Ocular Pharmacokinetic/Pharmacodynamic Modeling for Multiple Anti-glaucoma Drugs

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We have constructed a new ocular pharmacokinetic pharmacodynamic (PK/PD) model for anti-glaucoma drugs to describe ocular hypotensive effects on intraocular pressure (IOP) after instillation of a combination of an α_1-adrenergic antagonist, bunazosin, and a β-adrenergic antagonist, timolol, into rabbits. This model was constructed by the combination of two ocular PK/PD models for bunazosin and timolol by including aqueous humor dynamics based on both action mechanisms. We also verified the reliability of this model by confirming the drug concentrations in aqueous humor and ocular hypotensive effects after instillation of the drug combination. The aqueous humor concentrations of timolol and bunazosin were determined by an HPLC, and ocular hypotensive effect-time profiles were measured using a telemetry system, which was able to record automatically detailed effects. The combined model could simulate the aqueous humor concentrations of both drugs and the additive IOP-lowering effect after instillation of the combination using the MULTI (RUNGE) program and PK/PD parameters which were obtained from ocular hypotensive effects after instillation of bunazosin alone or timolol alone. The theoretical concentration curves of both drugs in the aqueous humor and the theoretical ocular hypotensive effect curves almost agreed with both the observed concentrations and ocular hypotensive effects after instillation of the drug combination. These results indicate the reliability and usefulness of PK/PD modeling considering aqueous humor dynamics to predict IOP in multidrug therapy. This is the first study to develop a PK/PD model for multidrug therapy for the eye.

Key words timolol; bunazosin; pharmacokinetics; pharmacodynamics; eye; aqueous humor dynamics

The current mainstay of glaucoma treatment is topical medications of ocular hypotensive effect drugs in controlling intraocular pressure (IOP) and preserving visual field. IOP is mainly determined by the aqueous humor dynamics, which is the coupling of aqueous humor formation in the ciliary body and its drainage through the uveoscleral route and the trabecular meshwork route.[1)] The action mechanisms of anti-glaucoma drugs are well known and classified into several action mechanisms[2,3)]: β-adrenergic antagonists (*e.g.*, timolol, betaxolol), carbonic anhydrase inhibitors (*e.g.*, dorzolamide), and α_2-adrenergic agonists (*e.g.*, apraclonidine, brimonidine) that suppress aqueous humor formation; prostaglandin FP receptor agonists (*e.g.*, latanoprost, travoprost), prostamides (*e.g.*, bimatoprost), and α_1-adrenergic antagonists (bunazosin), which promote aqueous humor outflow through the uveoscleral route; and cholinomimetics (*e.g.*, pilocarpine) responsible for promoting aqueous humor outflow through the trabecular meshwork route as a result of contraction of the ciliary muscle.

It is also a common practice to use multiple anti-glaucoma drugs in combination to achieve target IOP lowering when a favorable response is not obtained by monotherapy.[4)] In order to comprehend the result of multidrug treatment, some comparative studies on the combined effects of drugs were reported.[4,5)] However, in multidrug therapy, without experiments it has been impossible to determine the strength and duration of the ocular hypotensive effect quantitatively.

Pharmacokinetic/pharmacodynamic (PK/PD) models allow us to predict quantitatively both the drug concentration and pharmacological effect after administration of drugs. In the previous reports, we have succeeded in developing two ocular PK/PD models for anti-glaucoma drugs after instillation into rabbits. One is the model for an α_1-adrenergic antagonist, bunazosin,[6)] and the other is for a β-adrenergic antagonist, timolol,[7)] but no ocular PK/PD model for multidrug therapy for the eye has been devised. The models for bunazosin and timolol each include aqueous humor dynamics based on each action mechanism, which are different from one another; therefore, a combined ocular PK/PD model after instillation of a combination of multiple drugs must be able to be constructed by including each action mechanism.

In the present study, we constructed a new combined ocular PK/PD model by including both the action mechanisms of bunazosin and timolol, and simulated the theoretical drug concentrations and ocular hypotensive effects after instillation of a combination of bunazosin and timolol into rabbits using the combined model and PK/PD parameters for each drug. In addition, to verify the reliability of the combined model, we confirmed the drug concentrations and ocular hypotensive effects measured by a telemetry system after instillation of the drug combination. The PD parameters for bunazosin were recalculated from the ocular hypotensive effects measured by the telemetry system in order to obtain values in the same experimental conditions as other IOP measurement studies.

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MATERIALS AND METHODS

Animals Male Japanese White rabbits (2.0—3.6 kg) were housed individually in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4 or LRC4, Oriental Yeast Co., Ltd., Tokyo, Japan). The rabbits had free access to water and were maintained in a 12-h light–dark cycle (light-on was defined as 7:00 am, and light-off occurred at 7:00 pm). All experiments in the present study conformed to the "Principles of Laboratory Animal Care" (NIH publication #85-23, received 1985).

Materials Timolol maleate was purchased from Wako Pure Chemical Ind., Ltd. (Osaka, Japan). Bunazosin hydrochloride was kindly supplied from Eizai Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals used were reagent grade. Drug solutions were prepared with pH 7.4 phosphate-buffered saline (PBS). Each concentration of drug solution (%) is indicated as the free form for timolol and the salt form for bunazosin.

Drug Disposition in Aqueous Humor The ocular instillation and aqueous humor collection methods were as described previously.[6,7] Briefly, 25 μl of drug solution (mixture containing 0.01% bunazosin and 0.5% timolol in PBS) was carefully instilled in the middle of the lower conjunctival sac of unanaesthetized normal rabbits placed in restraint boxes. The rabbits were sacrificed with an overdose of sodium pentobarbital at the designated time after drug instillation. After thoroughly rinsing the corneal and conjunctival surfaces with 0.9% NaCl, the aqueous humor was collected and stored at -20 °C until drug determination.

Drug Determination Drug determination of bunazosin and timolol was performed as previously reported.[6,7] Briefly, for bunazosin determination a 50 μl aliquot of the aqueous humor was added to 1 ml acetonitrile containing prazosin hydrochloride (20 nM) as an internal standard. After mixing, each mixture was centrifuged at 8000×g for 5 min, and the supernatant (100 μl) was diluted with 200 μl of water. The sample (50 μl) was then injected into an HPLC system (LC-10AD, Shimadzu Co., Ltd., Kyoto, Japan) using a reversed-phase mode for the assay. The stationary phase was a TSKgel ODS-80T$_M$ packed column (250 mm length×4.6 mm i.d., Tosoh Corporation, Tokyo, Japan). A mixture of acetonitrile and 66 mM NaH_2PO_4 (3 : 7, v/v) was used as the mobile phase with a flow rate of 0.8 ml/min. Elution of the drug was monitored with a spectrofluorometric detector (RF-10A, Shimadzu Co., Ltd.; excitation wavelength 350 nm, emission wavelength 405 nm).

For timolol determination, a 50 μl aliquot of the aqueous humor was added to 50 μl HCl (0.1 M) and 100 μl methanol including propranolol (50 μM) as an internal standard. After mixing, each mixture was centrifuged at 2000×g for 10 min, and 50 μl of supernatant was injected into an HPLC system (LC-10AD) using a reversed-phase mode for the assay. The stationary phase was a Cosmosil 5C18-MS packed column (150 mm length×4.6 mm i.d., Nacalai Tesque Inc., Kyoto, Japan). A mixture of methanol and 3 mM diethylamine (3 : 7 v/v) was used as the mobile phase with a flow rate of 1.0 ml/min. Elution of the drug was monitored with a UV spectrophotometric detector at 290 nm (SPD-10A, Shimadzu Co., Ltd.).

IOP Measurement by a Telemetry System Implantation of a telemetry transmitter was performed as described in previous reports.[8,9] Briefly, the telemetry transmitter (Model TA11PA-C40; Data Science International, St. Paul, MN, U.S.A.) was inserted into a subcutaneous pocket prepared in the cheek of an anesthetized rabbit, and its pressure catheter was tunneled subcutaneously to an exit site near the superior conjunctival sac of the right eye. The sensor catheter was inserted into the midvitreous through a small hole in the sclera, and was tied with nylon sutures. After surgery, 0.3% ofloxacin solution and 0.1% sodium diclofenac solution were instilled into the right eye three times per day for approximately 7 d.

Data management of IOP was performed as in a previous report.[7] Briefly, the implanted telemetry transmitter sent IOP information to a receiver (RPC-1; Data Science International). IOP values were determined by the 15 min-average of IOP data. The rabbits were used in this study after confirming their IOP values, which displayed a stable circadian rhythm across the dark and light phase and correlated with the value measured by a pneumatonometer.

IOP Measurement Rabbits implanted with the telemetry transmitter and trained enough to be handled without causing undue stress were placed in restraint boxes. Twenty-five microliters of each drug solution (0.005%, 0.01%, 0.05%, or 0.1% bunazosin alone in PBS, or a mixture containing 0.01% bunazosin and 0.5% timolol in PBS) was carefully instilled into the middle of the lower conjunctival sac of the right eye of the unanesthetized rabbits at 11:00 pm under reduced red-colored lighting in the dark. The IOP value was obtained every 15 min until 7 h after instillation. IOP measurement was performed by the telemetry system as described above. Implanted rabbits were used repeatedly after cessation of each drug. This instillation time and measurement period were the same as our previous study,[7] in which IOP values (base line value; 20.7 mmHg) did not alter after vehicle instillation.

Combined Ocular PK/PD Model A combined ocular PK/PD model (Fig. 1) was constructed by including the aqueous humor dynamics based on the action mechanisms of bunazosin and timolol. An equilibrium of aqueous humor inflow (F_{in}) to the uveoscleral outflow (F_{us}) and trabecular outflow (F_{tra}). F_{tra} is expressed as follows[1]:

$$F_{tra} = C_{of} \cdot (\mathrm{IOP} - P_V) \tag{1}$$

where P_V is the episcleral venous pressure; C_{of} is the outflow facility.

Based on these relationships, IOP was expressed by the aqueous humor flow as follows:

$$\mathrm{IOP} = P_V + \frac{F_{in} - F_{us}}{C_{of}} \tag{2}$$

In rabbits, bunazosin reduces IOP by promoting aqueous humor outflow through the uveoscleral route alone,[3] and timolol reduces IOP by suppressing aqueous humor formation alone.[10] Therefore, the differential equation of IOP after instillation of timolol can be expressed as follows:

$$\frac{d\mathrm{IOP}}{dt} = \frac{dF_{in}}{dt} \cdot \frac{1}{C_{of}} - \frac{dF_{us}}{dt} \cdot \frac{1}{C_{of}} \tag{3}$$

According to our previous reports on the PK/PD model for bunazosin,[6] the differential equation of F_{us} was expressed as

follows:

$$\frac{dF_{us}}{dt} = K_{in\,BZ} - K_{out\,BZ} \cdot \left(1 - \frac{I_{max\,BZ} \cdot C_{A\,BZ}}{IC_{50\,BZ} + C_{A\,BZ}}\right) \cdot F_{us} \quad (4)$$

Furthermore, according to our previous reports on the PK/PD model for timolol,[7] the differential equation of F_{in} and regulator (M) were expressed as follows:

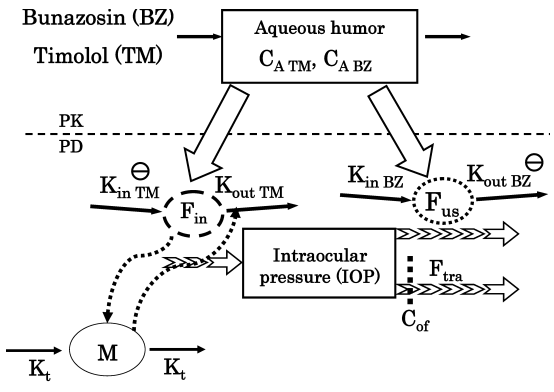
$$\frac{dF_{in}}{dt} = K_{in\,TM} \cdot \left(1 - \frac{I_{max\,TM} \cdot C_{A\,TM}}{IC_{50\,TM} + C_{A\,TM}}\right) - K_{out\,TM} \cdot F_{in} \cdot (1 + M) \quad (5)$$

$$\frac{dM}{dt} = K_t \cdot F_{in} - K_t \cdot M \quad (6)$$

where $K_{in\,BZ}$ is the zero-order rate constant for the production of F_{us}, $K_{out\,BZ}$ is the first-order rate constant for the loss of F_{us}, $I_{max\,BZ}$ is the maximum inhibitory effect attributed to bunazosin, and $IC_{50\,BZ}$ is the bunazosin concentration that exhibits 50% of the maximum inhibitory effect. $K_{in\,TM}$ is the zero-order rate constant for the production of F_{in}, and $K_{out\,TM}$ is the first-order rate constant for the loss of F_{in}. A decrease in F_{in} causes a decrease in regulator governed by the first-order rate constant (K_t). M is assumed to stimulate the loss of F_{in}. It is assumed that $K_{in\,TM}$ and $K_{out\,TM}$ account for the production and loss of F_{in}. $I_{max\,TM}$ is the maximum inhibitory effect attributed to timolol, and $IC_{50\,TM}$ is the timolol concentration that exhibits 50% of the maximum inhibitory effect. $C_{A\,BZ}$ and $C_{A\,TM}$ are the bunazosin and timolol concentrations in the aqueous humor. Each concentration is defined as drug amount in the aqueous humor (X_A) of bunazosin and timolol divided by the aqueous humor volume (V_A), respectively. X_A values are generated by Eq. A5 and Eq. A10 described in Appendix from ocular PK models for bunazosin and timolol, respectively.

Simultaneous Eq. 3 to 6 can be expressed as a combined PK/PD model (Fig. 1) after instillation of a mixture of bunazosin and timolol.

Estimation of PD Parameters for Bunazosin The ocular PK/PD model and PK parameters for bunazosin from our previous report[6] were adopted as estimations of PD parameters from ocular hypotensive effect–time profiles measured by the telemetry system. The estimations were performed using MULTI (RUNGE), a nonlinear least-squares computer program based on the Runge–Kutta–Gill method.[11] The derived equations in our previous reports were described in Ap-



$$\frac{dIOP}{dt} = \frac{dF_{in}}{dt} \cdot \frac{1}{C_{of}} - \frac{dF_{us}}{dt} \cdot \frac{1}{C_{of}}$$

$$\frac{dF_{us}}{dt} = K_{in\,BZ} - K_{out\,BZ} \cdot \left(1 - \frac{I_{max\,BZ} \cdot C_{A\,BZ}}{IC_{50\,BZ} + C_{A\,BZ}}\right) \cdot F_{us}$$

$$\frac{dF_{in}}{dt} = K_{in\,TM} \cdot \left(1 - \frac{I_{max\,TM} \cdot C_{A\,TM}}{IC_{50\,TM} + C_{A\,TM}}\right) - K_{out\,TM} \cdot F_{in} \cdot (1 + M)$$

$$\frac{dM}{dt} = K_t \cdot F_{in} - K_t \cdot M$$

Fig. 1. Combined Ocular Pharmacokinetic/Pharmacodynamic Model for Bunazosin and Timolol

($C_{A\,BZ/TM}$) drugs concentration of bunazosin and timolol in the aqueous humor, (IOP) intraocular pressure, (F_{in}) aqueous humor inflow, (F_{us}) uveoscleral outflow, (F_{tra}) trabecular outflow, (C_{of}) outflow facility, ($K_{in\,BZ/TM}$) zero-order rate constant of bunazosin and timolol, ($K_{out\,BZ/TM}$) first-order rate constant of bunazosin and timolol, (M) regulator, (K_t) first-order rate constant, ($I_{max\,BZ/TM}$) maximum inhibitory effect of bunazosin and timolol, and ($IC_{50\,BZ/TM}$) the drug concentration of bunazosin and timolol that exhibits 50% of the maximum inhibitory effect.

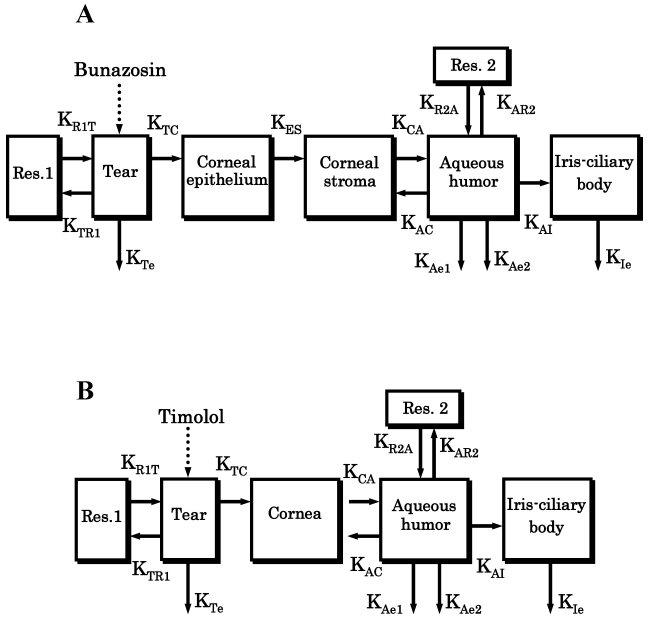


Fig. 2. Pharmacokinetic Models for (A) Bunazosin and (B) Timolol[6,7]

Res. 1 and Res. 2 are reservoir-1 and 2, K_{TR1} and K_{R1T} are the transfer rate constants between the tear fluid and Res. 1, K_{Te} is the elimination rate constant from the tear fluid, K_{TC} is the transfer rate constant from the tear fluid to the corneal epithelium or cornea, K_{ES} is the transfer rate constant from the corneal epithelium to the corneal stroma, K_{AI} is the transfer rate constant from the aqueous humor to the iris-ciliary body, K_{CA} and K_{AC} are the transfer rate constants between the corneal stroma or cornea and aqueous humor, K_{AR2} and K_{R2A} are the transfer rate constants between the aqueous humor and Res. 2, K_{Ie} is the elimination rate constant from the iris-ciliary body, and K_{Ae1} and K_{Ae2} are the elimination rate constants from the aqueous humor by aqueous humor outflow and other routes.

Table 1. Ocular Pharmacodynamic Parameters

	Bunazosin		Timolol
	Value	S.D.	Value
PD parameters			
$K_{in\,BZ/TM}$ (μl/min·min^{-1})	27.6×10^{-3}	4.5×10^{-3}	0.148[a]
$K_{out\,BZ/TM}$ (min^{-1})	0.133[b]	—	0.0663[a]
$I_{max\,BZ/TM}$	0.791	0.025	0.268[a]
$IC_{50\,BZ/TM}$ (nmol/ml)	6.32×10^{-3}	1.90×10^{-3}	5.71×10^{-3} [a]
K_t (min^{-1})	—	—	0.0152[a]
Physiological parameters			
F_{in} (μl/min)	2.239[c,d]		—
F_{us} (μl/min)	—		0.25[d,e]
C_{of} (μl/min/mmHg)	0.170[e]		0.170[e]
P_V (mmHg)	9.00[e]		9.00[e]

a) Each value was referenced from reported data.[7] *b*) K_{out} was estimated as K_{in} and F_{in} at time zero. *c*) Value was calculated from the baseline IOP value and other physiological parameters. *d*) Each value was used as an initial value at time zero after instillation in combination. *e*) Each value was referenced from reported data.[13]

pendix.

Simulation of Theoretical Concentration Curves of Bunazosin and Timolol Curves The ocular PK model (Fig. 2) and PK parameters of bunazosin and timolol from our previous report[6,7] were adopted in the simulation of a theoretical concentration curves of bunazosin and timolol in aqueous humor after instillation of a mixture containing 0.01% bunazosin and 0.5% timolol solution. The simulation was performed using MULTI (RUNGE).[11] The derived equations in our previous reports were described in Appendix.

Simulation of Theoretical Ocular Hypotensive Effect Curves The theoretical ocular hypotensive effect curves after instillation of 0.01% bunazosin alone, 0.5% timolol alone, and a mixture containing 0.01% bunazosin and 0.5% timolol were simulated by the combined ocular PK/PD model described above with PK parameters and PD parameters estimated by the telemetry system (Table 1) and physiological parameters (Table 1). The PK parameters for bunazosin and timolol and PD parameters for timolol were from our previous reports.[6,7] The PD parameters for bunazosin were from the values estimated in this study. The aqueous humor volume V_A (0.28 ml) was obtained from the data reported by Conrad and Robinson.[12] Physiological parameters P_V, F_{in}, and C_{of} were obtained from the data reported by Sakurai *et al.*[13] F_{in} was calculated from the baseline IOP value and other physiological parameters. The simulation was performed using MULTI (RUNGE).[11]

RESULTS

IOP Measurement after Instillation of Bunazosin by a Telemetry System Figure 3 shows the observed ocular hypotensive effect–time profiles measured by the telemetry system and their fitted curves using the PK/PD model for bunazosin after instillation of 0.005%, 0.01%, 0.05%, and 0.1% bunazosin. Table 1 shows the estimated PD parameters. In the observed data, the IOP reductions reached a maximum at 60 min after instillation of 0.005%, 0.01%, and 0.05% bunazosin and at 135 min after instillation of 0.1% bunazosin. Thereafter, the IOP values returned to the baseline more gradually with escalating concentrations of drug solution. In the fitted curves, the times of maximum IOP reduction were prolonged with escalating concentrations of drug solution. The fitted curves using the PK/PD model and parameters for bunazosin almost agreed with the observed ocular hypotensive effects.

Drug Concentrations after Instillation of a Combination of Bunazosin and Timolol Figure 4 shows the observed aqueous humor concentrations of bunazosin and timolol, and their theoretical curves simulated by each PK model (Fig. 2) and parameters after instillation of a mixture containing 0.01% bunazosin and 0.5% timolol. The theoretical concentration curves of both drugs in the aqueous humor almost agreed with the observed concentrations.

Theoretical Ocular Hypotensive Effects Simulated by a Combined Ocular PK/PD Model Figure 5A shows the theoretical ocular hypotensive effect curves simulated by the combined ocular PK/PD model (Fig. 1) and parameters after instillation of 0.01% bunazosin alone, 0.5% timolol alone,

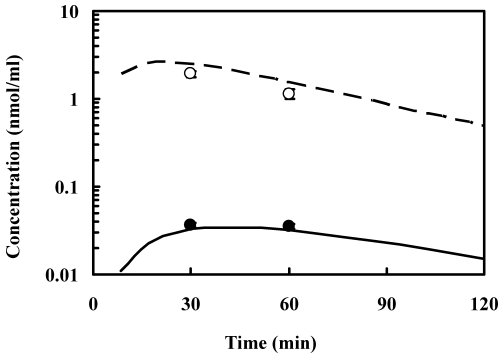


Fig. 4. Concentrations of Bunazosin and Timolol in Aqueous Humor after Instillation of a Mixture Containing 0.01% Bunazosin and 0.5% Timolol into Rabbits

(●) experimental data of bunazosin, (○) experimental data of timolol, (—) theoretical curve of bunazosin, and (– – –) theoretical curve of timolol. Each point represents the mean±S.E. of 4 experiments.

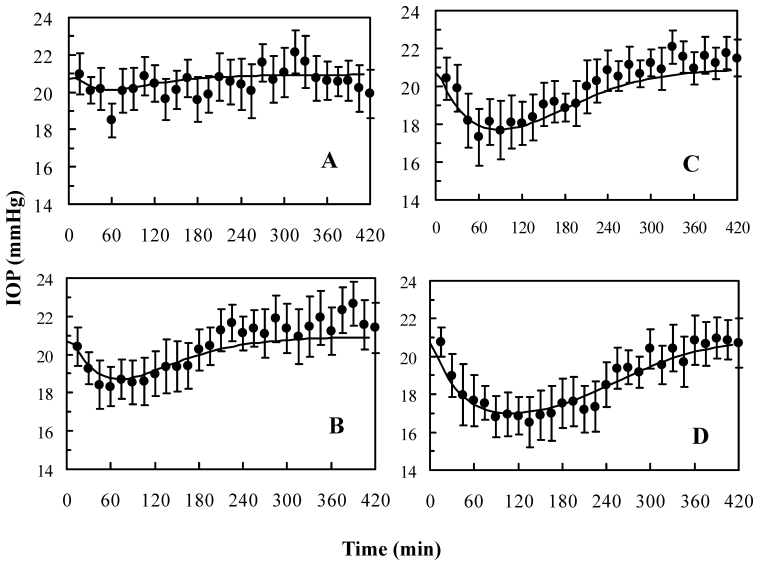


Fig. 3. Intraocular Pressures after Instillation of Bunazosin into Rabbits

(A) 0.005%, (B) 0.01%, (C) 0.05%, (D) 0.1%. (●) experimental data and (—) fitted curves. Each point represents the mean±S.E. of 8 experiments.

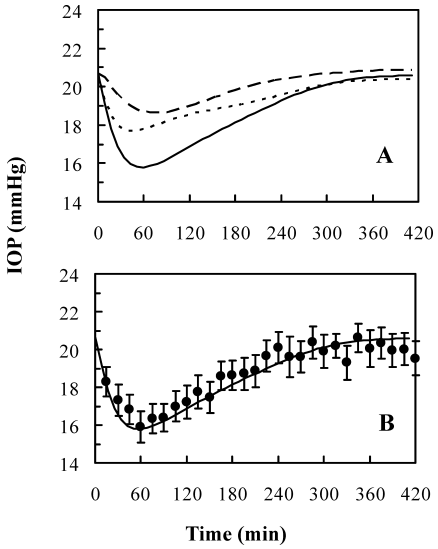


Fig. 5. Ocular Hypotensive Effect Curves after Instillation of Bunazosin Alone, Timolol Alone, and Both Drugs into Rabbits

(A) Theoretical curves simulated by PK/PD model. (– – –) 0.01% bunazosin alone, (⋯) 0.5% timolol alone, and (—) combined instillation of 0.01% bunazosin and 0.5% timolol. (B) (—) Theoretical curve and (●) experimental data after combined instillation of 0.01% bunazosin and 0.5% timolol. Each point represents the mean±S.E. of 8 experiments.

and a mixture of both drugs. The IOP value of the theoretical ocular hypotensive effect curve after combined instillation was more intense than that after instillation of each drug alone.

Observed Ocular Hypotensive Effect after Instillation in Combination Figure 5B shows the observed ocular hypotensive effect–time profile and theoretical curve after instillation of a mixture containing 0.01% bunazosin and 0.5% timolol. The theoretical curve after combined instillation almost agreed with the observed profile.

DISCUSSION

Some studies of PK/PD modeling of the combination of the effect of two drugs have been reported previously. Yassen *et al.* developed a mechanism-based PK/PD model for the combination of buprenorphine and fentanyl for respiratory-depressant effects in rats and human.[14,15] Arakawa *et al.* developed a PK/PD analysis of combined chemotherapy with carboplation and paclitaxel for patients with ovarian cancer.[16] These studies indicated that PK/PD modeling is applicable to the effect of two drugs in combination. In the present study, we tried to construct an ocular PK/PD model for the combination of two anti-glaucoma drugs.

A recently developed telemetry system[9] is able to obtain IOP values automatically, and allows us to observe detailed ocular hypotensive effects more easily than conventional methods such as using a pneumatonometer. Previously, we reported that ocular hypotensive effects on IOP measured by this telemetry system were applicable to ocular PK/PD analysis with timolol in rabbits.[7] In this study, we could detect ocular hypotensive effects after instillation of bunazosin using this system. We had previously developed an ocular PK/PD model for bunazosin using conventional a pneumatonometer.[6] In order to simulate the ocular hypotensive effect curves after instillation of the combination of timolol and bunazosin in the same conditions, PD parameters for bunazosin (Table 1) was recalculated from ocular hypotensive effect–time profiles obtained by the telemetry system.

PK models for bunazosin and timolol[6,7] are composed of 7 and 6 compartments, respectively (Fig. 2). The corneal part of bunazosin consists of two sub compartments, the corneal epithelium and its stroma, but that of timolol consists of one compartment. Physiologically, corneal epithelium is relatively impermeant to polar or hydrophilic compounds, and stromal permeability is high.[17] It seems that the difference in corneal compositions depends on the physicochemical properties of the drugs. Figure 2 shows a combined ocular PK/PD model after instillation of a combination of bunazosin and timolol. This model combines two ocular PK/PD models for bunazosin and timolol and includes the action mechanisms of both drugs involved in aqueous humor dynamics. The action mechanism of timolol in suppressing aqueous humor formation is different from that of bunazosin, which promotes aqueous humor outflow through the uveoscleral route. This model was able to simulate the additive IOP-lowering effect after instillation of a combination of timolol and bunazosin (Fig. 5A). The simulated result was compatible with previously reported results that bunazosin had additive effect on IOP-lowering by timolol in rabbit[4] and human.[18] This combined PK/PD model can quantitatively express the additive effect under any circumstances after combined instillation of both drugs, of which doses have ocular hypotensive effects after instillation alone, because this model consists of independent action mechanism of each drug.

To verify the theoretical value simulated by this model, the aqueous humor concentrations of bunazosin and timolol and ocular hypotensive effect–time profiles were measured after instillation of a combination of both drugs. The theoretical concentration curve of each drug in the aqueous humor almost agreed with each observed concentration (Fig. 4). The result indicated that the disposition of each drug in the eye did not interact with each other after instillation in combination. The theoretical ocular hypotensive effect curve after instillation of the combination of drugs almost agreed with the observed profile (Fig. 5B). The result supported the hypothesis that the ocular hypotensive effects of both drugs on IOP did not interact with each other after instillation in combination. These findings suggest that our approach of combined PK/PD modeling is reliable and enables us to provide logically-based estimations and predictions of both PK and PD after instillation of the combination of timolol and bunazosin.

Another action mechanism of drugs involved in IOP lowering is the promotion of aqueous humor outflow through the trabecular meshwork route. According to the equation of aqueous humor dynamics, Eq. 2, the combined ocular PK/PD model can be expanded as a generalized model based on three mechanisms of action of multiple drugs. In the generalized model, the differential equation of IOP in combination can be expressed as follows:

$$\frac{d\mathrm{IOP}}{dt} = \frac{1}{C_{\mathrm{of}}} \cdot \frac{dF_{\mathrm{in}}}{dt} + F_{\mathrm{in}} \cdot \frac{d\dfrac{1}{C_{\mathrm{of}}}}{dt} - \left(\frac{1}{C_{\mathrm{of}}} \cdot \frac{dF_{\mathrm{us}}}{dt} + F_{\mathrm{us}} \cdot \frac{d\dfrac{1}{C_{\mathrm{of}}}}{dt} \right) \quad (7)$$

where functional differential equations of F_{in}, F_{us}, and $1/C_{of}$ can be obtained from appropriate experimental results of ocular hypotensive effect and drug concentration–time profiles in the ocular tissues after instillation of each drug alone. In the case of instillation of two drugs, a functional differential equation of no corresponding mechanism of action, F_{in}, F_{us}, or $1/C_{of}$, may be defined as 0.

CONCLUSIONS

We have constructed a new combined ocular PK/PD model by including both the action mechanisms of bunazosin and timolol after instillation of a combination of both drugs into rabbits. In addition, we confirmed the drug concentrations and ocular hypotensive effects after instillation of the combination of drugs, and the reliability of this model was verified. This is the first study to show a ocular PK/PD model for multidrug therapy.

Acknowledgment The author wishes to thank Dr. Yamaoka for supplying MULTI (RUNGE).

APPENDIX

Ocular PK/PD Model for Bunazosin PK part of the ocular PK/PD model for bunazosin (Fig. 2A),[6] mass balance equations in the ocular tissues are expressed as follows[6]:

$$\frac{dX_T}{dt} = K_{R1T} \cdot X_{R1} - (K_{TC} + K_{TR1} + K_{Te}) \cdot X_T \quad \text{(A1)}$$

$$\frac{dX_{R1}}{dt} = K_{TR1} \cdot X_T - K_{R1T} \cdot X_{R1} \quad \text{(A2)}$$

$$\frac{dX_{CE}}{dt} = K_{TC} \cdot X_T - K_{ES} \cdot X_{CE} \quad \text{(A3)}$$

$$\frac{dX_{CS}}{dt} = K_{ES} \cdot X_{CE} + K_{AC} \cdot X_A - K_{CA} \cdot X_{CS} \quad \text{(A4)}$$

$$\frac{dX_A}{dt} = K_{CA} \cdot X_{CS} + K_{R2A} \cdot X_{R2} - (K_{AC} + K_{AR2} + K_{AI} + K_{Ae1} + K_{Ae2}) \cdot X_A \quad \text{(A5)}$$

$$\frac{dX_I}{dt} = K_{AI} \cdot X_A - K_{Ie} \cdot X_I \quad \text{(A6)}$$

$$\frac{dX_{R2}}{dt} = K_{AR2} \cdot X_A - K_{R2A} \cdot X_{R2} \quad \text{(A7)}$$

where X_T, X_{R1}, X_{CE}, X_{CS}, X_A, X_I, and X_{R2} are the drug amount in the tear fluid, reservoir-1 (Res. 1), corneal epithelium, corneal stroma, aqueous humor, the iris-ciliary body, and Res. 2, respectively. K_{TR1} and K_{R1T} are the transfer rate constants between the tear fluid and Res. 1, K_{Te} is the elimination rate constant from the tear fluid; K_{TC} is the transfer rate constant from the tear fluid to the corneal epithelium, K_{ES} is the transfer rate constant from the corneal epithelium to the corneal stroma, K_{AI} is the transfer rate constant from the aqueous humor to the iris-ciliary body, K_{CA} and K_{AC} are the transfer rate constants between the corneal stroma and aqueous humor, K_{AR2} and K_{R2A} are the transfer rate constants between the aqueous humor and Res. 2, K_{Ie} is the elimination rate constant from the iris-ciliary body, and K_{Ae1} and K_{Ae2} are the elimination rate constants from the aqueous humor by aqueous humor outflow and other routes.

PD part of the PK/PD model for bunazosin,[6] derived equations of F_{us} (aqueous humor outflow through the uveoscleral route) and IOP including aqueous humor dynamics theory are expressed as Eq. 4 and follows[6]:

$$\frac{d\mathrm{IOP}}{dt} = -\left[K_{in\,BZ} - K_{out\,BZ} \cdot \left(1 - \frac{I_{max\,BZ} \cdot C_{A\,BZ}}{IC_{50\,BZ} + C_{A\,BZ}} \right) \times \{F_{in} - C_{of} \cdot (\mathrm{IOP} - P_V)\} \right] \cdot \frac{1}{C_{of}} \quad \text{(A8)}$$

where denotations are the same as in the combined ocular PK/PD model.

Ocular PK Model for Timolol PK part of the PK/PD model for timolol (Fig. 2B), mass balance equations in the ocular tissues are expressed as Eqs. A1, A2, A6, A7 and follows[7]:

$$\frac{dX_C}{dt} = K_{TC} \cdot X_T + K_{AC} \cdot X_A - K_{CA} \cdot X_C \quad \text{(A9)}$$

$$\frac{dX_A}{dt} = K_{CA} \cdot X_C + K_{R2A} \cdot X_{R2} - (K_{AC} + K_{AR2} + K_{AI} + K_{Ae1} + K_{Ae2}) \cdot X_A \quad \text{(A10)}$$

where X_C is the drug amount in the cornea; K_{TC} is the transfer rate constant from the tear fluid to the cornea; K_{CA} and K_{AC} are the transfer rate constants between the cornea and aqueous humor. Other denotations are the same as in the PK model for bunazosin.

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