Formulation Influence on Conjunctival Penetration of Four Beta Blockers in the Pigmented Rabbit: A Comparison with Corneal Penetration

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The objective of this study was to compare the influence of pH, tonicity, benzalkonium chloride, and EDTA on the conjunctival and corneal penetration of four beta blockers—atenolol, timolol, levobunolol, and betaxolol. Drug penetration was evaluated using the isolated pigmented rabbit conjunctiva and cornea in the modified Ussing chamber. The conjunctiva was more permeable than the cornea to all four beta blockers. Formulation changes caused larger changes in corneal than in conjunctival drug penetration, especially for the hydrophilic beta blockers, atenolol and timolol. Raising the solution pH to 8.4 caused the largest increase in corneal penetration for all drugs except atenolol. This increase was greater than that obtained by removing the corneal epithelium. The same formulation also increased conjunctival drug penetration, although to a lesser extent. In the case of timolol, the formulation changes evaluated brought about similar changes in its ocular and systemic absorption with good *in vitro–in vivo* correlations. The above findings indicate that in making formulation changes to maximize corneal drug penetration, it is necessary to evaluate possible changes in conjunctival drug penetration, hence systemic absorption. Moreover, because the conjunctiva plays an active role in the noncorneal route of ocular drug absorption, the relative contribution of the noncorneal to the corneal routes to ocular drug absorption may also be altered by formulation changes.

KEY WORDS: conjunctival drug penetration; corneal drug penetration; beta blockers; ophthalmic formulation; chelating agents; preservatives; pH; tonicity; *in vitro–in vivo* correlation.

INTRODUCTION

The conjunctiva is a thin mucous membrane lining the inside of the eyelids and the anterior sclera. It is a vascularized tissue which, together with the nasal mucosa, permits the absorption of topically applied drugs into the bloodstream (1). Because the conjunctiva occupies 17 times the surface area of the cornea in the human (2), conjunctival drug absorption of an ocularly applied dose should theoretically be more likely to occur than corneal drug absorption. Nevertheless, the influence of formulation composition on conjunctival drug penetration has never been reported, although its influence on corneal drug penetration has been evaluated by various investigators (3–5). Typical changes in ophthalmic formulations for stability and sterility reasons include adjustment in pH and tonicity as well as incorporation of preservatives and chelating agents.

The objectives of the present study were (a) to determine how changes in formulation composition would affect conjunctival drug penetration relative to corneal drug penetration, (b) to identify the formulation composition that would maximize the ratio of corneal to conjunctival penetration, (c) to determine how such a formulation influence would be affected by drug lipophilicity, and (d) to determine the correlation between corneal drug penetration and ocular drug absorption and between conjunctival drug penetration and systemic drug absorption. The model drugs used were atenolol, timolol, levobunolol, and betaxolol. These compounds have similar molecular weights and pK_a's (about 9.2) but have different lipophilicities. The logarithm of the *n*-octanol/pH 7.4 buffer partition coefficient is 0.15, 2.64, 3.22, and 3.65, respectively. All except atenolol have been used in the treatment of open-angle glaucoma. The majority of the experiments were conducted *in vitro* using the isolated cornea and conjunctiva in order to study corneal and conjunctival drug penetration independently and to avoid the complicating factor of precorneal drug clearance (6). In addition, ocular and systemic absorption experiments were conducted with timolol in order to determine how predictive were the *in vitro* data of the *in vivo* situation. Table I lists the various formulations tested.

MATERIALS AND METHODS

Materials

Male, Dutch-belted pigmented rabbits, 1.8–2 kg, were purchased from Irish Farm Rabbitry (Los Angeles, CA). Atenolol HCl, timolol maleate, benzalkonium chloride, and reduced glutathione were purchased from Sigma Chemical Co. (St. Louis, MO). Betaxolol HCl and levobunolol HCl were kindly supplied by Professor Hans Bundgaard of the Royal Danish School of Pharmacy (Copenhagen, Denmark) and by Warner-Lambert (Ann Arbor, Michigan), respectively. All reagents were used as received.

Assays

All beta blockers were assayed by HPLC under isocratic conditions on a reversed-phase Beckman Ultrasphere ODS C-18 column (4.6 × 250 mm; particle size, 5 μm). The mobile phase was a mixture of methanol and 0.2% triethylamine HCl solution adjusted to pH 3.0 with H_3PO_4. The flow rate was 1.0 ml min^{-1}. Table II lists the concentration of methanol in the mobile phase, internal standard, detection wavelength, retention time, and detection limit of each beta blocker. Samples from the corneal and conjunctival penetration experiments were injected directly into the HPLC. Samples from the ocular and systemic absorption experiments were processed prior to HPLC, as described elsewhere (9).

Corneal and Conjunctival Penetration of Beta Blockers

The corneal and conjunctival penetration of beta block-

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Table I. Formulations Tested in the Corneal and Conjunctival Penetration Experiments[a]

Parameter/additive	Value/concentration
pH	6.0, 7.4, 8.4
Osmolarity (mOsm/kg)	80 ± 10, 300 ± 10, 610 ± 30
Benzalkonium chloride	0.005, 0.0125, 0.025, 0.05%
EDTA	0.1, 0.5%

[a] Unless otherwise indicated, all formulations were at pH 7.4 and 300 mOsm/kg, containing neither benzalkonium chloride nor EDTA.

ers was evaluated in modified Ussing chambers as described by Lee *et al.* (7). The effect of epithelial integrity on the corneal penetration of beta blockers was investigated by conducting the experiment with corneas whose epithelial layers had been removed with a No. 11 scalpel prior to the start of the experiment.

Ocular and Systemic Absorption of Topically Applied Timolol

The dosing and sample processing procedures were as previously described (7,9). In both the ocular and the systemic absorption experiments, 25 μl of a 15 m*M* timolol maleate solution was instilled to each eye of four to six rabbits. Timolol concentrations at 30 min postdosing were used as an index of ocular timolol absorption. The area under the plasma timolol concentration–time curve over 120 min was used as an index of systemic timolol absorption.

RESULTS

The conjunctiva was significantly more permeable than the cornea to every compound studied. The penetration of beta blockers through the cornea and, to a lesser extent, the conjunctiva from isotonic GBR solution at pH 7.4 was found to increase with increasing lipophilicity (Fig. 1). Levobunolol was partially reduced to dihydrolevobunolol during penetration across the cornea. In isotonic pH 7.4 solutions containing neither benzalkonium chloride nor EDTA, dihydrolevobunolol contributed about 22% toward the total levobunolol flux (Fig. 2). Consistent with previous results (7), levobunolol was not metabolized during penetration across the deepithelized cornea or the conjunctiva.

Effect of Corneal Epithelial Integrity on Penetration of Beta Blockers

Removal of the corneal epithelium increased the corneal permeability to atenolol, the most hydrophilic compound studied, by 44 times. But this penetration enhancement effect decreased sharply with increasing lipophilicity of the penetrant. The penetration of betaxolol, the most lipophilic compound studied, was reduced (Fig. 1).

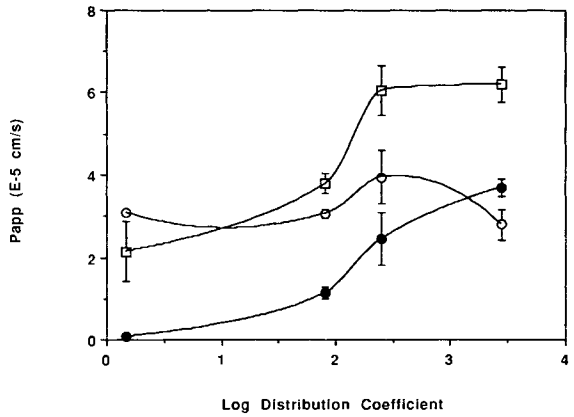

Fig. 1. Influence of drug lipophilicity on the permeability coefficients of beta blockers across the intact cornea (●), deepithelized cornea (○), and conjunctiva (□) of the pigmented rabbit. Error bars represent standard deviations for n = 4. From left to right: atenolol, timolol, levobunolol, and betaxolol.

Effect of pH on Corneal and Conjunctival Penetration of Beta Blockers

Generally, corneal penetration was more sensitive than conjunctival penetration to changes in the pH of the bathing medium, although the magnitude of these effects was dependent on the lipophilicity of the drug concerned (Fig. 3).

The corneal penetration of atenolol was not significantly affected by a pH over the range of 6.0 to 8.4 ($P = 0.08$ by ANOVA). Its conjunctival penetration was unchanged when the pH was raised from 6.0 to 7.4 but was more than doubled when the pH was raised from 7.4 to 8.4 ($P < 0.0003$).

Increasing the pH from 6 to 7.4 increased the corneal penetration of timolol 13.6 times ($P < 0.0001$) and its conjunctival penetration 15% ($P < 0.03$). Further increasing the pH to 8.4 brought about another 3.7 times increase in corneal penetration ($P < 0.0001$) and a 30% increase in conjunctival penetration ($P < 0.005$).

The corneal penetration of levobunolol followed a similar trend to that of timolol. As the pH was raised from 6.0 to 7.4, the corneal permeability coefficient was increased 20.7 times ($P < 0.001$). At pH 8.4 the permeability coefficient was 48.5 times higher than at pH 6.0 ($P < 0.001$). As can be seen in Fig. 2, the flux of the metabolite formed, dihy-

Table II. Mobile Phase Composition, Internal Standard, Detection Wavelength, Retention Time (t_R), and Detection Limit of Beta Blockers as Assayed by Reversed-Phase HPLC

Compound	% methanol	Internal standard	Wavelength (nm)	t_R (min)	Detection limit (n*M*)
Atenolol	20	Nadolol	225	11.8	35
Timolol	45	Propranolol	294	5.7	20
Levobunolol	55	Propranolol	225	5.3	30
Betaxolol	80	Benzoic acid	275	5.9	15

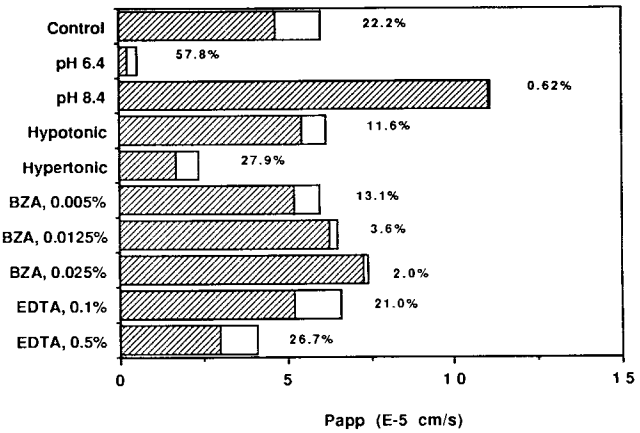

Fig. 2. Flux of levobunolol (▨) and dihydrolevobunolol formed (□) across the pigmented rabbit cornea from various formulations. The value next to each bar represents the percentage contribution of dihydrolevobunolol flux to total levobunolol flux.

drolevobunolol, was 4.3 times lower at pH 6.0 than at pH 7.4 ($P < 0.001$), but this represented a higher percentage of the total levobunolol transported, 58% at pH 6.0 as opposed to 22% at pH 7.4. Increasing the pH to 8.4 drastically reduced the flux of dihydrolevobunolol ($P < 0.001$), which contributed merely 0.6% toward the total levobunolol transported. The conjunctival penetration of levobunolol did not vary significantly with pH over the range of 6.0 to 8.4 ($P = 0.10$ by ANOVA), and no dihydrolevobunolol was formed.

The corneal penetration of betaxolol was increased 20.3 times as the pH was raised from 6.0 to 7.4 ($P < 0.0001$) but was not increased further when the pH was raised to 8.4 ($P = 0.04$). Its conjunctival penetration was reduced by 22% when the pH was raised from 6 to 7.4 but was increased by 17% upon raising the pH to 8.4 ($P < 0.03$).

Effects of Tonicity on Corneal and Conjunctival Penetration of Beta Blockers

The effects of varying solution tonicity on the penetration of beta blockers were more pronounced in the cornea than in the conjunctiva, although the nature of these effects was dependent on drug lipophilicity. As can be seen in Fig. 4, the corneal penetration of atenolol was unaffected by raising the tonicity from 284 to 582 mOsm/kg ($P = 0.05$) but was increased six times by lowering the tonicity to 83 mOsm ($P < 0.001$). The conjunctival penetration of atenolol followed a similar pattern, with no difference in penetration between isotonic and hypertonic conditions ($P = 0.05$) but with a doubling of penetration from hypotonic solutions.

The corneal penetration of timolol was doubled at low tonicity ($P < 0.01$) but was reduced by 1.6 times upon increasing the tonicity to 587 mOsm/kg ($P < 0.003$). Its conjunctival penetration was, however, increased by only 30% by lowering the tonicity to 80 mOsm/kg ($P < 0.001$) but, unexpectedly, was almost doubled by increasing the tonicity to 587 mOsm/kg ($P < 0.001$).

The corneal penetration of levobunolol was not as sensitive to a tonicity decrease from 300 to 67 mOsm/kg as atenolol or timolol ($P = 0.05$). There was a modest decrease in the flux of the dihydrolevobunolol formed ($P < 0.007$), resulting in a reduction in its contribution to the total flux from 22 to 12% (Fig. 2). At 637 mOsm/kg the flux of

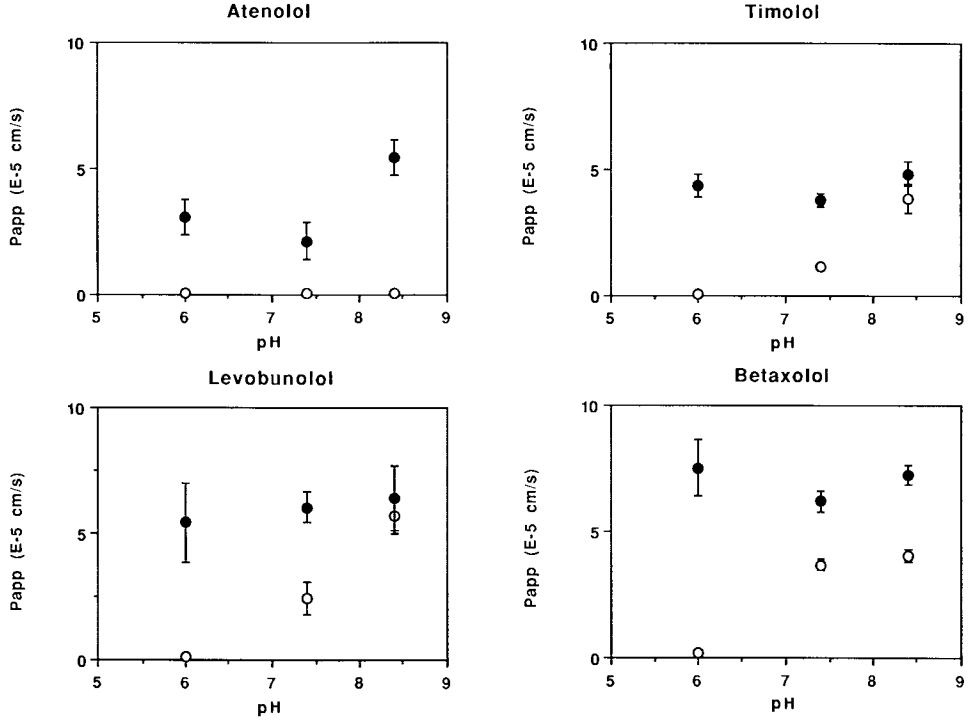

Fig. 3. pH influence on the corneal (○) and conjunctival (●) permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol. Error bars represent standard deviations for $n = 4$. Where not shown, the error bar is smaller than the size of the symbol.

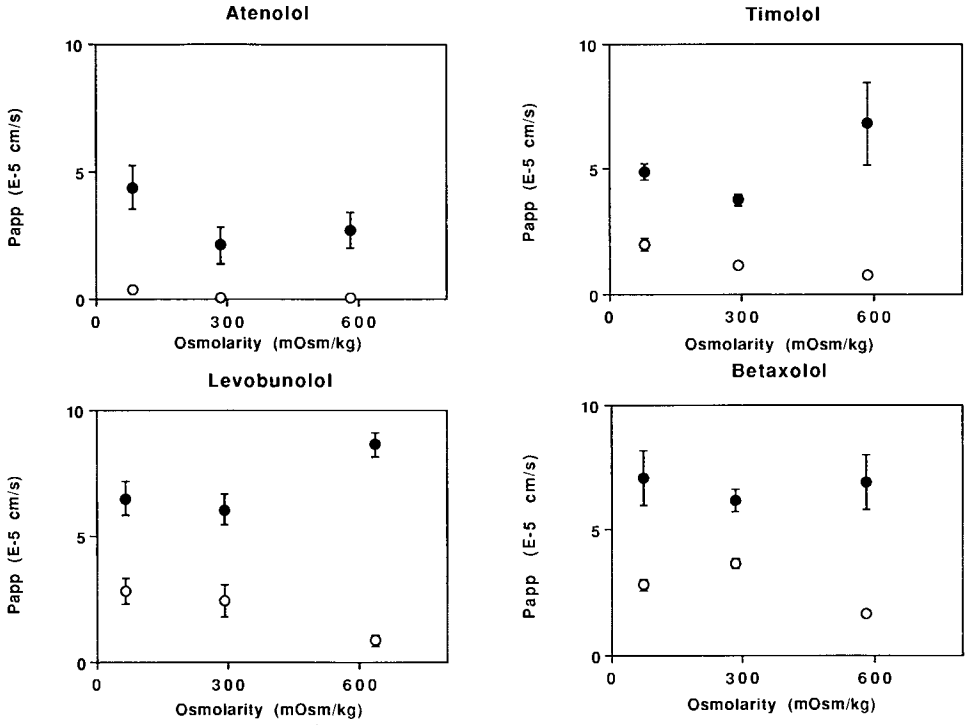

Fig. 4. Tonicity influence on the corneal (○) and conjunctival (●) permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol. Error bars represent standard deviations for $n = 4$. Where not shown, the error bar is smaller than the size of the symbol.

levobunolol was three times less than its flux under isotonic conditions ($P < 0.0008$), with a twofold reduction in the flux of the metabolite formed ($P < 0.001$). In contrast, the same increase in tonicity increased rather than decreased the conjunctival penetration of levobunolol, albeit by only 33% ($P < 0.001$). Lowering the tonicity from 292 to 67 mOsm/kg did not affect the conjunctival penetration of levobunolol ($P = 0.05$).

In the case of betaxolol, its corneal penetration was decreased by 23 and 55% under hypotonic and hypertonic conditions ($P < 0.006$), respectively, while its conjunctival penetration was not significantly affected by changes in tonicity over the range studied ($P = 0.05$ by ANOVA).

Effect of Benzalkonium Chloride on Corneal and Conjunctival Penetration of Beta Blockers

Benzalkonium chloride increased the conjunctival penetration of all four beta blockers; however, it had little effect on the corneal penetration of the more lipophilic beta blockers levobunolol and betaxolol. As the benzalkonium chloride concentration was raised from 0 to 0.025%, the corneal penetration of atenolol was increased 35 times, while its conjunctival penetration was increased 3.2 times ($P < 0.04$) (Fig. 5). In contrast, at a 0.025% benzalkonium chloride concentration, the corneal and conjunctival penetration of timolol was increased to a similar extent, 3.1 and 2.4 times ($P < 0.001$), respectively. Compared with timolol, both the corneal and the conjunctival penetration of levobunolol was less sensitive to changes in benzalkonium chloride concentration. The corresponding increase was 55 and 44% ($P < 0.003$). As shown in Fig. 2, the contribution of dihydrolevobunolol formed during corneal penetration to the total flux diminished very rapidly when the benzalkonium chloride concentration was increased from 0.005 to 0.0125%. The corneal penetration of betaxolol was not affected by adding 0.025% benzalkonium chloride to the formulation ($P = 0.05$), although its conjunctival penetration was increased by 41% ($P < 0.017$).

Effect of EDTA on the Corneal and Conjunctival Penetration of Beta Blockers

Depending on its concentration and drug lipophilicity, EDTA either increased, decreased, or left unaffected corneal and conjunctival penetration. EDTA at 0.05% increased the conjunctival penetration of atenolol 2.2 times and its corneal penetration 8.8 times ($P < 0.004$) (Fig. 6). At 0.5%, the conjunctival and corneal fluxes were increased by 3.3 and 31 times, respectively ($P < 0.001$). The corneal and conjunctival penetration of timolol was less sensitive to changes in EDTA concentration in the formulation. At 0.5% EDTA the corneal and conjunctival fluxes of timolol were, respectively, 1.9 and 1.6 times that of the control ($P < 0.001$). The corneal penetration of levobunolol, unaffected by 0.1% EDTA ($P = 0.10$), was reduced by 36% at 0.5% EDTA ($P < 0.005$). There was, however, essentially no change in the contribution of dihydrolevobunolol to the total levobunolol flux (Fig. 2). Conjunctival levobunolol penetration was also unaffected ($P = 0.10$). The same was true of the conjunctival flux of betaxolol ($P = 0.10$ by ANOVA), although 0.1% EDTA reduced its corneal flux by 31% ($P < 0.002$).

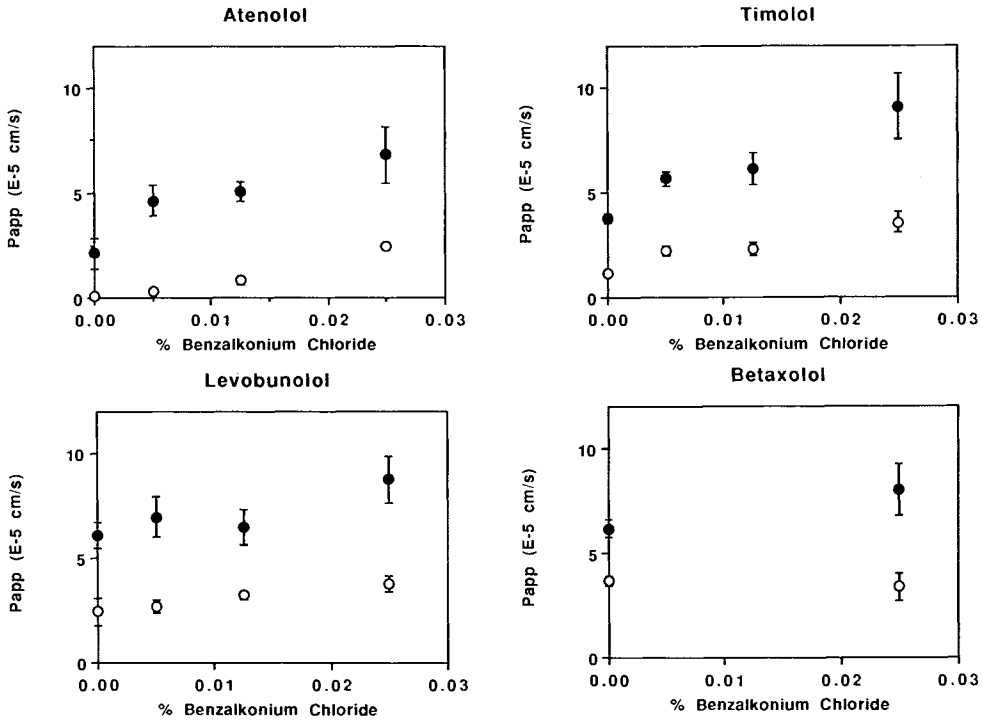

Fig. 5. Influence of benzalkonium chloride on the corneal (○) and conjunctival (●) permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol. Error bars represent standard deviations for $n = 4$. Where not shown, the error bar is smaller than the size of the symbol.

Effect of Formulation Changes on the Ratio of Corneal to Conjunctival Penetration of Beta Blockers

Figure 7 summarizes the effect of drug lipophilicity and formulation changes on the ratio of corneal to conjunctival penetration (the C/J ratio) of beta blockers. The C/J ratio increased in order of increasing lipophilicity: atenolol < timolol < levobunolol < betaxolol. In the case of betaxolol, this ratio was reduced by all formulation changes. Regardless of drug lipophilicity, lowering the formulation pH to 6 and increasing the tonicity of the formulation led to a lower C/J ratio, while lowering the tonicity of the formulation and adding benzalkonium chloride to the formulation led to a higher ratio. The direction in which the C/J ratio was altered by other formulation changes was a function of drug lipophilicity. Thus, raising the formulation pH to 8.4 decreased the C/J ratio for atenolol and betaxolol while increasing it for timolol and levobunolol. Adding EDTA to the formulation increased the C/J ratio for atenolol and timolol while reducing it for levobunolol and betaxolol.

Effect of Formulation Changes in the Ocular and Systemic Absorption of Topically Applied Timolol

Figure 8 shows the effect of formulation changes on the aqueous humor timolol concentration at 30 min postinstillation of a 0.65% timolol maleate solution, the maximum timolol concentration in plasma, and the area under the concentration–time curve in plasma. Both the aqueous humor timolol concentration and the maximum timolol concentration in plasma were decreased by lowering the solution pH to 6.4 and increasing the solution tonicity to 600 mOsm/kg but were increased by raising the solution pH to 8.4, decreasing the tonicity to 80 mOsm/kg, and adding 0.025% benzalkonium chloride or 0.1% EDTA to the formulation. A similar trend was seen in the plasma AUC, except that decreasing the tonicity brought about a reduction rather than an increase in the AUC.

As can be seen in Fig. 9, there is a good correlation between corneal Papp and aqueous humor timolol concentration from various formulations, suggesting that the *in vitro* model is predictive of the *in vivo* situation. A good correlation is also found between conjunctival Papp and plasma timolol AUC. The increase in conjunctival permeability due to an increase in the tonicity of the formulation did not, however, lead to an increase in absorption of timolol into the bloodstream.

DISCUSSION

This study demonstrates that the conjunctival and corneal permeabilities to beta blockers generally respond to changes in formulation composition in a similar way. These formulation changes are an increase in solution pH from 7.4 to 8.4 (Fig. 3), a decrease in solution tonicity from about 290 to 75 mOsm/kg (Fig. 4), and the incorporation of benzalkonium chloride (Fig. 5) and EDTA (Fig. 6) in the formulation. Conjunctival permeability is, however, less sensitive to a given formulation change than is corneal permeability. This is to be expected from the higher permeability of the conjunctiva than the cornea to drug penetration. At pH 7.4 under isotonic conditions and in the absence of benzalkonium chloride and EDTA, the ratio of conjunctival to corneal drug permeability is 30 for atenolol, 3 for timolol, 2.5 for levobunolol, and 1.7 for betaxolol (Fig. 7).

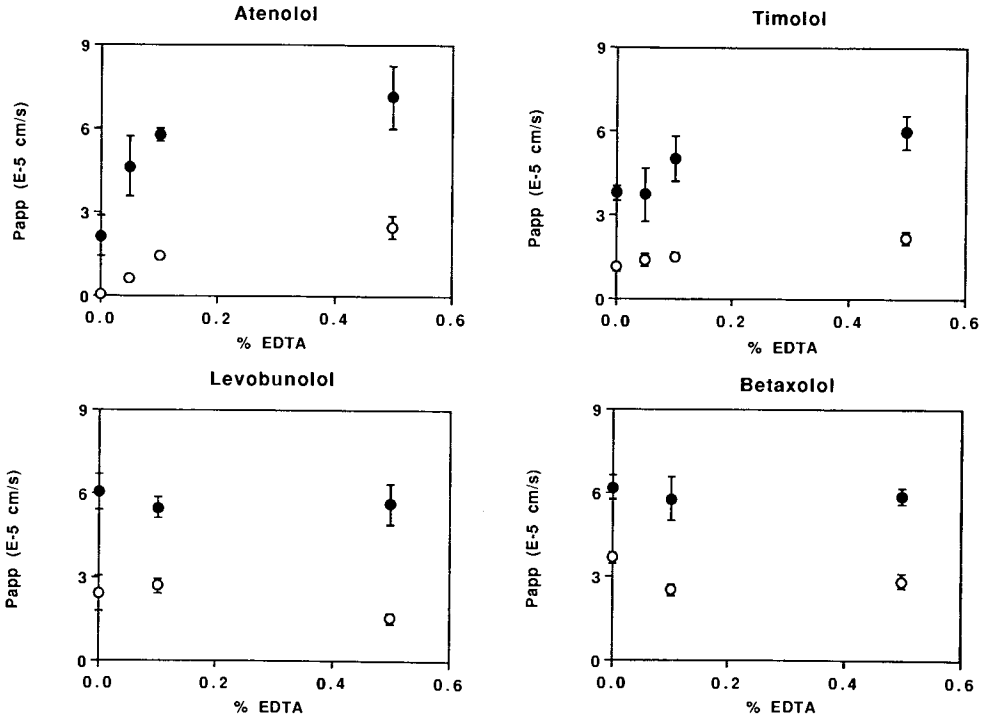

Fig. 6. Influence of EDTA on the corneal (○) and conjunctival (●) permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol. Error bars represent standard deviations for $n = 4$. Where not shown, the error bar is smaller than the size of the symbol.

Differences between conjunctival and corneal drug permeability do exist. These differences are seen in the response to reduction in pH from 7.4 to 6.0 (Fig. 3) and increase in tonicity from about 290 to 600 mOsm/kg (Fig. 4). Thus, lowering the formulation pH from 7.4 to 6.0, while expectedly reducing corneal permeability, caused a 16–43% increase in conjunctival permeability to all the beta blockers studied except levobunolol (Fig. 3). Moreover, increasing the formulation tonicity from 290 to 600 mOsm/kg, while reducing the corneal permeability to beta blockers by 30–55%, increased the conjunctival permeability by 11–80% (Fig. 4). These two findings point to some as yet unknown subtle biochemical and histological differences between the conjunctival and the corneal epithelial cells that become prominent when the pH is lowered and the tonicity is increased but remain unaffected by the other formulation

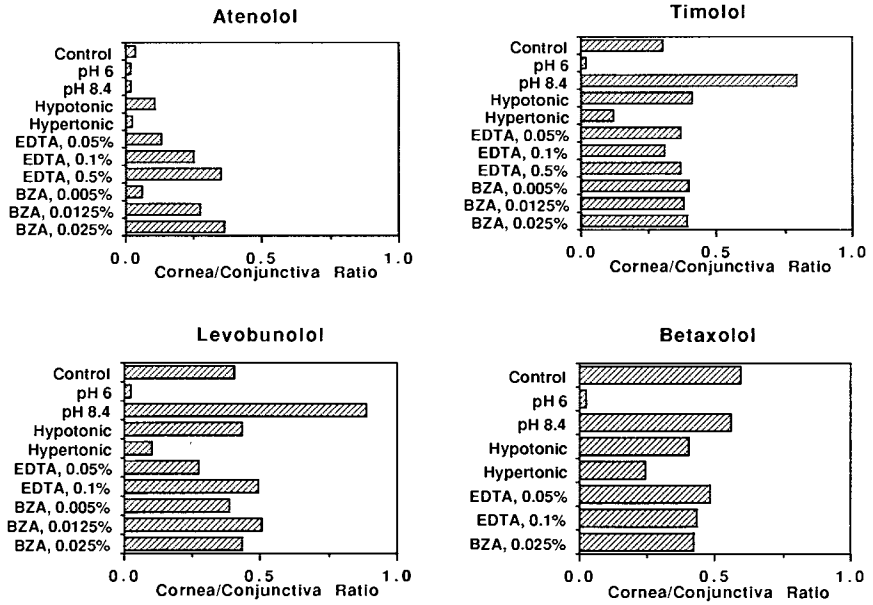

Fig. 7. Influence of formulation composition on the ratio of corneal to conjunctival permeability coefficients of atenolol, timolol, levobunolol, and betaxolol.

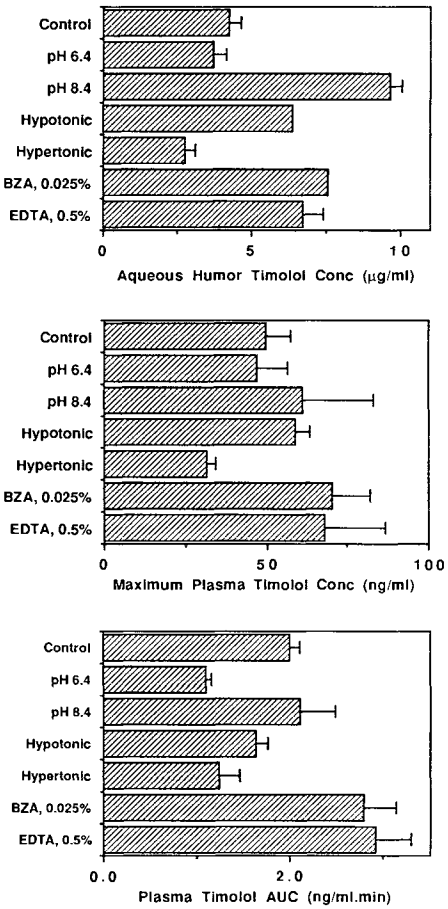

Fig. 8. Influence of formulation composition on the aqueous humor timolol concentration at 30 min (top plot), the maximum timolol concentration in plasma (middle plot), and the area under the concentration–time curve (AUC) in plasma (bottom plot) following the topical instillation of 25 μl of a 15 m*M* timolol maleate solution in the pigmented rabbit eye. Error bars represent standard deviations for n = 6–8 in the ocular absorption experiment and n = 4–6 in the systemic absorption experiment.

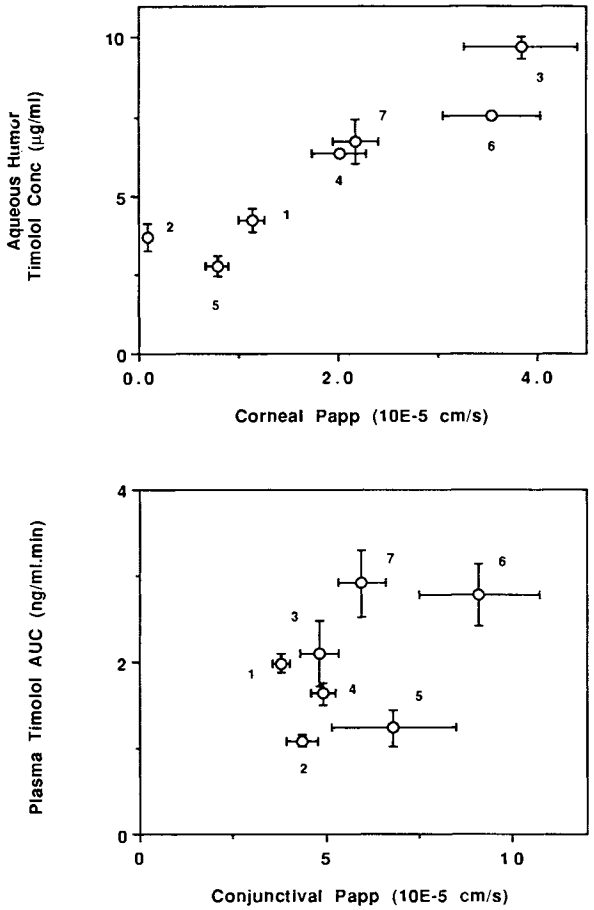

Fig. 9. *In vitro–in vivo* correlations of aqueous humor timolol concentration at 30 min and corneal permeability coefficient (top plot) and of plasma AUC and conjunctival permeability coefficient (bottom plot). Error bars represent standard deviations for n = 4–8. (1) Control (pH 7.4, isotonic, 0% benzalkonium chloride, and 0% EDTA); (2) pH 6.4; (3) pH 8.4; (4) hypotonic solution; (5) hypertonic solution; (6) 0.025% benzalkonium chloride; (7) 0.5% EDTA.

changes. It is likely that such differences reside in the mucus layer and the glycocalyx (10,11), whose polyelectrolyte characteristics are subject to perturbation by changes in pH and tonicity in much the same manner as an ion-exchange matrix, including collapse of the matrix in the extreme. In the case of hypertonicity-induced increase in conjunctival permeability, there is the additional possibility of underlying changes in the conjunctival epithelium, including discharge of goblet cell mucin and reduced goblet cell density (12).

The first hint that the conjunctiva and cornea are different from the standpoint of drug penetration is the different magnitude by which a given pH change alters the corneal and conjunctival permeability to the four beta blockers. For instance, whereas raising the pH from 7.4 to 8.4 increased the corneal permeability to timolol 2.4 times, it increased the conjunctival permeability by only 28% (Fig. 3). The magnitude of increase in both instances was much less than the factor of 8 increase in the fraction of timolol (pK_a, 9.21) in the nonionized, preferentially absorbed form. Clearly, changes in pH affect not only the fraction of drug in its nonionized form but also the biochemical and morphological features of the corneal and conjunctival epithelial cells.

An important finding in this study is that, regardless of drug lipophilicity, any change in formulation composition will change both corneal and conjunctival penetration and, in turn, ocular and systemic absorption (1,13) as well as the relative contribution of the corneal to the noncorneal pathways to ocular drug absorption (13). Except for reduction in solution pH and increase in solution tonicity, all formulation changes appear to alter corneal more than conjunctival permeability. Moreover, with few exceptions, the C/J ratio is more sensitive to formulation changes for the hydrophilic atenolol than for the more lipophilic levobunolol (Fig. 7). For instance, incorporating 0.1% EDTA into the formulation increased the C/J ratio of atenolol 6.5 times while increasing that of levobunolol only 20%. The corresponding values were 21 times and 25% upon incorporating 0.0125% benzalkonium chloride into the formulation. Betaxolol is the only beta blocker studied whose C/J ratio is reduced with all formulation changes (Fig. 7).

Conjunctival drug penetration is most effectively increased by incorporating 0.025% benzalkonium chloride into

the formulation (Fig. 11). Corneal drug penetration, on the other hand, is most effectively increased by raising the pH to 8.4, although lowering the solution tonicity and adding 0.025% benzalkonium chloride and 0.5% EDTA to the formulation are also effective. None of the above treatments is, however, as effective as deepithelizing the cornea (Fig. 10). These effects can be attributed to an increase in the fraction of drug in a nonionized, preferentially absorbed form (14); to an increased influx of water across the corneal epithelium (15)—the so-called solvent drag effect (16); and to changes in the integrity of the corneal epithelial cells (17,18). In the case of levobunolol, these formulation changes effect not only its corneal penetration, but also the formation of its reductive metabolite, dihydrolevobunolol (Fig. 2). There exists an inverse relationship in the extent between drug penetration and metabolism.

An obvious concern with using diffusion chambers to evaluate formulation effects on corneal and conjunctival penetration is the continuous bathing of the corneal and conjunctival surfaces by the drug, which is unrealistic in comparison with the minutes of contact time seen *in vivo* (6). Moreover, permeability is determined under pseudo steady-state conditions which are not established *in vivo*. Nevertheless, reasonably good *in vitro–in vivo* correlations are to be expected since the terms which comprise Papp—buffer/cornea partition coefficient and diffusion coefficient within the cornea—also determine absorption *in vivo*. Moreover, provided that corneal epithelium is saturated with drug within minutes to establish the concentration gradient for drug diffusion, the *in vitro* model would mimick the *in vivo* situation. The reasonably good *in vitro–in vivo* correlations shown in Fig. 9 suggest that, at least for timolol, this may be the case. The utility of the *in vitro* results is that, when used in conjunction with the *in vivo* results, they allow the delineation of the relative role of the formulation-induced changes in corneal and conjunctival penetration and of the changes in precorneal retention to the changes seen in the ocular and systemic drug absorption. For instance, in the case of timolol, the pH and tonicity effects on its ocular and systemic absorption, as shown in Fig. 8, must be due more to the positive changes in corneal and conjunctival (and perhaps nasal) penetration than to the negative changes in residence time in the conjunctival sac (6).

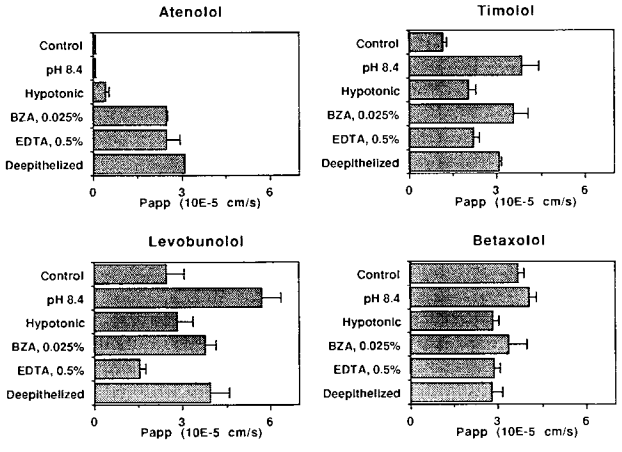

Fig. 10. Corneal permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol from the best formulation of each group relative to the control and the deepithelized cornea. Error bars represent standard deviations for $n = 4$.

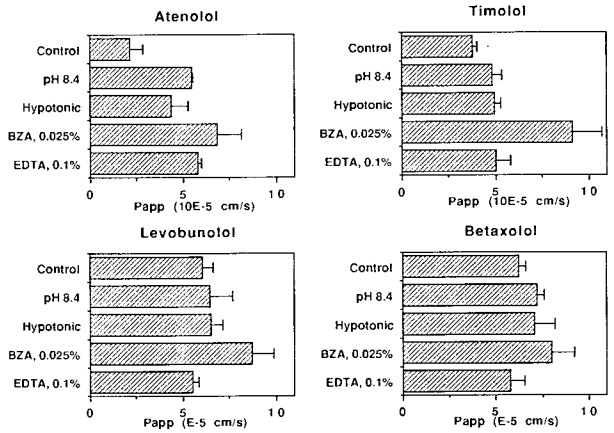

Fig. 11. Conjunctival permeability coefficients (Papp) of atenolol, timolol, levobunolol, and betaxolol from the best formulation of each group relative to the control. Error bars represent standard deviations for $n = 4$.

In conclusion, the possibility that not only corneal but also conjunctival penetration may be altered must be considered in making changes in ophthalmic formulations, even though conjunctival penetration is usually not as sensitive in this regard. Depending on the formulation change, corneal and conjunctival penetration may be affected in either the same or the opposite direction. At least for timolol, this information has provided useful insight on the relative importance of changes in corneal and conjunctival penetration and in precorneal drug retention in determining the ocular and systemic drug absorption from various formulations. Finally, because the conjunctiva plays an active role in the noncorneal route of ocular drug absorption, the relative importance of the noncorneal to the corneal routes in contributing to ocular drug absorption may also be altered by formulation changes.

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