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Penetration of β-Blockers through Ocular Membranes in Albino Rabbits

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Abstract

The purpose of this study was to investigate the barrier properties of ocular membranes for controlling the extent and pathway of ocular absorption of instilled β-blockers.

The penetration of β-blockers was measured across the isolated corneal, conjunctival and scleral membranes of the albino rabbit using a two-chamber glass diffusion cell. β-Blockers tested were atenolol, carteolol, tilisolol, timolol and befunolol.

Corneal penetration of befunolol was much higher than that of atenolol. Scraping the epithelium increased corneal penetration of β-blockers. Conjunctival membranes showed higher permeability than corneal and scleral membranes. The penetration parameters were estimated according to Fick's equation. The corneal permeability coefficient showed an apparent linear relationship with penetrant lipophilicity. The lipophilic character of the corneal barrier was determined by the partition coefficient of drug to corneal surface, not by the diffusion coefficient. Conjunctival and scleral permeability coefficients were not determined by the lipophilicity of β-blockers.

These results indicate that the conjunctiva, sclera and cornea of the rabbit eye are sufficiently different in permeation character to control the extent and pathway for ocular absorption.

Glaucoma is one of the most serious ocular diseases, which is characterized by high intraocular pressure, hardening of the eyeball and partial or complete loss of vision. β-Blockers, which decrease aqueous humour formation by the ciliary processes, are commonly used as instillation droplets (Görlich 1987; Katz et al 1987; Novack 1987).

Instilled drug is eliminated from the precorneal area due to drainage by the naso-lacrimal duct and dilution by tear turnover (Chrai et al 1973; Himmelstein et al 1978; Shell 1982; Lee & Robinson 1986). The drug in the precorneal area is also eliminated through cornea, conjunctiva and sclera. The rate and extent of intraocular absorption and therapeutic effectiveness of topically applied drugs are dependent on the transport characteristics of the cornea, the sclera and the conjunctiva (Ahmed et al 1987). The conjunctival and scleral penetrations may constitute an important route for some drugs which are poorly absorbed through the cornea and for some drug delivery systems such as conjunctival inserts (Lee & Robinson 1986; Schoenwald 1990; Sasaki et al 1993a). However, little information on barrier properties is available for the conjunctiva and sclera compared with that for the cornea.

The purpose of this study was to investigate the barrier properties of ocular membranes for controlling the extent and pathway of ocular absorption of instilled β-blockers. The penetration of β-blockers was measured across the isolated corneal, conjunctival and scleral membranes of the albino rabbit using a two-chamber glass diffusion cell.

Materials and Methods

Materials

Tilisolol hydrochloride was kindly supplied by Nisshin Flour Milling Co. Ltd (Tokyo, Japan). Befunolol and carteolol were also kindly supplied from Kaken Pharmaceutical Co. Ltd (Tokyo, Japan) and Otsuka Pharmaceuticals Co. Ltd (Tokyo, Japan), respectively. Timolol, atenolol, salicylic acid and *o*-ethoxybenzamide were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Pindolol hydrochloride was purchased from Sigma Chemical Co. (St Louis, MO, USA). Methyl-*p*-hydroxybenzoate was purchased from Kishida Chemicals Co. Ltd (Tokyo, Japan). Salicyl methionine was prepared by standard methods in our laboratory (Nakamura et al 1992). All other chemicals were of reagent grade obtained from Nacalai Tesque Inc. Phosphate-buffered saline (pH 7·4) was prepared by mixing an isotonic phosphate buffer with an equal volume of saline.

Apparent partition coefficients (PC) of compounds were determined in 1-octanol : 50 mM phosphate buffer (pH 7·4) (1 : 1, v/v) after shaking for 5 min at 22 ± 1°C. Before use, 1-octanol and buffer solution were saturated with the relevant aqueous or organic phase. The PC values were calculated from drug concentrations in the aqueous phase before and after addition of 1-octanol. The initial concentration of compounds was 0·2 mM in buffer.

The lipophilic indices ($\log k'$) were determined by an HPLC system (LC-6A, Shimadzu Co., Ltd, Kyoto, Japan) with a stationary phase of Cosmosil $5C_{18}$-P packed column (150 mm length × 4·6 mm i.d., Nacalai Tesque Inc.) and a mobile phase of methanol : 50 mM NaH_2PO_4 (35 : 65, v/v) with a flow rate of 1·0 mL min^{-1}. β-Blockers were detected spectrometrically or fluorometrically. The indices were calculated from the equation: $\log k' = \log [(t_r - t_0)/t_0]$, where t_r is the retention time and t_0 is the elution time of the solvent.

In-vitro penetration experiment

Male Nippon albino rabbits, 2·0–3·0 kg, were individually

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housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co. Ltd, Tokyo, Japan). The rabbits were starved for 24 h before use but had free access to water. All experiments in the present study conformed to the Guideline for Animal Experimentation in Nagasaki University.

The glass apparatus for in-vitro diffusion and the procedure for preparing ocular membranes were according to the previous report (Lee et al 1988; Sasaki et al 1993b). Rabbits were killed with an overdose of a sodium pentobarbitone solution administered via a marginal ear vein. The ocular membranes were dissected and mounted in the diffusion chambers. In some experiments, the epithelium of cornea was scraped with a surgical knife to prepare a stromal and endothelial membrane (scraped cornea). Glutathione bicarbonated Ringer's solution was used throughout the diffusion studies (Grass & Robinson 1988).

Penetrant solution (5 mM β-blockers, 4 mL) and penetrant-free solution (4 mL) were added to the epithelial side (donor side) and the endothelial side (receiver side), respectively. The contents of each chamber were stirred gently and bubbled with a 95% O_2–5% CO_2 mixture. The diffusion apparatus was jacketed to maintain the ocular membrane, donor solution and receiver solution at 35 ± 0.5°C (cornea) and 37 ± 0.5°C (conjunctiva and sclera). At appropriate time intervals, a sample (50 μL) was withdrawn from the receiver side and the concentration of β-blocker was determined by HPLC.

Drug determination

The samples of β-blocker for in-vitro experiments (50 μL) were mixed with pH 7.4 isotonic phosphate-buffered saline or 0.1 M HCl (50 μL) and methanol (100 μL) including internal standard (300 $\mu g\,mL^{-1}$ *o*-ethoxybenzamide for tilisolol, 500 $\mu g\,mL^{-1}$ methyl-*p*-hydroxybenzoate for timolol, 20 $\mu g\,mL^{-1}$ pindolol for carteolol, 30 $\mu g\,mL^{-1}$ salicylic acid for atenolol, and 50 $\mu g\,mL^{-1}$ salicyl methionine for befunolol). The mixture was centrifuged at 12000 *g* for 10 min and 50 μL of supernatant was injected into an HPLC system.

An HPLC system (LC-6A, Shimadzu Co., Ltd) was used in the reverse-phase mode for assay. The stationary phase used was Cosmosil $5C_{18}$-P packed column (150 mm length × 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol and 50 mM NaH_2PO_4 (tilisolol 37:63; atenolol 20:80; timolol 40:60; befunolol 45:55; carteolol 25:75, v/v) were used as the mobile phase with a flow rate of 1.0 mL min^{-1}. Retention of drug was monitored with a UV spectrophotometric detector (SPD-10A, Shimadzu Co. Ltd; 220 nm for atenolol; 290 nm for timolol; 250 nm for carteolol) and a fluorescence HPLC monitor (RF-535, Shimadzu Co. Ltd; excitation wavelength 315 nm and emission wavelength 420 nm for tilisolol; excitation wavelength 300 nm and emission wavelength 500 nm for befunolol).

Data analyses

The penetration profiles were analysed based on a diffusion model for the infinite dose system which considers the ocular membrane to be a one-plane barrier membrane. This membrane model assumes a constant drug concentration in the donor solution and a sink condition in the receiver phase, since the cumulative amount of drug transferred to the receiver compartment was much smaller than the donor amount. According to the model, the Laplace transform for the total amount of drug appearing in the receiver cell (Q) is expressed as follows:

$$Q = K' \cdot D'^{0.5} \cdot A \cdot C_0 / s^{1.5} / \sinh(s^{0.5}/D'^{0.5}) \qquad (1)$$

where: $K' = K \cdot L$; $D' = D/L/L$; $Kp = K' \cdot D'$; $LT = 1/6/D'$ and D is the diffusion coefficient in membrane, K is the partition coefficient of drug between membrane and donor solution, L is the effective length of diffusion through membrane, s is the Laplace variable with respect to time, A is the effective diffusion area, C_0 is the drug concentration in the donor phase, Kp is the permeability coefficient and LT is the lag time. Since it is difficult to determine correctly the real diffusion length (L) for each penetrant, the diffusion parameter (D′) and the partition parameter (K′) were defined.

In the same manner, corneal penetration profiles of β-blockers were also analysed according to the two-layer model (Hashida et al 1988). The Laplace transform for the total amount of drug appearing in the receiver cell (Q) is expressed as follows:

$$Q = K'_2 \cdot K'_1 \cdot A \cdot C_0 / s^{1.5} / (L_1/D'^{0.5}_2 \cdot \sinh(s^{0.5}/D'^{0.5}_2) \cdot \cosh(s^{0.5}/D'^{0.5}_1) + K'_2/D'^{0.5}_1 \cdot \sinh(s^{0.5}/D'^{0.5}_1) \cdot \cosh(s^{0.5}/D'^{0.5}_2)) \qquad (2)$$

where: $K'_1 = K_1 \cdot L_1$; $D'_1 = D_1/L_1/L_1$; $Kp_1 = K'_1 \cdot D'_1$; $LT_1 = 1/6/D'_1$; $K'_2 = K_2 \cdot L_2$; $D'_2 = D_2/L_2/L_2$; $Kp_2 = K'_2 \cdot D'_2$; $LT_2 = 1/6/D'_2$ and subscripts 1 and 2 for K, D, L, K′, D′, Kp and LT express the epithelial layer and the stromal and endothelial layer, respectively. The penetration parameters for the two-layer model were calculated by fitting the penetration profiles of β-blockers through cornea and scraped cornea to equation 1 and equation 2, simultaneously. The diffusion parameters (D_2) and partition parameters (K_2) of the stromal and endothelial layers is assumed to be almost equal to those of scraped cornea. The thickness of cornea used in the present study was 0.03708 cm; Huang et al (1983) reported the thickness of epithelium layer to be 0.00385 cm. The diffusion lengths were defined as thicknesses of the epithelial layer and of the stromal and endothelial layer, 0.00385 (L_1) and 0.03323 cm (L_2), respectively.

The penetration parameters were estimated by fitting the penetration profiles to equations using MULTI(FILT), a nonlinear least-squares computer program based on a fast inverse Laplace transform algorithm (Yano et al 1989). This program was written in MS-FORTRAN and run on a personal computer (PC-9801 BX, NEC, Tokyo, Japan).

Results and Discussion

The physicochemical properties of β-blockers are summarized in Table 1. Befunolol, timolol and carteolol are used clinically as ophthalmic droplets for glaucoma in Japan. Tilisolol was reported to decrease intraocular pressure after its instillation in rabbit eye (Nakagawa et al 1984; Tadokoro et al 1990). The β-blockers chosen were

Table 1. Physicochemical properties of β-blockers.

β-Blocker	Mol. wt	Log PC[a]	Log k'[b]
Atenolol	266·3	−1·5	−0·46
Carteolol	292·4	−0·81	0·067
Tilisolol	304·4	−0·24	0·43
Timolol	316·4	−0·16	0·50
Befunolol	291·4	−0·009	0·59

[a]Apparent partition coefficient between 1-octanol and pH 7·4 buffer at 22°C. [b]Lipophilic indices determined by HPLC.

similar in mol. wt and pK_a value (Schoenwald & Huang 1983; Novack 1987). However, the logarithmic values of apparent partition coefficient (PC) of the β-blockers varied between −1·5 and −0·009. There is a linear relationship between log PC and the lipophilic index (log k') in HPLC (correlation coefficient = 0·999).

Penetration of β-blockers through cornea and scraped cornea was examined in in-vitro experiments. The penetration profiles are shown in Fig. 1. Penetration parameters according to Fick's equation were calculated from the profiles. The results are summarized in Table 2. β-Blockers showed various permeability coefficients (Kp) through cornea. The differences of permeability coefficients among drugs were determined by the partition parameter (K') rather than diffusion parameter (D'). Scraping the epithelium of cornea increased the permeability coefficients of β-blockers and shortened the lag time. The scraping increased both partition parameter and diffusion parameter. The penetration improvement by scraping (ScCR/CR) was greater for hydrophilic drugs than for lipophilic drugs.

The corneal composite structure is characterized by three primary layers: epithelium, stroma and endothelium. The stratified epithelial cells with tight junctions are considered to comprise the corneal penetration barrier (Huang et al 1983; Chien et al 1988; Huang et al 1989). Therefore, the penetration profiles of cornea were analysed as a two-layer membrane, an epithelial layer and a stromal and endothelial layer, to characterize the barrier function. Diffusion coefficients (D_1, D_2) and partition coefficients (K_1, K_2) in each layer are summarized in Table 3. The epithelial layer showed lower diffusion coefficients than the stromal and endothelial layer. Partition coefficients of lipophilic drugs in the epithelial layer were higher than those of hydrophilic drugs. In the stromal and endothelial layer, partition coefficients of β-blockers were close to unity (0·72–1·6).

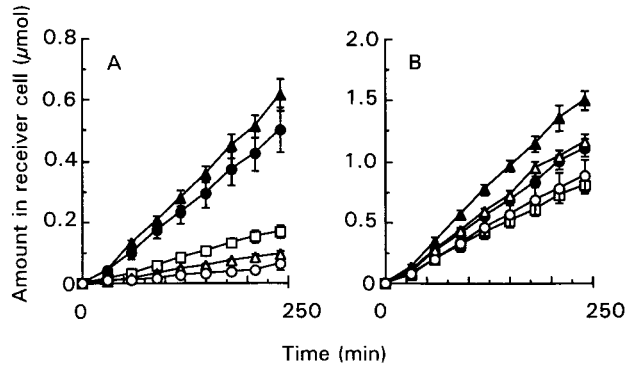

FIG. 1. Penetration profile of β-blockers through cornea (A) and scraped cornea (B). ○ Atenolol, △ carteolol, □ tilisolol, ● timolol, ▲ befunolol. Each point represents the average of at least three experiments ± s.e.m.

Fig. 2 shows the conjunctival and scleral penetration of β-blockers. The penetration parameters are summarized in Table 4. The conjunctival permeabilities of β-blockers were much larger than corneal or scleral permeabilities. For most β-blockers, both diffusion and partition parameters in conjunctiva were higher than those in cornea. The scleral permeability was higher than corneal permeability in hydrophilic drugs but lower in lipophilic drugs. All β-blockers showed a long lag time for scleral penetration. The diffusion parameter in sclera was lower than that in cornea, although the partition parameter was higher than that in cornea.

The importance of the corneal route rather than the non-corneal route (conjunctival and scleral routes) has been reported in intraocular absorption of ocularly applied drugs (Doane et al 1978; Ahmed & Patton 1987). The ratios of permeability coefficient of cornea to that of conjunctiva (CR/CJ) or sclera (CR/SC) showed lower values for hydrophilic β-blockers (Table 4). These results suggest that the non-corneal route contributes more to the intraocular absorption of hydrophilic drugs in comparison with lipophilic drugs.

Conjunctiva as well as cornea has stratified and squamous epithelia. Huang et al (1989) demonstrated a predominant contribution of the paracellular pathway to conjunctival permeability. The conjunctival paracellular permeability of β-blockers can explain the high penetration, regardless of

Table 2. Permeation parameters of β-blockers through cornea and scraped cornea using a one-layer membrane model.

β-Blocker	Cornea					Scraped cornea					ScCR/CR[a]
	n	Lag time (h)	D' (h^{-1})	K' × 10^3 (cm)	Kp × 10^3 (cm h^{-1})	n	Lag time (h)	D' (h^{-1})	K' × 10^3 (cm)	Kp × 10^3 (cm h^{-1})	
Atenolol	6	0·2	0·78	4·2	3·3	3	0·1	1·8	29	53	16
Carteolol	6	0·3	0·53	12	6·1	6	0·1	1·7	42	70	11
Tilisolol	6	0·2	0·91	11	10	3	0·1	2·0	24	48	4·8
Timolol	6	0·2	0·74	41	30	6	0·1	1·5	45	66	2·2
Befunolol	4	0·3	0·66	37	37	3	0·1	1·8	50	90	2·4

D' (D/L/L): diffusion parameter, K' (K · L): partition parameter, Kp: permeability coefficient. [a]The ratio of permeability coefficient of scraped cornea (ScCR) to that of cornea (CR).

Table 3. Permeation parameters of β-blockers through epithelial layer and stromal and endothelial layer of cornea using a two-layer membrane model.

β-Blocker	Epithelial layer				Stromal and endothelial layer			
	Lag time (h)	$D_1 \times 10^3$ ($cm^2 h^{-1}$)	K_1	$Kp_1 \times 10^3$ ($cm\,h^{-1}$)	Lag time (h)	$D_2 \times 10^3$ ($cm^2 h^{-1}$)	K_2	$Kp_2 \times 10^3$ ($cm\,h^{-1}$)
Atenolol	0·1	0·026	0·054	0·36	0·1	2·1	0·84	53
Carteolol	0·1	0·032	0·066	0·55	0·1	1·9	1·3	70
Tilisolol	0·1	0·046	0·11	1·3	0·1	2·2	0·72	48
Timolol	0·1	0·026	1·5	9·7	0·1	1·6	1·4	67
Befunolol	0·1	0·023	0·87	5·2	0·1	1·9	1·6	90

D: diffusion coefficient, K: partition coefficient, Kp: permeability coefficient. Subscripts 1 and 2 for K, D and Kp express the epithelial layer and the stromal and endothelial layer, respectively.

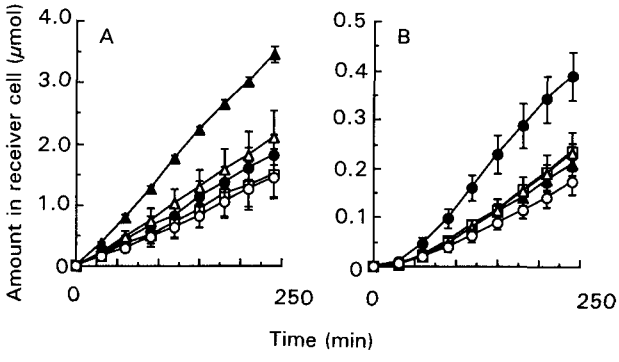

FIG. 2. Penetration profile of β-blockers through conjunctiva (A) and sclera (B). ○ Atenolol, △ carteolol, □ tilisolol, ● timolol, ▲ befunolol. Each point represents the average of at least three experiments ± s.e.m.

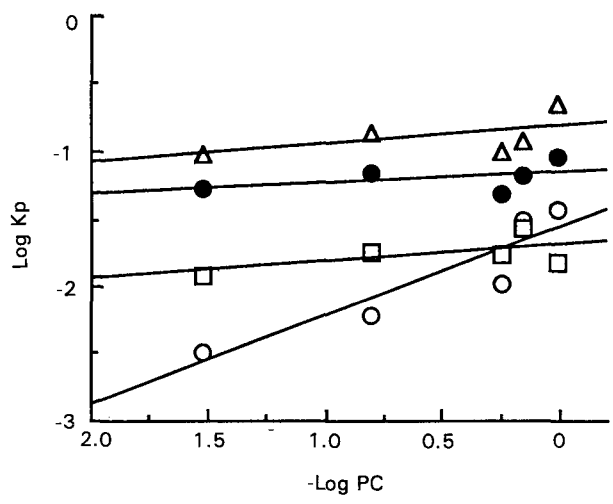

FIG. 3. Relationship between logarithmic values of apparent partition coefficient (PC) between 1-octanol and pH 7·4 buffer and permeability coefficient (Kp). ○ Cornea, log Kp = 0·663 × log PC − 1·564, correlation coefficient = 0·915; △ conjunctiva; □ sclera; ● scraped cornea.

lipophilicity. On the other hand, in-vivo conjunctival absorption may be affected by the thickness of the underlying connective tissues and by the permeability of the blood capillaries (Huang et al 1989).

Sclera is a tough and thick fibrous tissue composed primarily of collagen and mucopolysaccharides. The long lag time for scleral penetration is due to a slow diffusion of drug through a dense and thick tissue. It was suggested that most of the drug permeating the sclera may be carried away by the blood circulation before diffusion to other intraocular sites in-vivo (Doane et al 1978).

Fig. 3 shows the relationship between the logarithmic value of PC and the permeability coefficient. The corneal permeability showed a significant correlation ($P < 0.05$) with penetrant lipophilicity. Conjunctival and scleral penetrations were not affected by penetrant lipophilicity. In a wider range of drug lipophilicity, a parabolic relationship between lipophilicity and corneal permeability or conjunctival permeability has been reported (Schoenwald & Huang 1983; Chien et al 1991; Suhonen et al 1991; Wang et al 1991). The relationship between logarithmic values of PC and diffusion parameter or partition parameter is shown in Fig. 4. The dependency of corneal permeability on drug lipophilicity was predominantly determined by a partition

Table 4. Permeation parameters of β-blockers through conjunctiva and sclera using a one-layer membrane model.

β-Blocker	Conjunctiva					CR/CJ[a]	Sclera					CR/SC[b]
	n	Lag time (h)	D′ (h^{-1})	K′ × 10^3 (cm)	Kp × 10^3 ($cm\,h^{-1}$)		n	Lag time (h)	D′ (h^{-1})	K′ × 10^3 (cm)	Kp × 10^3 ($cm\,h^{-1}$)	
Atenolol	5	0·2	0·76	130	95	0·035	4	0·8	0·21	59	12	0·28
Carteolol	5	0·1	1·4	100	140	0·044	3	1·0	0·16	110	18	0·34
Tilisolol	6	0·1	1·1	88	100	0·10	6	0·9	0·18	97	17	0·59
Timolol	6	0·1	1·2	97	120	0·25	4	0·6	0·26	110	27	1·1
Befunolol	4	0·1	2·5	90	230	0·16	4	0·9	0·20	78	15	2·5

D′ (D/L/L): diffusion parameter, K′ (K · L): partition parameter, Kp: permeability coefficient. [a]The ratio of permeability coefficient of cornea (CR) to that of conjunctiva (CJ). [b]The ratio of permeability coefficient of cornea (CR) to that of sclera (SC).

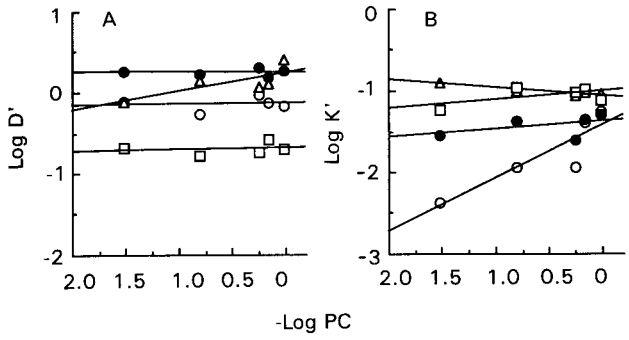

FIG. 4. Relationship between logarithmic values of apparent partition coefficient (PC) between 1-octanol and pH 7·4 buffer and diffusion parameter (D′, A) and partition parameter (K′, B). ○ Cornea, $\log K' = 0{\cdot}651 \times \log PC - 1{\cdot}423$, correlation coefficient = 0·882; △ conjunctiva; □ sclera; ● scraped cornea.

process, not by its diffusion of the drug into the corneal surface.

Thus, these results indicate that the conjunctiva, sclera and cornea of the rabbit eye are sufficiently different in permeation character to control the extent and pathway for ocular absorption. Further investigation may be necessary to control the in-vivo absorption of ocularly applied drug.

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References

Ahmed, I., Patton, T. F. (1987) Disposition of timolol and inulin in the rabbit eye following corneal versus non-corneal absorption. Int. J. Pharm. 38: 9–21

Ahmed, I., Gokhale, R. D., Shah, M. V., Patton, T. F. (1987) Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea. J. Pharm. Sci. 76: 583–586

Chien, D.-S., Bundgaard, H., Lee, V. H. L. (1988) Influence of corneal epithelial integrity on the penetration of timolol prodrugs. J. Ocular Pharmacol. 4: 137–146

Chien, D.-S., Sasaki, H., Bundgaard, H., Buur, A., Lee, V. H. L. (1991) Role of enzymatic lability in the corneal and conjunctival penetration of timolol ester prodrugs in the pigmented rabbit. Pharm. Res. 8: 728–733

Chrai, S. S., Patton, T. F., Mehta, A., Robinson, J. R. (1973) Lacrimal and instilled fluid dynamics in rabbit eyes. J. Pharm. Sci. 62: 1112–1121

Doane, M. G., Jensen, A. D., Dohlman, C. H. (1978) Penetration routes of topically applied eye medications. Am. J. Ophthalmol. 85: 383–386

Görlich, W. (1987) Experiences in clinical research with beta blockers in glaucoma. Glaucoma 9: 21–27

Grass, G. M., Robinson, J. R. (1988) Mechanisms of corneal drug penetration I: in vivo and in vitro kinetics. J. Pharm. Sci. 77: 3–14

Hashida, M., Okamoto, H., Sezaki, H. (1988) Analysis of drug penetration through skin considering donor concentration decrease. J. Pharmacobiodyn. 11: 636–644

Himmelstein, K. J., Guvenir, I., Patton, T. F. (1978) Preliminary pharmacokinetic model of pilocarpine uptake and distribution in the eye. J. Pharm. Sci. 67: 603–606

Huang, A. J. W., Tseng, S. C. G., Kenyon, K. R. (1989) Paracellular permeability of corneal and conjunctival epithelia. Invest. Ophthalmol. Vis. Sci. 30: 684–689

Huang, H.-S., Schoenwald, R. D., Lach, J. L. (1983) Corneal penetration behavior of β-blocking agents II: assessment of barrier contributions. J. Pharm. Sci. 72: 1272–1279

Katz, I. M., Kulaga, S. F., Gould, A. L., Miller, I. M., Clineschmidt, C. M., Wittreich, J. M. (1987) Long-term tolerability and efficacy of timolol ophthalmic solution. Glaucoma 9: 84–88

Lee, V. H. L., Robinson, J. R. (1986) Topical ocular drug delivery: recent developments and future challenges. J. Ocular Pharmacol. 2: 67–108

Lee, V. H. L., Chien, D.-S., Sasaki, H. (1988) Ocular ketone reductase distribution and its role in the metabolism of ocularly applied levobunolol in the pigmented rabbit. J. Pharmacol. Exp. Ther. 246: 871–878

Nakagawa, Y., Sugai, T., Chin, W.-P., Shibuya, T., Hashimoto, K., Imai, S. (1984) Pharmacological profile of a new β-adrenoceptor blocker, 4-[3-(tert-butylamino)-2-hydroxypropoxy]-*N*-methylisocarbostyril hydrochloride (N-696). Arzneim. Forsch. 34: 194–199

Nakamura, J., Kido, M., Nishida, K., Sasaki, H. (1992) Hydrolysis of salicylic acid-tyrosine and salicylic acid-methionine prodrugs in the rabbit. Int. J. Pharm. 87: 59–66

Novack, G. D. (1987) Ophthalmic beta-blockers since timolol. Surv. Ophthalmol. 31: 307–327

Sasaki, H., Tei, C., Nishida, K., Nakamura, J. (1993a) Drug release from an ophthalmic insert of a beta-blocker as an ocular drug delivery system. J. Contr. Rel. 27: 127–137

Sasaki, H., Igarashi, Y., Nishida, K., Nakamura, J. (1993b) Ocular delivery of the β-blocker, tilisolol, through the prodrug approach. Int. J. Pharm. 93: 49–60

Schoenwald, R. D. (1990) Ocular drug delivery. Pharmacokinetic considerations. Clin. Pharmacokinet. 18: 255–269

Schoenwald, R. D., Huang, H.-S. (1983) Corneal penetration behavior of β-blocking agents I: physicochemical factors. J. Pharm. Sci. 72: 1266–1272

Shell, J. W. (1982) Pharmacokinetics of topically applied ophthalmic drugs. Surv. Ophthalmol. 26: 207–218

Suhonen, P., Järvinen, T., Peura, P., Urtti, A. (1991) Permeability of pilocarpic acid diesters across albino rabbit cornea in vitro. Int. J. Pharm. 74: 221–228

Tadokoro, Y., Sato, K., Hatakeyama, S., Kawase, S. (1990) Japan Patent 255618

Yano, Y., Yamaoka, K., Tanaka, H. (1989) A nonlinear least squares program, MULTI(FILT), based on fast inverse Laplace transform for microcomputers. Chem. Pharm. Bull. 37: 1035–1038

Wang, W., Sasaki, H., Chien, D.-S., Lee, V. H. L. (1991) Lipophilicity influence on conjunctival drug penetration in the pigmented rabbit: a comparison with corneal penetration. Curr. Eye Res. 6: 571–579