed to improve fifteenfold. Therefore, it is possible to significantly reduce the systemic absorption of timolol without reducing its ocular absorption by using a low dose of its O-butyryl prodrug. Invest Ophthalmol Vis Sci 29:626-629, 1988

Timolol prodrugs are bioreversible derivatives of timolol designed to improve its ocular absorption through increased lipophilicity.1 As a result, either a smaller topical dose or a reduced dosing frequency may be used, thereby reducing the extent of systemic timolol absorption and hence the risk of systemic side effects. Recently, we reported that four prodrug esters of timolol—O-acetyl, O-propionyl, O-butyryl, and O-pivalyl timolol—did not alter the plasma timolol concentration in the pigmented rabbit even though they caused a four- to sixfold increase in the timolol concentration in the aqueous humor at 5 and 30 min post-instillation of 15 mM solutions. These findings suggest that at least a two- to fourfold reduction in the topical dose of timolol would be possible by using such prodrugs, perhaps achieving the same intraocular pressure lowering effect as a higher dose of timolol while significantly reducing the systemic drug load.

The purpose of this study was to investigate whether it was possible to reduce the systemic absorption of timolol in the pigmented rabbit by reducing the topical dose of O-butyryl timolol in proportion to the degree of enhancement in corneal absorption observed previously.2 This prodrug was selected for further study because it best improved the corneal absorption of timolol and was the most susceptible to ocular hydrolysis, which generates the parent drug.1,2 In this study, timolol concentrations in the aqueous humor and plasma were monitored periodically for up to 120 min following the topical instillation of 15 mM solutions of O-butyryl timolol or timolol. These were then compared with those obtained from 3.75, 7.5, and 11.5 mM solutions of each compound. In each case, the area under the concentration-time curve (AUC) was used as an index of ocular or systemic drug absorption. The AUC was calculated using the trapezoidal rule with extrapolation to infinity.3

Materials and Methods

Materials

Male, Dutch belt pigmented rabbits, weighing 2.5–3.0 kg, were purchased from American Rabbity (Los Angeles, CA). Timolol maleate and propranolol HCl were purchased from Sigma Chemicals (St. Louis, MO). O-Butyryl timolol HCl was synthesized and purified as described elsewhere.1 Solutions of this prodrug were prepared immediately before each experiment in a 10 mM TRIS HCl buffer (pH 7.4) and rendered isotonic by adding NaCl. Timolol and O-butyryl timolol were assayed using an HPLC procedure as described previously.2

Systemic Absorption of Topically Applied O-Butyryl Timolol

Rabbits, six in a group, were placed in polycarbonate restraining boxes with no restriction in head or
eye movement. Twenty-five microliters of a 15 mM O-butyryl timolol solution were instilled in the conjunctival sac of both eyes of each rabbit. At 3, 6, 10, 15, 30, 45, 60, 90 and 120 min post-dosing, 2 ml of blood was collected from a precannulated ear artery, transferred into centrifuge tubes, and immediately centrifuged at 4°C to yield plasma. The plasma samples were frozen immediately and stored at -20°C until assayed by HPLC. The volume of blood aspirated was replenished with an equal volume of dextrose solution. After a 7-day washout period, the experiment was repeated with the same rabbits using a 15 mM timolol solution.

Ocular Absorption of Topically Applied O-Butyryl Timolol

The rabbit handling procedure, the composition of the dosing solution, and the instilled dose volume were as described in the systemic absorption experiment. At 5, 15, 20, 30, 45, 60, 90 or 120 min, the rabbits were 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. The aqueous humor samples were frozen immediately and stored at -20°C until assayed by HPLC. At least three rabbits (six eyes) were used per time point. A separate group of rabbits was used for dosing with timolol.

Effect of Instilled Dose Reduction on Ocular and Systemic Absorption of O-Butyryl Timolol

The experiments described for the ocular and systemic absorption of 15 mM O-butyryl timolol were repeated using 3.75, 7.5 and 11.5 mM O-butyryl timolol solutions. Parallel experiments were conducted with timolol for comparison.

The investigations utilizing animals, as described in this report, conformed to the ARVO Resolution on the Use of Animals in Research.

Results

The time course of total concentration of timolol, i.e., prodrug plus timolol formed, in the aqueous humor and plasma over the 120 min post-instillation of 15 mM O-butyryl timolol solutions is displayed in Figure 1. Intact prodrug was never recovered in the plasma and was recovered in the aqueous humor to the extent of 15% or less only within the first 20 min of solution instillation.

Compared with timolol instillation, O-butyryl timolol provided higher drug concentrations in the aqueous humor but did not alter the apparent time at which peak drug concentration was achieved. Based on the area under the concentration-time curve, O-butyryl timolol provided a 5.5-fold enhancement in the ocular absorption of timolol. In contrast, the time course of total timolol concentration in the plasma was virtually superimposable to that seen after dosing with timolol, although the drug concentration at 3 min post-instillation of O-butyryl timolol appeared to be slightly higher than that from timolol instillation (Fig. 1).

When the instilled concentration of O-butyryl timolol was reduced to 3.75 mM, the aqueous humor timolol concentration was still about 75% higher than that obtained with 15 mM timolol, while the extent of systemic absorption was reduced ten times (P < 0.05 by a one-tailed student t-test). Although O-butyryl timolol was similar to timolol in the dependence of systemic absorption upon instilled dose, it penetrated the cornea into the aqueous humor better than timolol at each instilled dose (Fig. 2, Table 1).
Finally, the first order elimination rate constants of timolol and O-butyryl timolol from the aqueous humor and plasma were independent of the instilled dose concentration. These were calculated from the declining portion of a drug concentration-time profile using one-compartment pharmacokinetic analysis. In the aqueous humor, the elimination rate constant was 0.012 ± 0.001 min⁻¹ for timolol and 0.013 ± 0.002 min⁻¹ for O-butyryl timolol. In the plasma, it was 0.047 ± 0.002 min⁻¹ for timolol and 0.049 ± 0.005 min⁻¹ for O-butyryl timolol.

Discussion

Timolol is the most widely prescribed medication for control of open-angle glaucoma in the United States. It owes its popularity to its long duration of action and low incidence of ocular side effects when compared with other glaucoma medications, such as pilocarpine and epinephrine. Nonetheless, its therapeutic usefulness is occasionally limited by cardiovascular and respiratory side effects. These occur as a result of absorption of the topically applied drug into the systemic circulation. Although one may reduce the systemic absorption of topically applied timolol by reducing the instilled dose or by occluding the puncta following topical solution instillation, neither approach is fully satisfactory.

A more satisfactory solution appears to be the administration of a lipophilic prodrug of timolol. Because of its improved corneal absorption characteristics, more timolol would reach the aqueous humor, thereby permitting a reduction in either the dosing frequency or the instilled dose size. The results obtained with O-butyryl timolol in this study indicate that these desirable attributes of prodrugs can be achieved.

The supporting evidence is as follows. At an equivalent dose as timolol, O-butyryl timolol afforded a 5.5-fold increase in timolol concentration in the aqueous humor (Fig. 1, Table 1). Since the duration of action increases by one half-life with each doubling of dose, the duration of intraocular pressure lowering attainable with O-butyryl timolol would conceivably be extended by two and one-half half-lives or about 130 min (the half-life of elimination of timolol from the aqueous humor of the pigmented rabbit is 53 min). As a result, the dosing frequency of O-butyryl timolol may be reduced, thereby reducing the risk of suffering from the cardiovascular and respiratory side effects of timolol.

A companion strategy to reduce systemic drug load is to administer a lower dose of O-butyryl timolol in proportion to the degree of enhancement in ocular drug absorption. A fourfold reduction of the instilled...
dose of O-butyryl timolol resulted in a more than proportional (tenfold) reduction in systemic drug absorption (Fig. 1, Table 1). This finding suggests that reducing the instilled dose of O-butyryl timolol may be more advantageous than reducing its dosing frequency insofar as improving its therapeutic index is concerned.

The nonlinear reduction in the ratio of systemic to ocular absorption of timolol when the instilled concentration of O-butyryl timolol was decreased cannot yet be explained (Table 1). A possible basis for nonlinearity is saturation in either the specific uptake mechanisms by the cornea and the conjunctiva or the metabolic capacity of these ocular membranes. However, the lack of curvilinear dependency of ocular or systemic uptake on instilled dose for timolol, as shown in Figure 2, did not support specific uptake mechanisms being involved in the absorption of this drug. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12 In fact, no intact prodrug was recovered in either the aqueous humor or plasma at higher prodrug concentrations. This is also expected to be the case for O-butyryl timolol on the basis of similarities in chemical structure. Similarly, the lack of increases in the fraction of intact prodrug recovered in the aqueous humor or plasma at higher prodrug concentrations did not support the notion of saturation of corneal and conjunctival esterases.12

In conclusion, it is possible to significantly reduce the systemic absorption of timolol without reducing its ocular absorption by using a low dose of its O-butyryl prodrug. Work is in progress to determine the extent as well as duration of intraocular pressure lowering following the topical instillation of varying doses of O-butyryl timolol and other timolol prodrugs in pigmented rabbits with experimentally induced ocular hypertension.

**Key words:** timolol, prodrugs, O-butyryl timolol, ocular absorption, systemic absorption

**References**