Characterization of Ocular Pharmacokinetics of Tilisolol after Instillation into Anesthetized Rabbits

Hitoshi Sasaki, Kenzo Yamamura, Takahiro Mukai, Koyo Nishida, Junzo Nakamura, Mikiro Nakashima, and Masataka Ichikawa

Department of Hospital Pharmacy, Nagasaki University School of Medicine, 1–7–1 Sakamoto, Nagasaki 852–8501, Japan, and School of Pharmaceutical Sciences, Nagasaki University, 1–14 Bunkyo-machi, Nagasaki 852–8521, Japan.

Received June 14, 1999; accepted August 5, 1999

The purpose of this study was to characterize the ocular pharmacokinetics of a beta-blocker, tilisolol, after instillation into anesthetized rabbits using a mathematical model including a diffusion process. The samples were analyzed by HPLC. Anesthetized rabbit was used as a model of tear secretion deficiency. Anesthetized rabbits showed higher drug concentration in the tear fluid and aqueous humor after instillation than unanesthetized rabbits. A mathematical model including a diffusion process and in vivo penetration parameters well described the concentrations of tilisolol in the aqueous humor after instillation in anesthetized rabbits.

Key words: ocular pharmacokinetics; tilisolol; diffusion model; ocular penetration; drug delivery system; tear deficiency

Upon instillation of an ophthalmic drug, most of the instilled amount is rapidly eliminated from the precorneal area due to drainage through the nasolacrimal duct and dilution by tear turnover. On the other hand, instilled drugs in the tear fluid are commonly slowly absorbed through the cornea. Such behavior can result in poor bioavailability to the anterior segment and an increase in systemic side effects. There are a number of factors influencing the bioavailability and therapeutic effect of instilled drugs. One of the important factors is turnover of the tear fluid. The conjunctival cul-de-sac normally contains about 7.5 µl of tear fluid. The physiological turnover rate is 6.3–8.8%/min. The tear flow associated with a loss of instilled drug is also influenced by various factors. Non-physiological pH or tonicity in solution can stimulate a transient tear flow and enhanced drug loss. On the other hand, dry eye, which is the most common cause of chronically red and irritated eyes, decreases the tear fluid and shortens break up time. Visual display terminal work generates this lacrimal abnormality that has become a social problem. Sjogren's syndrome is a systemic autoimmune disease characterized by decreased lacrimal and salivary gland function causing dry eye and dry mouth. Anesthetization was also reported to decrease a production of tear fluid. These deficiencies of tear secretion may increase the intraocular bioavailability of instilled drug although there is little information about ocular pharmacokinetics.

We previously developed an in vivo kinetic model that accounts for the corneal diffusion process to analyze the pharmacokinetics of instilled drugs. In the present study, we characterized the ocular pharmacokinetics of a beta-blocker, tilisolol, using an in vivo kinetic model after instillation into anesthetized albino rabbits.

MATERIALS AND METHODS

Materials and Animals Tilisolol hydrochloride was kindly supplied by Nissin Flour Milling Co., Ltd. (Tokyo, Japan). o-Butylxenobenzamide was purchased from Nacalai Tesque Inc. (Kyoto, Japan). All other chemicals of reagent grade were obtained from Nacalai Tesque Inc. Phosphate-buffered saline (pH 7.4) was prepared by mixing isotonic phosphate buffer with an equal volume of 0.9% NaCl.

Male Nippon albino rabbits (2.0–3.0 kg) were used throughout the study. All experiments in the present study conformed to the Principles of Laboratory Animal Care (NIH publication #85-23, revised 1985).

Drug Disposition after Instillation Anesthetized rabbits by administration of sodium pentobarbital solution via marginal ear vein were kept in a prone position on a wooden plate. Twenty-five microliters of tilisolol solution (100 μm) in phosphate-buffered saline (pH 7.4) was carefully instilled with a micropipette (Gison 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 samples (0.5 µl) were 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 solution 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. The sample was subjected to a HPLC assay.

Drug Determination The tear fluid and aqueous humor involving tilisolol were mixed with 0.1 M HCl and methanol including an internal standard (o-butyroxybenzamide). The mixture was centrifuged at 12000 g for 15 min and 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 5C18-P packed column (150 mm length × 4.6 mm i.d., Nacalai Tesque Inc.). A mixture of methanol and 50 mM Na2HPO4 (37:63 v/v) was used as the mobile phase with a flow rate of 1.0 ml/min. Retention of drug was monitored with a fluorescence HPLC monitor (RF-535, Shimadzu Co., Ltd.; excitation wave length 315 nm, emission wave length 420 nm).

Data Analysis The apparent distribution volume and elimination rate constant in the tear fluid were estimated by a mono-exponential equation from the drug concentration-
time profile in the tear fluid after instillation.

The simulation was based on a pharmacokinetic model for the finite dose system which considers the cornea to be a one-plane barrier membrane. As shown in Fig. 1, instilled drug diffuses to the cornea from the tear fluid compartment to the aqueous humor compartment with the reservoir compartment. In the figure, \( X_0 \) is the initially instilled dose, \( C_{TF} \) is the drug concentration in the tear fluid, \( C_{CR} \) is the drug concentration in the cornea, \( C_{AHI} \) is the drug concentration in the aqueous humor, \( V_{AHI} \) is the drug concentration in the reservoir, \( V_{TF} \) is the apparent distribution volume in the tear fluid, \( V_{CR} \) is the cornea volume, \( V_{AHI} \) is the apparent distribution volume in the reservoir, \( D_{AHI} \) is the diffusion coefficient of drug in the cornea, \( K_{CR} \) is the partition coefficient of drug between the cornea and tear fluid, \( A \) is the effective diffusion area, \( L \) is the effective diffusion length in the cornea, \( K_{E_{TF}} \) is the elimination rate constant in the tear fluid, \( K_{E_{AHI}} \) is the elimination rate constant in the aqueous humor, \( K_{t_{fp}} \) is the transfer rate constant from the aqueous humor to the reservoir, and \( K_{t_{fp}} \) is the transfer rate constant from the reservoir to the aqueous humor.

Based on this model, the following differential equation was obtained for the cornea at time \( t \) (\( t>0 \)) and distance \( x \) (\( 0<X<L \)).

\[
\frac{d(C_{CR}/\partial_t)}{V_{CR}\partial(C_{CR}/\partial X^2)} = \frac{D_{AHI}}{V_{AHI}\partial(C_{AHI}/\partial X^2)}
\]

(1)

To solve the differential equation, the following initial condition and boundary conditions are necessary.

\[ t=0, \quad 0<X<L, \quad (C_{CR}/\partial t)_t=0 \]  
(2)

\[ t>0, \quad X=0, \quad C_{CR}=K_{CR}C_{TF} \]  
(3)

\[ t>0, \quad X=L, \quad C_{AHI}=C_{CR}/K_{CR} \]  
(4)

In the same manner, the following differential equations are obtained in the tear fluid, aqueous humor and the reservoir.

\[ t=0, \quad V_{TF}(\partial(C_{TF}/\partial t))=X_0 \]  
(5)

\[ t>0, \quad V_{TF}(\partial(C_{TF}/\partial t))=\frac{-K_{E_{TF}}(V_{TF}+D_{TF}(\partial(C_{TF}/\partial X)))}{V_{TF}+D_{TF}} \]  
(6)

\[ t=0, \quad V_{AHI}(\partial(C_{AHI}/\partial t))=0 \]  
(7)

\[ t>0, \quad V_{AHI}(\partial(C_{AHI}/\partial t))=K_{t_{fp}}V_{TF}C_{TF}+\frac{-K_{E_{AHI}}(V_{AHI}+D_{AHI}(\partial(C_{AHI}/\partial X)))}{V_{AHI}+D_{AHI}} \]  
(8)

\[ t=0, \quad V_{AHI}(\partial(C_{AHI}/\partial t))=0 \]  
(9)

\[ t>0, \quad V_{AHI}(\partial(C_{AHI}/\partial t))=K_{t_{fp}}V_{TF}C_{TF}+\frac{-K_{E_{AHI}}(V_{AHI}+D_{AHI}(\partial(C_{AHI}/\partial X)))}{V_{AHI}+D_{AHI}} \]  
(10)

These differential equations were solved using Laplace transforms. The Laplace transform for the amount of drug appearing in the aqueous humor (\( AH_{amount} \)) was expressed as follows.

\[
AH_{amount} = sX_0V_{TF}(s+K_{t_{fp}})W
\]

\[
W = V_{TF}V_{AHI}(s+K_{E_{TF}})\left((s+K_{CR}+K_{TF}+K_{t_{fp}})(s+K_{t_{fp}})\sinh d+szV_{AHI}(s+K_{CR}+K_{TF})\sinh d+szV_{AHI}(s+K_{CR}+K_{TF})\cosh d+szV_{AHI}(s+K_{CR}+K_{TF})\cosh d+s^2z(s+K_{t_{fp}})\sinh d\right)
\]

\[
d = L/sD_{TF} \]

\[
Z = K_{E_{AHI}}V_{AHI}/d
\]

where \( s \) is the Laplace variable with respect to time. The Laplace transforms for the amount of drug appearing in the tear fluid (\( TF_{amount} \)) and cornea (\( CR_{amount} \)) were expressed as follows:

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. Apparent distribution volumes, elimination rate constant and transfer rate constants in the aqueous humor and reservoir, and in vivo penetration parameters (\( D′ \) and \( K′ \) ) were previously reported in unanesthetized rabbits. These parameters were used for a simulation of drug concentrations in the aqueous humor and cornea after instillation. The simulation was carried out by the equations using MULTI(FILT)SIM, a simulation computer program. This program was written by MS-FORTRAN and run on a personal computer (PC-9821, NEC, Tokyo, Japan).

RESULTS AND DISCUSSION

Tilisolol was synthesized as a non-selective and hydrophilic beta-blocker and has been reported to reduce intraocular pressure after instillation in rabbit eye. The physicochemical properties, such as molecular weight and \( pK_a \) value, of tilisolol were similar to those of other ophthalmic beta-blockers.

The concentration-time profile of tilisolol in the tear fluid after instillation into anesthetized rabbits showed an almost mono-exponential curve as shown in Fig. 1. The profile of anesthetized rabbits was higher than that of unanesthetized rabbits. The elimination rate constant and the apparent distribution volume were estimated according to the one-compartment model because cornea permeability was negligible (Table 1). It was previously reported that anesthetized rabbits showed a similar elimination rate constant and apparent distribution volume of tilisolol to those of fluorescein-isothiocyanate(FITC)-dextran, indicating that the tear fluid turnover mainly contributes to the drug disposition in the precorneal area. Anesthetized rabbits decreased the production of the tear fluid to 47.2% compared to unanesthetized rabbits. Patton and Robinson reported that pilocarpine elimination in
Table 1. Pharmacokinetic Parameters in Tear Fluid after Instillation of Tilisolol (100 mg/25 μl) into Anesthetized Rabbits

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ke12 (min⁻¹)</th>
<th>Ve12 (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anesthetized rabbit</td>
<td>0.068±0.011</td>
<td>0.068±0.014</td>
</tr>
<tr>
<td>Unanesthetized rabbit</td>
<td>0.144±0.031</td>
<td>0.051±0.007</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. of 4 experiments. a) Data were reported previously.8)

The concor area decreased in anesthetized rabbits and resulted in an increase of maximum concentration and area under the concentration-time profile of pilocarpine in the aqueous humor.

The concentration-time profile of tilisolol in the aqueous humor after instillation is shown in Fig. 2A. Anesthetized rabbits showed higher aqueous concentrations of tilisolol than those observed in the unanesthetized rabbits reported previously.8) The calculation of tilisolol concentration in the aqueous humor was performed in anesthetized rabbits assuming that their drug behaviors in the cornea and aqueous humor were not different from those of unanesthetized rabbits. The calculation was carried out using the parameters in Table 1, in vivo penetration parameters (D' = 0.24 h⁻¹, K' = 0.008 cm²), and in vivo kinetic parameters (Kt12 = 0.032 min⁻¹, Kr12 = 0.037 min⁻¹, KeAH = 0.033 min⁻¹, V12HA = 0.485 ml, V12AH = 0.406 ml) that were reported previously.8)

The calculated lines in the aqueous humor and cornea are shown in Figs. 2A and B. The calculated line of drug concentrations in the aqueous humor was almost consistent with the experimental data in anesthetized rabbits. This agreement supports the validity of the in vivo pharmacokinetic model as well as the validity of in vivo parameters in the eyes of albino rabbits. A decrease in the elimination rate constant in the tear fluid enhanced a maximum concentration of tilisolol and slightly prolongs the time it takes for drug to reach the maximum concentration in the aqueous humor. However, chronic tear deficiency will influence not only the tear flow but also the corneal permeability by the induction of inflammation. Although further investigation is necessary for applying the present mathematical model to the clinical medication, it may be useful for predicting and evaluating the absorption of instilled drugs.

Acknowledgments The authors wish to thank Nisshin Flour Milling Co., Ltd. for kindly supplying tilisolol hydrochloride. This research was supported by a Grant-in-Aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, and by a Grant-in-Aid from Uehara Memorial Foundation.

REFERENCES