# Characterization of Ocular Pharmacokinetics of Beta-Blockers Using a Diffusion Model After Instillation

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***Purpose.*** To characterize the ocular pharmacokinetics of beta-blockers (timolol and tilisolol) after instillation in the albino rabbit using a mathematical model that includes a diffusion process.

***Methods.*** The disposition of fluorescein isothiocyanate-dextran (FITC-dextran, molecular weight 4400), timolol, and tilisolol was determined in tear fluid and aqueous humor after instillation or ocular injection in rabbits. The *in vivo* penetration parameters were estimated by fitting the concentration-time profiles to the Laplace equations based on a diffusion model using MULTI(FILT) program. The *in vitro* permeability of drugs was measured across cornea using a two-chamber diffusion cell.

***Results.*** Concentration-time profiles of drugs in the tear fluid after instillation showed a monoexponential curve. Although a monoexponential curve was observed in the aqueous humor concentration of FITC-dextran after injection into the aqueous chamber, timolol and tilisolol showed a biexponential curve. On the basis of these results, an *in vivo* pharmacokinetic model was developed for estimation of penetration parameters. The *in vitro* partition parameters were higher than those of the *in vivo* parameters.

***Conclusions.*** The ocular absorption of timolol and tilisolol was characterized using an *in vivo* pharmacokinetic model and *in vivo* penetration parameters.

**KEY WORDS:** diffusion model; drug delivery system; ocular penetration; pharmacokinetics.

## INTRODUCTION

The bioavailability and pharmacokinetics of instilled drug in the anterior segment of the eye are mainly controlled by the following factors: disposition of the drug in the precorneal area (tear fluid); penetration of the drug into the cornea; and disposition of the drug in the aqueous chamber. Several compartment models have been applied to describe the pharmacokinetics of ophthalmic drugs in the eye (1,2). However, the application of compartment models to the corneal penetration of drugs is of limited use.

Most penetration profiles of ophthalmic drugs through the thick cornea show lag time before displaying a steady state flux. The elimination of drug in the precorneal area and aqueous humor progresses under nonsteady state conditions, since drug elimination is much faster than corneal penetration. The diffusion process is described by Fick's second law. Therefore, a pharmacokinetic model that accounts for the diffusion process is more adequate than a simple compartment model for the characterization of the ocular pharmacokinetics of the instilled drug. This model is also useful for the evaluation of the *in vivo* penetration process, as it employs both diffusion and partition parameters, which are used in *in vitro* penetration experiments. However, there is little extant information about the application of the diffusion model to the ocular pharmacokinetics of instilled drugs.

Therefore, we developed an *in vivo* pharmacokinetic model that accounts for the diffusion process and characterizes the ocular pharmacokinetics of beta-blockers after instillation into the eyes of albino rabbits.

## MATERIALS AND METHODS

### Materials and Animals

Timolol maleate and tilisolol hydrochloride were kindly supplied by Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan) and Nisshin Flour Milling Co., Ltd. (Tokyo, Japan). All chemicals were of reagent grade. Male Nippon albino rabbits (2.0–3.0 kg) were used. All experiments conformed to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985).

### Drug Disposition After Instillation

Unanesthetized rabbits were kept in a prone position on a wooden plate. Twenty-five μl of drug solution (timolol and tilisolol: 100 mM, FITC-dextran: 50 mM) in phosphate-buffered saline (pH 7.4) were carefully instilled with a micropipette (Gilson Medical Electronics, Villiers-le-Bel, France) in the middle of the lower conjunctival sac of the eye. At the appropriate time after instillation, tear fluid (0.5 μl) was collected by a glass capillary (EM minicaps®, Hirschmann Laborgerate, Germany) from the middle of the lower marginal tear strip and was diluted by 50 μl of phosphate-buffered saline (pH 7.4). In the remaining experiments, rabbits were sacrificed by an overdose of sodium pentobarbital at the appropriate time after instillation. After thoroughly rinsing the corneal and conjunctival surfaces with 0.9% NaCl and blotting them dry, the aqueous humor was aspirated from the anterior chamber using a 1.0 ml disposable syringe with a 27-gauge needle.

### Drug Disposition After Ocular Injection

Anesthetized rabbits were placed on a wooden plate in a prone position. About 10 min before the administration of the drug, the eyes were anesthetized locally with 0.4% oxybuprocaine hydrochloride. Five μl of drug solution (timolol and tilisolol: 3 mM, FITC-dextran: 1 mM) were injected into the aqueous chamber using a microsyringe fitted with a 30-gauge needle. In order to prevent leakage of the drug solution from the aqueous humor after administering the drug, the injected needle was cut off and was fixed by surgical adhesion (Aron Alfa A®, Sankyo

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Co., Ltd., Tokyo, Japan). The protocol for sampling the aqueous humor is described above. The sample was subjected to HPLC assay.

### *In Vitro* Penetration Experiment

The glass apparatus for the *in vitro* diffusion experiment and the procedure for preparing ocular membranes have been described in the previous reports (3). Penetration solution (5 mM timolol and tilisolol, 4 ml) and penetrant-free solution (4 ml) were added to the epithelial side (donor side) and endothelial side (receiver side), respectively. At appropriate time intervals (at first 5 min and afterward at 10 min intervals for 120 min), a sample (50 μl) was withdrawn from the receiver side and was assayed by HPLC.

### Drug Determination

The samples involving timolol and tilisolol were mixed with 0.1 M HCl and methanol including an internal standard (methyl-*p*-hydroxybenzoate for timolol and *o*-ethoxybenzamide for tilisolol). After centrifugation, the supernatant was injected into a HPLC system. The HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) was used in a reverse-phase mode for the assay. The stationary phase used was a Cosmosil $5C_{18}$-P packed column (150 mm length × 4.6 mm i.d., Nacalai Tesque Inc.). A mixture of methanol and 50 mM $NaH_2PO_4$ (40:60 for timolol and 37:63 for tilisolol, v/v) was used as the mobile phase with a flow rate of 1.0 ml/min. Retention of drug was monitored with a UV spectro-photometric detector for timolol (SPD-10A, Shimadzu Co., Ltd.; 295 nm) and with a fluorescence monitor for tilisolol (RF-535, Shimadzu Co. Ltd.; excitation wave length 315 nm, emission wave length 420 nm). The sample for FITC-dextran was determined with a spectrofluorophotometer (RF-1500, Shimadzu Co., Ltd.; excitation wavelength 489 nm, emission wavelength 515 nm).

### Data Analysis

The *in vivo* drug behavior after instillation was analyzed by a pharmacokinetic model for the finite dose system which considers the cornea to be a one-plane barrier membrane. In this model, instilled drug diffuses into the cornea from the tear fluid compartment to the aqueous humor compartment with the reservoir compartment. The *in vivo* penetration parameters were estimated from the drug concentrations in the aqueous humor after instillation. Based on this model, the Laplace transforms for the amount of drug appearing in the aqueous humor ($AH_{amount}$) are expressed as follows:

$$AH_{amount} = sZX_0V_{AHc}(s + Kt_{pc})/W$$

$$\begin{aligned} W = {} & V_{TF}V_{AHc}(s + Ke_{TF})((s + Ke_{AH} + Kt_{cp})(s + Kt_{pc}) \\ & - Kt_{cp}Kt_{pc})\sinh d + sZV_{AHc}((s + Ke_{AH} \\ & + Kt_{cp})(s + Kt_{pc}) - Kt_{cp}Kt_{pc})\cosh d \\ & + sZV_{TF}(s + Ke_{TF})(s + Kt_{pc})\cosh d \\ & + s^2Z^2(s + Kt_{pc})\sinh d \end{aligned} \quad (1)$$

$$d = L(s/D_{CR})^{0.5}$$

$$Z = K_{CR}V_{CR}/d,$$

where $X_0$ is the initially instilled dose, $V_{TF}$ is the apparent distribution volume in the tear fluid, $V_{AHc}$ is the apparent distribution volume in the aqueous humor, $Ke_{TF}$ is the elimination rate constant in the tear fluid, $Ke_{AH}$ is the elimination rate constant in the aqueous humor, $Kt_{pc}$ and $Kt_{cp}$ are the transfer rate constants between the aqueous humor and reservoir, $D_{CR}$ is the diffusion coefficient of drug in the cornea, $K_{CR}$ is the partition coefficient of drug between the cornea and donor solution, L is the effective diffusion length in the cornea, $V_{CR}$ is the corneal volume, $s$ is the Laplace variable with respect to time. Since it is difficult to determine correctly the real diffusion length for the penetrant, the diffusion parameter ($D' = D_{CR}/L/L$) and the partition parameter ($K' = K_{CR}V_{CR}$) were defined. Kp (=K'D'/the effective diffusion area) is the permeability coefficient. Apparent distribution volume and elimination rate constant in the tear fluid were estimated by the concentration-time profile in the tear fluid after instillation. Apparent distribution volumes, elimination rate constants, and transfer rate constants in the aqueous humor and reservoir were estimated by the concentration-time profile in the aqueous humor after injection into the aqueous chamber.

Based on this model, the Laplace transforms for the amount of drug appearing in the tear fluid ($TF_{amount}$) and the cornea ($CR_{amount}$) are expressed as follows:

$$\begin{aligned} TF_{amount} = {} & X_0V_{TF}(V_{AHc}((s + Ke_{AH} + Kt_{cp})(s + Kt_{pc}) \\ & - Kt_{cp}Kt_{pc})\sinh d + sZ(s + Kt_{pc})\cosh d/W \end{aligned} \quad (2)$$

$$\begin{aligned} CR_{amount} = {} & X_0Z(V_{AHc}((s + Ke_{AH} + Kt_{cp})(s + Kt_{pc}) \\ & - Kt_{cp}Kt_{pc})(\cosh d - 1) + sZ(s + Kt_{pc})\sinh d)/W \end{aligned} \quad (3)$$

$$\begin{aligned} W = {} & V_{TF}V_{AHc}(s + Ke_{TF})((s + Ke_{AH} + Kt_{cp})(s + Kt_{pc}) \\ & - Kt_{cp}Kt_{pc})\sinh d + sZV_{AHc}((s + Ke_{AH} \\ & + Kt_{cp})(s + Kt_{pc}) - Kt_{cp}Kt_{pc})\cosh d \\ & + sZV_{TF}(s + Ke_{TF})(s + Kt_{pc})\cosh d \\ & + s^2Z^2(s + Kt_{pc})\sinh d \end{aligned}$$

$$d = L(s/D_{CR})^{0.5}$$

$$Z = K_{CR}V_{CR}/d$$

*In vitro* penetration profiles for timolol were analyzed by a diffusion model for the infinite dose system, which regards the cornea as a one-plane barrier membrane (4). This membrane model assumes a constant drug concentration in the donor phase and a sink condition in the receiver phase, since the cumulative amount of drug transferred to the receiver phase was much smaller than that of the donor phase. According to the model, the Laplace transform for the total amount of drug appearing in the receiver phase (Q) is expressed as follows:

$$\begin{aligned} Q &= ZC_0/s/\sinh d \\ d &= L(s/D_{CR})^{0.5} \\ Z &= K_{CR}V_{CR}/d, \end{aligned} \quad (4)$$

where $C_0$ is the constant drug concentration in the donor phase.

The *in vivo* and *in vitro* penetration parameters were estimated by fitting the penetration and concentration-time profiles

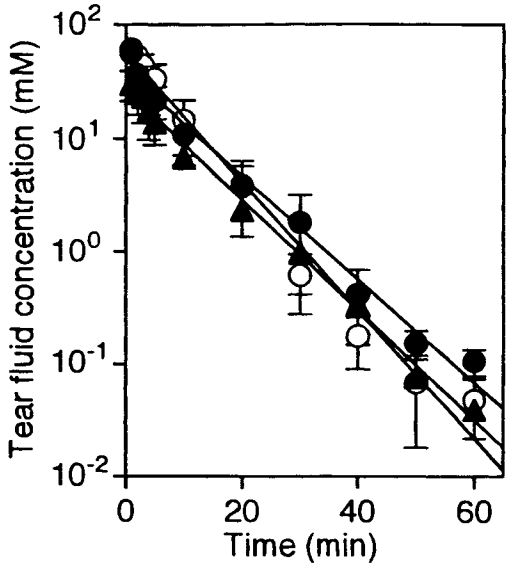


**Fig. 1.** Tear fluid concentration of FITC-dextran, timolol, and tilisolol after instillation in albino rabbits. (▲) FITC-dextran, (●) timolol, (○) tilisolol. Each point represents the mean ± S.E. of at least 4 experiments.

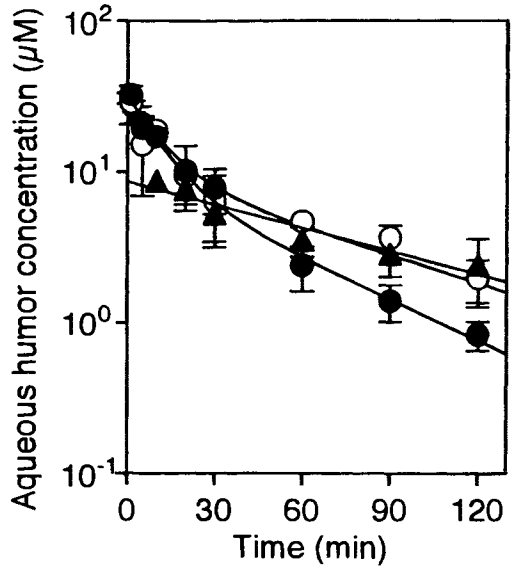


**Fig. 2.** Aqueous humor concentration of FITC-dextran, timolol, and tilisolol after injection into aqueous chamber in albino rabbits. (▲) FITC-dextran, (●) timolol, (○) tilisolol. Each point represents the mean ± S.E. of at least 3 experiments.

to the equations using MULTI (FILT), a nonlinear least-squares computer program based on a fast inverse Laplace transform algorithm (5). This program was written by MS-FORTRAN and run on a personal computer (PC-9821 V10, NEC, Tokyo, Japan).

## RESULTS

### Drug Disposition in Tear Fluid and Aqueous Humor

Figure 1 shows the concentration-time profiles of timolol, tilisolol, and FITC-dextran in the tear fluid after instillation. These profiles showed a monoexponential curve. The elimination rate constant and apparent distribution volume were estimated according to a one-compartment model as given in Table 1. The pharmacokinetic parameters of timolol and tilisolol were not significantly different from those of FITC-dextran.

Figure 2 shows the concentration-time profiles of timolol, tilisolol, and FITC-dextran in the aqueous humor after injection into the aqueous chamber. Although a monoexponential curve was observed in the concentration-time profile of FITC-dextran, timolol and tilisolol eliminated biexponentially. The eliminations of timolol and tilisolol took place faster than that of FITC-dextran. Pharmacokinetic parameters of these profiles were calculated by a two-compartment model as given in Table 2.

**Table 1.** Pharmacokinetic Parameters in Tear Fluid After Instillation of FITC-Dextran (50 mM/25 μl), Timolol (100 mM/25 μl), and Tilisolol (100 mM/25 μl)

| Parameter | $Ke_{TF}$ ($min^{-1}$) | $V_{TF}$ (ml) |
|---|---|---|
| FITC-dextran | 0.13 ± 0.02 | 0.059 ± 0.008 |
| Timolol | 0.17 ± 0.05 | 0.062 ± 0.012 |
| Tilisolol | 0.14 ± 0.03 | 0.051 ± 0.007 |

*Note:* Each value represents mean ± S.E. of 5 experiments. Pharmacokinetic parameters of timolol and tilisolol were not significantly different from that of FITC-dextran (Student's t-test, $P < 0.05$).

Based on these results, an *in vivo* pharmacokinetic model that accounted for the corneal diffusion process was developed in order to predict the concentration of ophthalmic drug in anterior tissues such as tear fluid, cornea, and aqueous humor after instillation (Fig. 3).

### *In Vivo* Ocular Absorption

The concentration-time profile of timolol in the aqueous humor after instillation is shown in Fig. 4A. Timolol and tilisolol showed maximum concentrations at 30 and 60 min, respectively, and thereafter gradually decreased. The *in vivo* penetration parameters of timolol and tilisolol were estimated from the aqueous humor concentrations after instillation by the pharmacokinetic model (Fig. 3) and model parameters (Tables 1 and 2). The fitting curves were consistent with the aqueous humor concentrations of timolol and tilisolol after instillation, as shown in Fig. 4A. The *in vivo* penetration parameters for timolol and tilisolol are listed in Table 3. Timolol showed higher values than tilisolol, namely, 1.6-fold in the diffusion parameter and 1.8-fold in the partition parameter. The simulation curves

**Table 2.** Pharmacokinetic Parameters in Aqueous Humor After Injection into Aqueous Chamber of FITC-Dextran (1 mM/5 μl), Timolol (3 mM/5 μl), and Tilisolol (3 mM/5 μl)

| Parameter | FITC-dextran[a] | Timolol[b] | Tilisolol[b] |
|---|---|---|---|
| $Ke_{AH}$ ($min^{-1}$) | 0.016 | 0.057 | 0.033 |
| $Kt_{cp}$ ($min^{-1}$) | — | 0.028 | 0.032 |
| $Kt_{pc}$ ($min^{-1}$) | — | 0.031 | 0.037 |
| $V_{AHc}$ (ml) | 0.477 | 0.446 | 0.485 |
| $V_{AHp}$ (ml) | — | 0.414 | 0.406 |

[a] Analyzed by 1-compartment model.
[b] Analyzed by 2-compartment model.

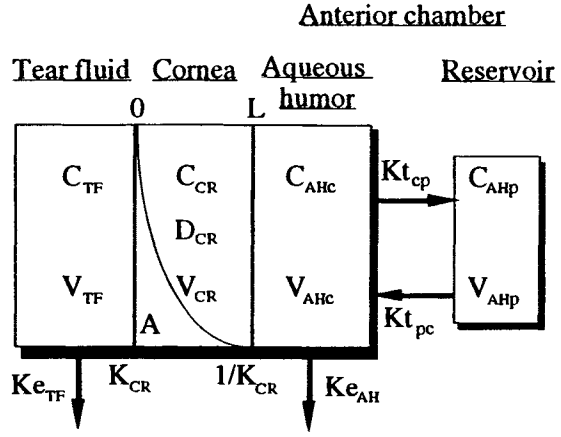


**Fig. 3.** Pharmacokinetic model including a diffusion process for in vivo experiment in albino rabbits. Abbreviation: $C_{TF}$, the drug concentration in the tear fluid; $C_{CR}$, the drug concentration in the cornea; $C_{AHc}$, the drug concentration in the aqueous humor; $C_{AHp}$, the drug concentration in the reservoir; $V_{TF}$, the apparent distribution volume in the tear fluid; $V_{CR}$, the corneal volume; $V_{AHc}$, the apparent distribution volume in the aqueous humor; $V_{AHp}$, the apparent distribution volume in the reservoir; $D_{CR}$, the diffusion coefficient of drug in the cornea; $K_{CR}$, the partition coefficient of drug between the cornea and tear fluid; A, the effective diffusion area; L, the effective diffusion length in the cornea; $Ke_{TF}$, the elimination rate constant in the tear fluid; $Ke_{AH}$, the elimination rate constant in the aqueous humor; $Kt_{cp}$, the transfer rate constant from the aqueous humor to the reservoir; $Kt_{pc}$, the transfer rate constant from the reservoir to the aqueous humor.

of drug concentrations in the cornea were also calculated from the model parameters and *in vivo* penetration parameters, and are shown in Fig. 4B.

### Permeability of Timolol Through Cornea

Permeability of timolol and tilisolol through an isolated cornea were studied by an *in vitro* technique using a diffusion device mounted with a rabbit cornea as a diffusion membrane. The penetration profile of timolol and tilisolol showed a steady-state diffusion, which took place after a lag time. *In vitro* penetration parameters were calculated from the profiles by Fick's equation and are listed in Table 3. Although the *in vitro* partition parameters were higher than those of the *in vivo* parameters, there was no difference between the *in vitro* and *in vivo* diffusion parameters.

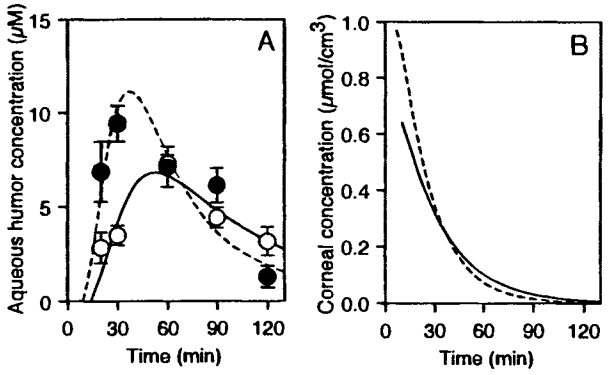


**Fig. 4.** Concentration of timolol and tilisolol in aqueous humor (A) and cornea (B) after instillation into albino rabbits. (●) Experimental data of timolol and (-----) simulation line, (○) experimental data of tilisolol and (——) simulation line. Each point represents the mean ± S.E. of at least 3 experiments. A simulation line in aqueous humor is equal to a fitting line.

**Table 3.** In Vivo and In Vitro Penetration Parameters of Timolol and Tilisolol Through Cornea

| Drugs | Parameter | In vivo | In vitro |
|---|---|---|---|
| Timolol | D′ ($hr^{-1}$) | 0.38 | 0.38 ± 0.05 |
| | K′ ($cm^3$) | 0.014 | 0.132 ± 0.012 |
| Tilisolol | D′ ($hr^{-1}$) | 0.24 | 0.24 ± 0.01 |
| | K′ ($cm^3$) | 0.008 | 0.048 ± 0.007 |

*Note:* D′ ($D_{CR}/L/L$), diffusion parameter; K′ ($K_{CR}V_{CR}$), partition parameter. In vitro penetration experiment value represents mean ± S.E. of 4 experiments.

## DISCUSSION

In ophthalmic chemotherapy, most instilled drugs are rapidly eliminated from the precorneal area due to drainage through the nasolacrimal duct and dilution by tear turnover (6). The corneal route is a dominant route for access to the aqueous humor (7). An understanding about the pharmacokinetics of instilled drugs will result in improvement of such chemotherapy.

In the present study, FITC-dextran, a hydrophilic and high molecular weight dye, was used to measure the physiological volume and turnover of tear fluid and aqueous humor because of its inability to permeate biological membranes. The elimination rate constant of FITC-dextran in tear fluid was nearly equal to a physiological turnover rate (6). The apparent distribution volume of FITC-dextran (0.059 ± 0.008 ml) was also close to the calculated initial volume (0.033 ml) that included an instillation volume (0.025 ml) and a normal volume of tear fluid, contained in a conjunctival cul-de-sac (0.008 ml) (6). A slight increase in the apparent distribution volume may be explained by increased tear flow accompanied by the stimulation caused by the drug instillation (6). In the aqueous humor, the apparent elimination rate constant and distribution volume of FITC-dextran were nearly equal to the physiological aqueous humor turnover rate and volume. The flow rate of aqueous humor in rabbit eyes is approximately from 1–1.5% of the chamber volume per minute (0.010–0.015 $min^{-1}$) and the total volume of the aqueous humor is about 300 μl (8).

Timolol is a nonselective and lipophilic beta-blocker that is widely used in the treatment of open-angle glaucoma. Tilisolol was synthesized as a nonselective and hydrophilic beta-blocker and has been reported to reduce intraocular pressure after instillation in rabbit eyes (9). Timolol and tilisolol are smaller and more lipophilic than FITC-dextran. In the tear fluid, the elimination rate constants and apparent distribution volumes of timolol and tilisolol were not significantly different from those of FITC-dextran. These results indicate that the turnover of the tear fluid mainly contributes to the drug disposition in the precorneal area.

The *in vitro* penetration of timolol and tilisolol was analyzed by a diffusion equation (Table 3). Several researchers have measured the corneal permeability of timolol (4,10). Chang *et*

*al.* (11) and Sasaki *et al.* (12) reported that the corneal permeability coefficients (Kp) of timolol were $18.2 \times 10^{-6}$ cm/sec and $7.6 \times 10^{-6}$ cm/sec, respectively. These values are comparable to the corneal Kp observed in the present study ($8.9 \times 10^{-6}$ cm/sec). Timolol has a higher permeability coefficient than does tilisolol; this difference leads to the higher lipophilicity of timolol because corneal penetration is dependent on drug lipophilicity (2). The drug concentration in the whole cornea was calculated by a mathematical model (Fig. 4B). After instillation, timolol showed higher cornea concentrations than tilisolol within about 30 min; slightly lower concentrations were then observed in timolol. This result agrees with findings that show a faster corneal penetration of timolol than of tilisolol.

In the aqueous humor, timolol and tilisolol showed rapid clearance and the existence of a peripheral compartment as a reservoir; neither of these were observed with FITC-dextran (Fig. 4A). Maximum concentration of timolol in the aqueous humor was reached at a faster rate than it was in the case of tilisolol. This phenomena was explained not only by the faster corneal penetration (D′ and K′) of timolol but also by the faster elimination into the aqueous humor ($Ke_{AH}$) than was observed in the case of tilisolol. The elimination of drugs through ocular tissues in the aqueous humor is dependent on drug lipophilicity (2). Drug clearance in the aqueous humor is significantly greater than aqueous humor turnover because of additional pathways such as metabolism and systemic uptake in vascular tissues (e.g., iris and ciliary body) (2). The tissues also act as a reservoir for drugs in the aqueous humor because they possess melanine granules, which strongly bind to drugs. Zane *et al.* (13) investigated physicochemical factors associated with binding and retention of drugs in the ocular melanin granules of rats. Such tissues are porous and possess a large surface, such that a distribution equilibrium with a drug dissolved in aqueous humor can be expected to occur rapidly. The lens also accumulates drugs by a diffusion process following instillation and systemic administration (14). On the other hand, it is well known that beta-blockers decrease aqueous humor formation by the ciliary processes. In preliminary experiments, however, the elimination rate of FITC-dextran in the aqueous humor after coinjection with timolol or tilisolol into the anterior chamber was not significantly different from the elimination rate of FITC-dextran injected alone. It has been reported that a single instillation of timolol did not show a significant change in the flow rate of the aqueous humor in rabbit eyes (15,16).

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. It was possible to develop a mathematical model representing the three layers of cornea according to the physiology of the cornea. However, the penetration parameters were too numerous to be determined with great accuracy by fitting using Laplace equations. The penetration profile with a lag time was previously described by a mono-layer model (4). Therefore, the present study showed a diffusion model using a mono-layer. This mathematical model is useful for predicting the aqueous humor concentration of a drug. The pharmacokinetic model uses *in vivo* penetration parameters to describe the concentrations of timolol and tilisolol in the aqueous humor after instillation (Fig. 4A). In the preliminary experiment to this study, this model and *in vivo* parameters were also able to predict tilisolol concentrations in the aqueous humor after instillation of tilisolol in the form of viscous formulation (data not shown). The present findings support the validity of the *in vivo* pharmacokinetic model as well as the validity of *in vivo* parameters. An increase in the diffusion parameter enhances maximum concentration rates and shortens the time it takes for a drug to reach a maximum concentration in the aqueous humor. The diffusion parameter can be increased by destruction of the epithelial barrier, as is the case in corneal ulcers and infections. The partition parameter enhances the maximum concentration. The partition parameter can be improved mainly by an increase of drug lipophilicity. An increase in the elimination rate constant in tears and aqueous humor decreases the drug concentration in the aqueous humor. The elimination rate of drugs in tears may be decreased by dry eye disease or by application with viscous solutions.

It is important to note that the *in vivo* partition parameters calculated by the present model were lower than those of the *in vitro* parameters. However, there was no difference between the *in vivo* and *in vitro* diffusion parameters. The smaller *in vivo* partition parameters may be explained by a smaller available corneal surface area under the *in vivo* conditions. Keister *et al.* (17) reported that the instilled drug was absorbed through approximately one third of the actual total corneal and conjunctival area in the rabbit eye. Precorneal tear film consists of three main layers, namely, the superficial oily layer, the middle aqueous layer, and the mucin layer. An unstirred layer is likely to form on a surface of cornea under *in vivo* conditions. Continuous tear flow might lower drug concentrations on the front of the cornea such that the concentration becomes apparently lower than that observed on the sampling portion. Mikkelson *et al.* (18) also reported that protein binding with drugs in tear fluid can decrease ocular bioavailability. Further investigation will be necessary to clarify the mechanism causing the difference between the *in vitro* and *in vivo* penetrations.

In conclusion, an *in vivo* pharmacokinetics of timolol and tilisolol were characterized using a mathematical model. This pharmacokinetic model, as well as *in vivo* penetration parameters, will be effective for the estimation of the regimen for ophthalmic chemotherapy; it will also aid the development of ocular drug delivery systems.

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