Pharmacokinetic Prediction of the Ocular Absorption of an Instilled Drug with Ophthalmic Viscous Vehicle

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We previously developed an *in vivo* pharmacokinetic model that accounts for the corneal diffusion in albino rabbits and predicts the concentration of beta-blockers in the anterior segments. The purpose of this study is to pharmacokinetically predict the ocular absorption and characterize the systemic absorption of instilled drug with ophthalmic viscous vehicle to assist in its design and evaluation. Tilisolol and carboxymethylcellulose sodium salt (CMC) were used as the model ophthalmic drug and viscous polymer, respectively. After instillation of tilisolol with CMC vehicle in rabbits, the disposition of the drug in tear fluid, aqueous humor, and plasma were determined by HPLC. The ocular and systemic absorption were analyzed by a mathematical model including a diffusion process and a two-compartment model with first-order absorption, respectively. CMC vehicle increased the area under the concentration–time curve (AUC) of tilisolol in the tear fluid and aqueous humor and slightly reduced the AUC in plasma. The concentrations of tilisolol in the aqueous humor after instillation with CMC vehicle were accurately predicted from the tear concentrations by using the *in vivo* ocular pharmacokinetic model. CMC vehicle improved the ocular delivery of tilisolol.

Key words eye; pharmacokinetics; diffusion equation; viscous vehicle; drug delivery system

Following instillation of an ophthalmic drug, most of it is rapidly eliminated from the precorneal area, due to drainage *via* the nasolacrimal duct and dilution by tear fluid turnover, and easily absorbed into the systemic circulation.^{1–3)} Such behavior can result in poor bioavailability in an anterior segment and increased severity of systemic adverse effects.^{4,5)} One traditional way to alleviate this problem is to increase the viscosity of the formulation by incorporation of water-soluble polymers such as polyvinyl alcohol, methylcellulose and hydroxymethyl cellulose.^{6–9)} A viscous vehicle may modify not only the ocular absorption but also the systemic absorption of the instilled drug.^{10,11)} For the rational design and evaluation of these formulations, mathematical models describing the ocular and systemic absorption are very useful although there have been few reports involving viscous formulations.

We previously developed an *in vivo* pharmacokinetic model that accounts for corneal diffusion in albino rabbits and predicted the concentrations of beta-blockers in the anterior segments.¹²⁾ The plasma concentration of the instilled drug can be analyzed by a compartmental model. The purpose of this study is to mathematically characterize the ocular and systemic absorption of an instilled ophthalmic drug in the form of a viscous formulation. Tilisolol, a non-selective beta-blocker,^{13,14)} and carboxymethylcellulose sodium salt (CMC) were used as the model ophthalmic drug and vehicle, respectively.

MATERIALS AND METHODS

Animals Male Nippon albino rabbits (2.0–3.0 kg) were used throughout the study. The animals were individually 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 pre-

sent study conformed to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised 1985).

Materials Tilisolol hydrochloride was kindly supplied by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan). CMC (High viscosity, C-5013) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Salicyl tyrosine was prepared according to a routine method in our laboratory.¹⁵⁾ *o*-Ethoxybenzamide and all other chemicals of reagent grade were purchased from Nacalai Tesque Inc. (Kyoto, Japan). Phosphate-buffered saline (pH 7.4) was prepared by mixing isotonic phosphate buffer with an equal volume of 0.9% NaCl.

Viscosity and Drug Release in CMC Vehicle CMC was dissolved in phosphate-buffered saline (0.3% or 1.0%, w/v). The viscosity of the CMC vehicles was measured by viscometer (B type viscometer, rotor HH-2, Tokyo Keiki, Tokyo, Japan).

The release of tilisolol from CMC vehicles was examined using a glass apparatus in the *in vitro* diffusion experiment. The cellulose membrane (Seamless Cellulose Tubing 8/32, Viskase Sales Corp, U.C.C.) was mounted in the diffusion chambers. Tilisolol solution with CMC (5 mM, 3 ml) and penetrant-free solution (3 ml) were added to the donor and receiver sides, respectively. The concentration of tilisolol in the receiver side was determined by HPLC.

Drug Disposition in Tear Fluid Unanesthetized albino rabbits were kept in a prone position on a wooden plate. Twenty-five microliters of CMC vehicle containing tilisolol (100 mM) was carefully instilled with a micropipette (Gilson Medical Electronics, Villiers-le-Bel, France) into the lower conjunctival sac of the eye. At appropriate times after instillation, tear fluid samples (0.5 μ l) were collected using a glass capillary (EM minicaps[®], Hirschmann Laborgerate, Germany) from the lower conjunctival sac and diluted with 50 μ l phosphate-buffered saline. The samples were subjected to HPLC assay.

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Drug Disposition in Aqueous Humor and Blood

Unanesthetized albino rabbits were kept in a prone position on a wooden plate. Twenty-five microliters of tilisolol solution (100 mM) in phosphate-buffered saline or CMC vehicle were carefully instilled with a micropipette into the lower conjunctival sac of both eyes. In the remaining experiments, tilisolol solution was administered intravenously to the rabbits. Blood samples were collected using a heparinized syringe at appropriate times from a marginal ear vein and centrifuged at $15000 \times g$ for 15 min. The plasma samples were subjected to HPLC assay.

Aqueous humor was collected after albino rabbits had been sacrificed by an overdose of sodium pentobarbital solution at 30, 60, 120, and 240 min following instillation of the tilisolol with CMC vehicles and buffer solution. After thoroughly rinsing the corneal and conjunctival surfaces with saline and blotting them dry, about 200 μ l of the aqueous humor was aspirated from the anterior chamber using a 1.0 ml disposable syringe with a 27-gauge needle. These samples were also subjected to HPLC assay.

Drug Determination The tear fluid sample of tilisolol (50 μ l) was mixed with 0.1 M HCl (50 μ l) and methanol (100 μ l) containing an internal standard (300 μ g/ml *o*-ethoxybenzamide). The mixture was centrifuged at $15000 \times g$ for 15 min and the supernatant (50 μ l) was injected into the HPLC system. The aqueous humor sample of tilisolol (200 μ l) was mixed with 1 M HCl (20 μ l) and methanol (300 μ l) containing an internal standard (100 μ g/ml *o*-ethoxybenzamide). The mixture was centrifuged at $15000 \times g$ for 15 min and 50 μ l supernatant was injected into the HPLC system. The plasma sample (700 μ l) was mixed with 300 μ l 2 M perchloric acid and centrifuged at $15000 \times g$ for 15 min. The supernatant (800 μ l) was shaken with 200 μ l 5 M sodium hydroxide and 6 ml chloroform for 15 min. After centrifugation at $650 \times g$ for 15 min, 5 ml of the organic layer was separated, mixed with methanol (100 μ l) containing an internal standard (20 μ g/ml salicyl tyrosine) and evaporated *in vacuo*. The residue was dissolved in 250 μ l 20% methanol in phosphate-buffered saline. The sample was injected into HPLC system.

The HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) was used in the reverse-phase mode for the assay. The stationary phase used was a Cosmosil 5C₁₈-P packed column (150 mm length $\times$ 4.6 mm i.d., Nacalai Tesque Inc.). Mixtures of methanol and 50 mM NaH₂PO₄ (37:63 (v/v) for samples of tear fluid and aqueous humor) or acetonitrile, methanol and 50 mM NaH₂PO₄ (12:8:80 (v/v) for plasma samples) were used as mobile phase, at a flow rate of 1.0 ml/min. Retention of the drug was monitored with a fluorescence HPLC monitor (RF-535, Shimadzu Co., Ltd.; excitation wavelength 315 nm, emission wavelength 420 nm).

Data Analysis Prediction of the aqueous humor concentration of tilisolol after instillation with CMC vehicles was performed based on a pharmacokinetic model that accounts for the diffusion of a finite dose by considering the cornea to be a one-plane layer. In this model, instilled drug diffuses into the cornea from the tear fluid containing a reservoir compartment and releases to the aqueous humor containing another reservoir compartment. Based on this model, the Laplace transforms for the amount of drug appearing in the aqueous humor (AH_{amount}) can be expressed as follows:

$$AH_{\text{amount}} = sZX_0V_{\text{AHc}}(s + Kt_{\text{TFpc}})(s + Kt_{\text{AHpc}})/W \quad (1)$$

$$\begin{aligned} W = & V_{\text{TFc}}V_{\text{AHc}}((s + Ke_{\text{TF}} + Kt_{\text{TFcp}})(s + Kt_{\text{TFpc}}) - Kt_{\text{TFcp}}Kt_{\text{TFpc}}) \\ & \times ((s + Ke_{\text{AH}} + Kt_{\text{AHcp}})(s + Kt_{\text{AHpc}}) - Kt_{\text{AHcp}}Kt_{\text{AHpc}})\sinh d \\ & + sZV_{\text{AHc}}((s + Ke_{\text{AH}} + Kt_{\text{AHcp}})(s + Kt_{\text{AHpc}}) - Kt_{\text{AHcp}}Kt_{\text{AHpc}})(s + Kt_{\text{TFpc}})\cosh d \\ & + sZV_{\text{TFc}}((s + Ke_{\text{TF}} + Kt_{\text{TFcp}})(s + Kt_{\text{TFpc}}) - Kt_{\text{TFcp}}Kt_{\text{TFpc}})(s + Kt_{\text{AHpc}})\cosh d \\ & + s^2Z^2(s + Kt_{\text{TFpc}})(s + Kt_{\text{AHpc}})\sinh d \\ d = & L(s/D_{\text{CR}})^{0.5} \\ Z = & K_{\text{CR}}V_{\text{CR}}/d \end{aligned}$$

where X_0 is the initially instilled dose, D_{CR} is the diffusion coefficient of drug in the cornea, K_{CR} is the partition coefficient of drug between the cornea and tear fluid, L is the effective diffusion length in the cornea, V_{CR} is the corneal volume, s is the Laplace variable with respect to time, V_{TFc} and V_{AHc} are the apparent distribution volumes in the tear fluid and aqueous humor, respectively, Ke_{TF} and Ke_{AH} are the elimination rate constants in the tear fluid and aqueous humor, respectively, Kt_{TFcp} and Kt_{TFpc} are the transfer rate constants between the tear fluid and reservoir of the precorneal area, and Kt_{AHcp} and Kt_{AHpc} are the transfer rate constants between the aqueous humor and reservoir of the aqueous chamber. The apparent distribution volume, elimination rate constant and transfer rate constants in the tear fluid were estimated by the concentration-time profile in the tear fluid after instillation with CMC vehicles. Other pharmacokinetic parameters and the *in vivo* penetration parameters have been previously reported.¹²⁾ Since it is difficult to determine accurately the real diffusion length for the penetrant, the diffusion parameter ($D' = D_{\text{CR}}/L/L$) and the partition parameter ($K' = K_{\text{CR}}V_{\text{CR}}$) were defined. The aqueous humor concentrations were simulated by equation (1) using MULTI(FILTS), a computer simulation program based on a fast inverse Laplace transform algorithm.¹⁶⁾ This program was written by MS-FORTRAN and run on a personal computer (PC-9821 V10, NEC, Tokyo, Japan).

The plasma concentration profiles after instillation with CMC vehicles and buffer solution were fitted to the two-compartment model with first-order absorption by the nonlinear least-squares method.¹⁷⁾ In this model, the equation for the plasma concentration of tilisolol is as follows:

$$\begin{aligned} C_p = & \text{Dose}K_aF/V_c \\ & \times [(K_a - K_{21})\exp(-K_a t)/\{(\beta - K_a)(K_a - \alpha)\} \\ & + (\alpha - K_{21})\exp(-\alpha t)/\{(K_a - \alpha)(\alpha - \beta)\} \\ & + (\beta - K_{21})\exp(-\beta t)/\{(\alpha - \beta)(\beta - K_a)\}] \end{aligned} \quad (2)$$

Hybrid parameters α and β are defined as $\alpha + \beta = K_{12} + K_{21} + K_{\text{el}}$ and $\alpha\beta = K_{21}K_{\text{el}}$. V_c is the volume of the central compartment. K_{el} is the elimination rate constant from the central compartment. K_{12} and K_{21} are the transfer rate constants between the central and peripheral compartments. These parameters were estimated from the plasma concentrations after intravenous injection of tilisolol.¹⁸⁾ K_a is the first-order rate constant for tilisolol absorption into the systemic circulation from the eye. F is the bioavailability of tilisolol after instillation with CMC vehicles and buffer solution.

The area under the concentration-time curve (AUC) of

Fig. 1. Release of Tilisolol from CMC Vehicle through the Dialysis Membrane

(○) solution, (□) 0.3% CMC, (■) 1.0% CMC. Each point represents the mean $\pm$ S.E. of at least four experiments.

Fig. 2. Tear Fluid Concentration of Tilisolol after Instillation of CMC Vehicles in Albino Rabbits

(○) solution, (□) 0.3% CMC, (■) 1.0% CMC. Each point represents the mean $\pm$ S.E. of at least five experiments.

tilisolol in the aqueous humor (AUC_{AH}) and plasma (AUC_{PL}) were calculated using a linear trapezoidal formula. The AUC_{AH} was calculated from the average points at 30, 60, 120, and 240 min. The AUC_{PL} was calculated from the points over the period 0–480 min.

RESULTS

Drug Release from CMC Vehicle The viscosity of CMC vehicle increased with an increase in the CMC concentration. The viscosity of the 0.3% and 1.0% CMC vehicles was 43 and 676 cps, respectively. Figure 1 shows the amount of tilisolol in the receiver side arising from the CMC vehicles through the dialysis membrane. The drug release decreased with an increase in the CMC concentration. The observed release rate constant without CMC (buffer solution), with 0.3% CMC, and with 1.0% CMC was 0.0198 ± 0.0008 ($\mu\text{mol}/\text{min}$), 0.0118 ± 0.0014 ($\mu\text{mol}/\text{min}$), and 0.0078 ± 0.0003 ($\mu\text{mol}/\text{min}$), respectively.

Drug Disposition in Tear Fluid Figure 2 shows the concentration–time profiles of tilisolol in tear fluid after instillation of solution and the CMC vehicles. It has been reported that the profile of such a solution is apparently mono-exponential.¹²⁾ However, the concentrations of tilisolol after instillation of the CMC vehicles were higher than those of the solution and the profile was a bi-exponential.

Based on these results, an *in vivo* pharmacokinetic model,

Fig. 3. *In Vivo* Pharmacokinetic Model Including a Diffusion Step after Instillation of CMC Vehicles in Albino Rabbits

Abbreviations: C_{TF} , drug concentration in the tear fluid; C_{TFp} , drug concentration in the reservoir of the precorneal area; C_{CR} , drug concentration in the cornea; C_{AHc} , drug concentration in the aqueous humor; C_{AHp} , drug concentration in the reservoir of the aqueous chamber; V_{TFc} , apparent distribution volume in the tear fluid; V_{TFp} , apparent distribution volume in the reservoir of the precorneal area; V_{CR} , corneal volume; V_{AHc} , apparent distribution volume in the aqueous humor; V_{AHp} , apparent distribution volume in the reservoir of the aqueous chamber; D_{CR} , diffusion coefficient of drug in the cornea; K_{CR} , partition coefficient of drug between the cornea and tear fluid; A , effective diffusion area; L , effective diffusion length in the cornea; Ke_{TF} , elimination rate constant in the tear fluid; Ke_{AH} , elimination rate constant in the aqueous humor; Kt_{TFcp} , transfer rate constant from the tear fluid to the reservoir of the precorneal area; Kt_{TFpc} , transfer rate constant from the reservoir of the precorneal area to the tear fluid; Kt_{AHcp} , transfer rate constant from the aqueous humor to the reservoir of the aqueous chamber; Kt_{AHpc} , transfer rate constant from the reservoir of the aqueous chamber to the aqueous humor.

Table 1. Pharmacokinetic Parameters for Ocular Absorption

Parameters	Solution ^{a)}	0.3% CMC	1.0% CMC
Parameters for tear fluid			
Ke_{TF} (min^{-1})	0.14 ± 0.03	0.12 ± 0.02	0.08 ± 0.01
Kt_{TFcp} (min^{-1})	—	0.43 ± 0.13	0.29 ± 0.14
Kt_{TFpc} (min^{-1})	—	0.27 ± 0.09	0.19 ± 0.06
V_{TFc} (ml)	0.051 ± 0.007	0.035 ± 0.004	0.043 ± 0.007
V_{TFp} (ml)	—	0.059 ± 0.011	0.049 ± 0.021
AUC_{TF} ($\text{min} \cdot \mu\text{M}$)	447.9 ± 134.6	664.7 ± 223.3	829.1 ± 92.8
Other parameters ^{b)}			
Ke_{AH} (min^{-1})		0.033	
Kt_{AHcp} (min^{-1})		0.032	
Kt_{AHpc} (min^{-1})		0.037	
V_{AHc} (ml)		0.485	
V_{AHp} (ml)		0.406	
$D' (=D_{CR}/L/L)$ (h^{-1})		0.24	
$K' (=K_{CR}V_{CR})$ (cm^3)		0.008	

Each value represents the mean $\pm$ S.E. of at least five experiments. ^{a)} The profile for tear fluid was analyzed as a mono-exponential curve.¹²⁾ ^{b)} Parameters obtained from data on the instillation of tilisolol were reported previously.¹²⁾

including corneal diffusion, was developed to predict the concentrations of tilisolol in aqueous humor after the instillation of CMC vehicles (Fig. 3). Pharmacokinetic parameters for the *in vivo* ocular model are shown in Table 1. The apparent elimination rate constant (Ke_{TF}), transfer rate constant (Kt_{TFcp} , Kt_{TFpc}) and distribution volumes (V_{TFc} , V_{TFp}) in the tear fluid were estimated using a two-compartment model. *In vivo* penetration parameters and pharmacokinetic parameters in the aqueous chamber have been previously reported.¹²⁾

***In Vivo* Ocular Absorption** Figure 4 shows the aqueous humor concentration of tilisolol after instillation with buffer solution and CMC vehicles. Tilisolol showed a maximum concentration of $8.8 \pm 2.5 \mu\text{M}$ and $9.3 \pm 0.7 \mu\text{M}$, 60 min after instillation of 0.3 and 1.0% CMC vehicles, respectively. These concentrations were higher than that of the buffer solution. The simulation curves using the *in vivo* pharmacokinetic model (Fig. 3) and pharmacokinetic parameters (Table

Fig. 4. Concentration of Tilisolol in Aqueous Humor after Instillation of CMC Vehicles in Albino Rabbits

(○) solution, (□) 0.3% CMC, (■) 1.0% CMC, (—) predicted line for 0.3% CMC, (---) predicted line for 1.0% CMC. Each point represents the mean $\pm$ S.E. of at least three experiments. Prediction was carried out using parameters in Table 1.

Fig. 5. Plasma Concentration of Tilisolol after Intravenous Injection and Instillation of CMC Vehicles in Albino Rabbits

(●) intravenous injection, (○) instillation in solution, (□) instillation in 0.3% CMC, (■) instillation in 1.0% CMC. Each point represents the mean $\pm$ S.E. of at least four experiments.

1) agreed well with these measurements.

In Vivo Systemic Absorption Figure 5 shows the plasma concentration of tilisolol after intravenous injection and instillation with buffer solution and CMC vehicles. The plasma profiles of tilisolol after intravenous injection followed a two-compartment model.¹⁸⁾ The CMC vehicles slightly reduced the plasma concentrations of tilisolol compared with the buffer solution. The plasma concentrations of tilisolol after instillation of CMC vehicles reached a maximum at 60 or 90 min after dosing and then gradually disappeared. Pharmacokinetic parameters were calculated from the plasma profile of tilisolol after intravenous injection. Using the pharmacokinetic parameters, the first-order absorption, K_a , and bioavailability, F , were estimated from the plasma concentration of tilisolol after instillation of the solution and CMC vehicles. The pharmacokinetic parameters of tilisolol for systemic absorption are listed in Table 2. The CMC vehicles slightly reduced the K_a and F values compared with the buffer solution. The AUC_{PL} and AUC_{AH} were calculated and are also shown in Table 2. The AUC_{PL} of the 0.3% and 1.0% CMC vehicles was 81% and 73% of the buffer solution, respectively, although the AUC_{AH} of the CMC vehicles was higher than that of the buffer solution.

Table 2. Pharmacokinetic Parameters for Systemic Absorption

Parameters	Solution	0.3% CMC	1.0% CMC
Parameters for absorption			
K_a (min^{-1})	0.032 ± 0.007	0.021 ± 0.003	0.027 ± 0.006
F (min^{-1})	0.69 ± 0.10	0.54 ± 0.09	0.50 ± 0.06
AUC_{PL}^a ($\text{min} \cdot \mu\text{M}$)	23.6 ± 4.8	19.1 ± 3.6	17.3 ± 1.9
AUC_{AH}^b ($\text{min} \cdot \mu\text{M}$)	813	1367	1362
Other parameters ^{c)}			
K_{el} (min^{-1})		0.06	
K_{12} (min^{-1})		0.10	
K_{21} (min^{-1})		0.03	
V_c (l)		3.4	
V_p (l)		9.8	

Each value represents the mean $\pm$ S.E. of at least four experiments. a) AUC_{PL} was calculated over the period 0–480 min. b) AUC_{AH} was calculated from average points at 30, 60, 120 and 240 min. c) Parameters were obtained from data following the intravenous injection of tilisolol solution.

DISCUSSION

Topical application of drugs to the eyes is significantly impeded by their efficient removal.^{3,19)} One effective approach for improving ocular bioavailability has been the use of vehicles that retard drug loss from the precorneal area.^{3,19,20)} Chrai and Robinson⁷⁾ have reported that the drug concentrations in the precorneal area after instillation with methylcellulose vehicle increased, and the rate of drug elimination decreased following an increase in viscosity. Smart *et al.*²¹⁾ have shown that CMC solution is very muco-adhesive compared with other polymer solutions *in vitro*. In the present study, inclusion of CMC suppressed drug release from the vehicle (Fig. 1) and prolonged its retention in the precorneal area (Fig. 2 and Table 1). The retention of CMC in the precorneal area may act as a reservoir for tilisolol and resulted in bi-exponential elimination from the precorneal area. There was little difference between 0.3% CMC and 1.0% CMC in terms of the AUC_{tr} . The viscous vehicle may induce lacrimation as a reflex secretion and increase drug elimination from the precorneal area.

The behavior of the instilled drug in the anterior chamber is complicated because it includes both slow processes, such as corneal penetration, and rapid processes, such as disposition and distribution in the tear fluid and aqueous humor. We have previously developed an *in vivo* pharmacokinetic model that accounts for corneal diffusion. Therefore, prediction of tilisolol concentrations in the aqueous humor after instillation with CMC vehicles was performed by examining the tilisolol concentrations in tear fluid using our *in vivo* pharmacokinetic model (Fig. 3) and *in vivo* parameters (Table 2). The predicted drug concentrations in the aqueous humor were basically similar to the experimental data (Fig. 4). This agreement supports the validity of the *in vivo* pharmacokinetic model as well as the validity of the *in vivo* parameters for the eyes of albino rabbits.

The systemic absorption of ocularly administered beta-blockers may cause respiratory and cardiovascular side-effects. Linn and Jones²²⁾ have reported that the systemic absorption of timolol may be reduced with an increase in viscosity because the viscous vehicles reach the nasal mucosa at a slower rate than a non-viscous solution. In the present study, the CMC vehicles did not reduce the absorption rate constant and absorption ratio significantly (Fig. 5, Table 2).

The slight reduction in the absorption rate of tilisolol with the CMC vehicles is presumably caused by the sustained release of tilisolol from the vehicles. A delay in nasal drainage could also contribute to the reduced absorption rate. The AUC_{AH} was increased 1.7-fold by 0.3% CMC vehicle and 1.0% CMC vehicle compared with the buffer solution.

It was concluded that these viscous vehicles are useful for ophthalmic pharmacotherapy. They can be mathematically evaluated and improved by using *in vivo* pharmacokinetic models and suitable pharmacokinetic parameters.

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