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In Vivo Ocular Pharmacokinetic Model for Designing Dosage Schedules and Formulations of Ophthalmic Drugs in Human

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The purpose of this study was to develop an in vivo pharmacokinetic model and parameters for predicting the concentrations of ophthalmic drugs in the anterior chamber after instillation into human eyes. We have already reported the usefulness of mathematical model including a diffusion process in rabbits[1)]. Timolol was used as a model ophthalmic drug. The concentrations of timolol and fluorescein in the tear fluid were determined after instillation into the eyes of human volunteers. The in vivo pharmacokinetic parameters in the tear fluid were estimated by the elimination profile according to a one-compartment model. The concentrations of timolol in the aqueous humor were obtained from the data previously reported[2,3)]. Other parameters of timolol were estimated from the concentration profiles of timolol in the aqueous humor according to a pharmacokinetic model including a corneal diffusion process. The parameters for human were almost equal to those for rabbits reported previously[1)]. This mathematical model and in vivo parameters will be effective to estimate the adequate regimen for ophthalmic chemotherapy and develop the ocular drug delivery systems.

Key words : timolol, diffusion model, ocular penetration, pharmacokinetic, drug delivery system

Introduction

In ophthalmic chemotherapy, topical application of drugs is the general method of choice under most circumstances because it gains a high drug concentration in the target site of anterior chamber with convenience and safety[4,5)]. However, rapid eliminations of instilled drugs in the tear and poor permeability of cornea result in a poor bioavailability in an anterior segment and increased severity in systemic adverse effects[6-8)]. Numerous attempts have been reported to improve the ocular delivery[9-14)].

In order to rationally design the adequate dosage regimen and formulation for patient, it is important to develop pharmacokinetic models that can evaluate the ocular absorption of instilled drugs[15,16)]. However, the behaviors of instilled drug in anterior chamber is complicated because it includes both slow process such as corneal penetration and rapid processes such as disposition and distribution in the tear fluid and aqueous humor[17,18)].

In the previous report, we have successfully established an in vivo pharmacokinetic model including a corneal diffusion process in rabbits and predicted the concentrations of beta-blockers in the anterior segments[1)]. Therefore, in the present study, we developed an in vivo pharmacokinetic parameters for human using the mathematical model including a corneal diffusion process. Timolol was used as a model ophthalmic drug. Although timolol is widely used in the treatment of open-angle glaucoma as instillation droplets[19)], its usefulness for glaucoma therapy has been limited by topical and systemic side effects such as cardiovascular and respiratory complications[19,20)]. It is especially necessary to control pharmacokinetic behavior of the instilled timolol for effective treatment.

Materials and Methods

Materials.

Timolol (0.5% Timoptol®, 11.6 mM timolol, Banyu Pharmaceutical Co. Ltd., Tokyo, Japan) and fluorescein (Fluorecite 1®, 12 mM fluorescein, Japan Alcon Co. Ltd., Tokyo, Japan) were purchased commercially. Methyl-p-hydroxybenzoate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals of reagent grade were obtained 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.

Drug Disposition in Tear Fluid.

Drug solution was instilled into six and three healthy volunteers (23-40 years) for fluorescein and timolol, respectively. All experiments in the present study con-

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formed to the "Declaration of Helsinki and The Guiding Principles in the care and Use of Animals (DHEW Publication, NIH 80-23). Twenty-five microliters of drug solutions (timolol : 11.6 mM, fluorescein : 12 mM) were carefully instilled with a micropipette (Gilson Medical Electronics, Villiers-Bel, France) in the middle of lower conjunctival sac of the eye. At appropriate time after instillation, tear fluid samples (0.5 μl) were collected by a glass capillary (EM minicaps®, Hirschman Laborgerate, Germany) from the middle of the lower marginal tear strip and were diluted by 50 μl of pH 7.4 phosphate-buffered saline. Drug concentrations in the samples were determined with a high performance liquid chromatography (HPLC) or fluorophotometer.

Drug Determination

The samples of the tear fluids for timolol (50 μl) were mixed with 0.1 M HCl (50 μl) and methanol (100 μl) including an internal standard (500 μg/ml methyl-p-hydroxybenzoate). The mixtures were centrifuged at 12000 x g for 15 min and the supernatants (50 μl) were injected into a HPLC system.

The HPLC system (LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) was used in a reverse-phase mode for assay. The stationary phase used was Cosmosil $5C_{18}$-P packed column (150 mm length x 4.6 mm i.d., Nacalai Tesque Inc., Kyoto, Japan). Mixtures of methanol and 50 mM NaH_2PO_4 (40 : 60 v/v) were used as the mobile phase with a flow rate of 1.0 ml/min. Retention of drug was monitored with a UV spectrophotometric detector (SPD-10A, Shimadzu Co., Ltd.; 295 nm for timolol). Fluorescein was determined with a spectrofluorophotometer (RF-1500, Shimadzu Co., Ltd.; excitation wave length 490 nm, emission wave length 511 nm).

Data Analysis

The concentration profiles for timolol and fluorescein in the tear fluid were analyzed by a one-compartment model. The concentrations in the tear fluid (C_{TF}) at time t are expressed as follows :

$$C_{TF} = X_0 \exp(-Ke_{TF}t) / V_{TF} \quad (1)$$

where X_0 is the initially instilled dose, V_{TF} is the apparent distribution volume in the tear fluid, Ke_{TF} is the elimination rate constant in the tear fluid.

The concentration profiles for timolol in the aqueous humor were obtained from the data reported by Phillips et al[2]. and Ellis et al.[3]. The data were analyzed by a pharmacokinetic model including a diffusion process for the finite dose system which considers the cornea to be a one-plane layer (Fig. 1). Based on this model, the Laplace transforms for the amount of drug appearing in the aqueous

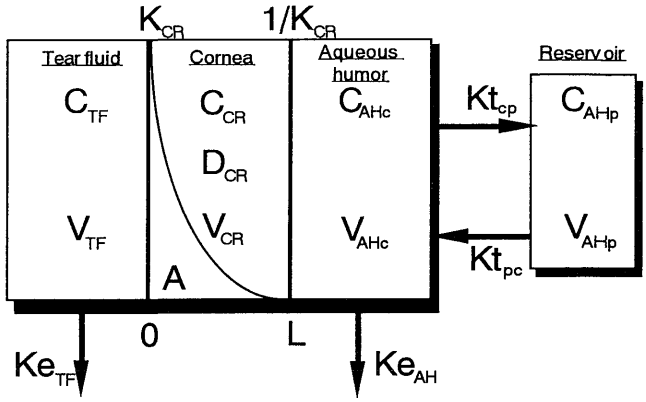

Fig. 1. In vivo pharmacokinetic model including a diffusion process for human. Abbreviation : CTF, the drug concentration in the tear fluid ; C_{CR}, the drug concentration in the cornea ; C_{AHc}, the drug concentration in the aqueous humor ; C_{AHp}, the drug concentration in the reservoir ; V_{TF}, the apparent distribution volume in the tear fluid ; V_{CR}, the effective diffusion volume in the cornea ; 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 donor solution ; 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.

humor (AH_{amount}) are expressed as follows :

$$AH_{amount} = sZX_0V_{AHc}(s+Kt_{pc}) / W \quad (2)$$

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

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

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

where 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, V_{AHc} is the apparent distribution volume in the aqueous humor, Ke_{AH} is the elimination rate constant in the aqueous humor, Kt_{cp} and Kt_{pc} are the transfer rate constants between the aqueous humor and reservoir.

Based on this model, the Laplace transforms for the amount of drug appearing in the tear fluid (TF_{amount}) and in

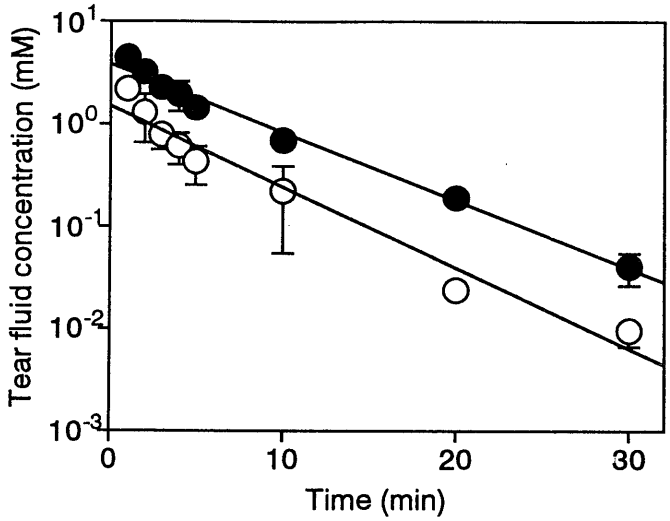

Fig. 2. Concentration of fluorescein and timolol in the tear fluid after instillation into human eyes. (●) fluorescein, (○) timolol. Each point represents the mean ± S.E. of at least three experiments.

the cornea (CR_{amount}) are expressed as follows :

$$TF_{amount} = X_0 V_{TF} (V_{AHc}((s+Ke_{AH}+Kt_{cp})(s+Kt_{pc}) - Kt_{cp}Kt_{pc}) \sinh d + sZ(s+Kt_{pc}) \cosh d/W \quad (3)$$

$$CR_{amount} = X_0 Z (V_{AHc}((s+Ke_{AH}+Kt_{cp})(s+Kt_{pc}) - Kt_{cp}Kt_{pc})(\cosh d-1) + sZ(s+Kt_{pc}) \sinh d)/W \quad (4)$$

Apparent distribution volume and elimination rate constant in the tear fluid were estimated by the concentration-time profile in the tear fluid after instillation (equation 1). The parameters were also calculated from an average concentration time-profile of three volunteers and were used for the estimation of in vivo corneal penetration parameters and pharmacokinetic parameters for timolol in the aqueous humor. The corneal elimination of drug in the tear fluid was negligible because the corneal permeability coefficient of timolol was much lower than the elimination rate constant in the tear fluid.

Since it is difficult to determine correctly the real diffusion length for penetrant, the diffusion parameter ($D' = D_{CR}/L/L$) and the partition parameter ($K' = K_{CR} V_{CR}$) were defined. The penetration parameters (D', K'), apparent distribution volume (V_{AHc}), elimination rate constant (Ke_{AH}) and transfer rate constants in the aqueous humor and reservoir (Kt_{cp}, Kt_{pc}) were estimated using the equation 2 from the concentration-time profiles in the aqueous humor. The estimations were carried out by using MULTI[21], a nonlinear least-squares computer program, and MULTI (FILT)[22], a nonlinear least-squares computer program based on a fast inverse Laplace transform algorithm. The simulation and fitting lines were calculated from equations 2, 3 and 4, and estimated parameters by using MULTI (FILTS)[22], a computer program based on a fast inverse Laplace transform algorithm. These programs were written by MS-FORTRAN and run on a personal computer (PC-9821 V10, NEC, Tokyo, Japan).

Table 1. Pharmacokinetic parameters for fluorescein and timolol in the tear fluid after instillation into human eyes

Parameter	Fluorescein	Timolol
Ke_{TF} (min^{-1})	0.16±0.01 (0.15)	0.18±0.01 (0.18)
V_{TF} (ml)	0.09±0.02 (0.08)	0.28±0.10 (0.19)

Pharmacokinetic parameters of timolol in the tear fluid were not significantly different from those of fluorescein (Welch's *t*-test).
The parameters in parenthesis were calculated from an average concentration time-profile of human. The parameters in parenthesis for timolol were used for the estimation of in vivo corneal penetration parameters and pharmacokinetic parameters for timolol in the aqueous humor.

Table 2. In vivo corneal penetration parameters and pharmacokinetic parameters for timolol in the aqueous humor after instillation into human eyes

Parameter	Human	Rabbit*
D' (hr^{-1})	0.32	0.33
K' (cm^3)	0.006	0.014
Ke_{AH} (min^{-1})	0.013	0.057
Kt_{cp} (min^{-1})	0.020	0.028
Kt_{pc} (min^{-1})	0.086	0.031
V_{AHc} (min^{-1})	0.142	0.446

* In vivo corneal penetration parameters and pharmacokinetic parameters in rabbit were previously reported.

Results

In Vivo Pharmacokinetic Model

Fig. 1 shows an in vivo pharmacokinetic model including a corneal diffusion process for predicting the concentrations of ophthalmic drugs in anterior tissues such as tear, cornea, and aqueous humor after an instillation. In this model, instilled drug diffuses the cornea from the tear fluid to the aqueous humor with the reservoir during a mono-exponential elimination in the tear fluid.

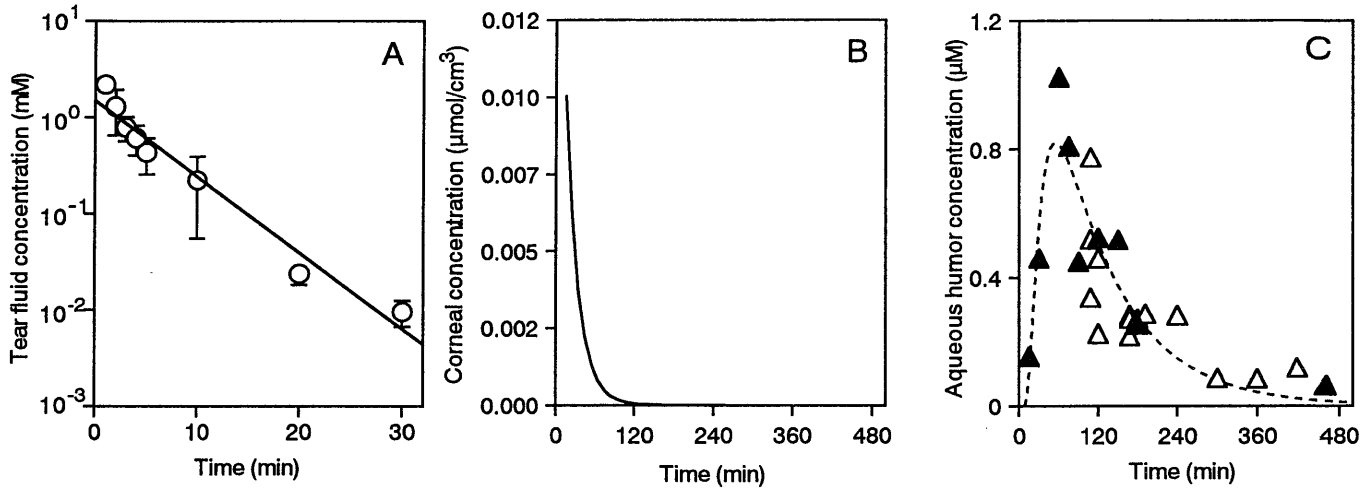

Fig. 3. Concentration of timolol in the tear fluid (A), cornea (B), and aqueous humor (C) after instillation into human eyes. (○) timolol, (△) experimental data according to Phillips et al.[2], (▲) experimental data according to Ellis et al.[3], (−) simulation line, (---) fitting line. Parameter values for simulation : Ke_{TF} (min^{-1}) = 0.18, V_{TF} (ml) = 0.19, Ke_{AH} (min^{-1}) = 0.013, Kt_{cp} (min^{-1}) = 0.020, Kt_{pc} (min^{-1}) = 0.086, V_{AHc} (ml) = 0.142, D' (= D_{CR}/L/L) (hr^{-1}) = 0.32, K' (= $K_{CR}V_{CR}$) (cm^3) = 0.006.

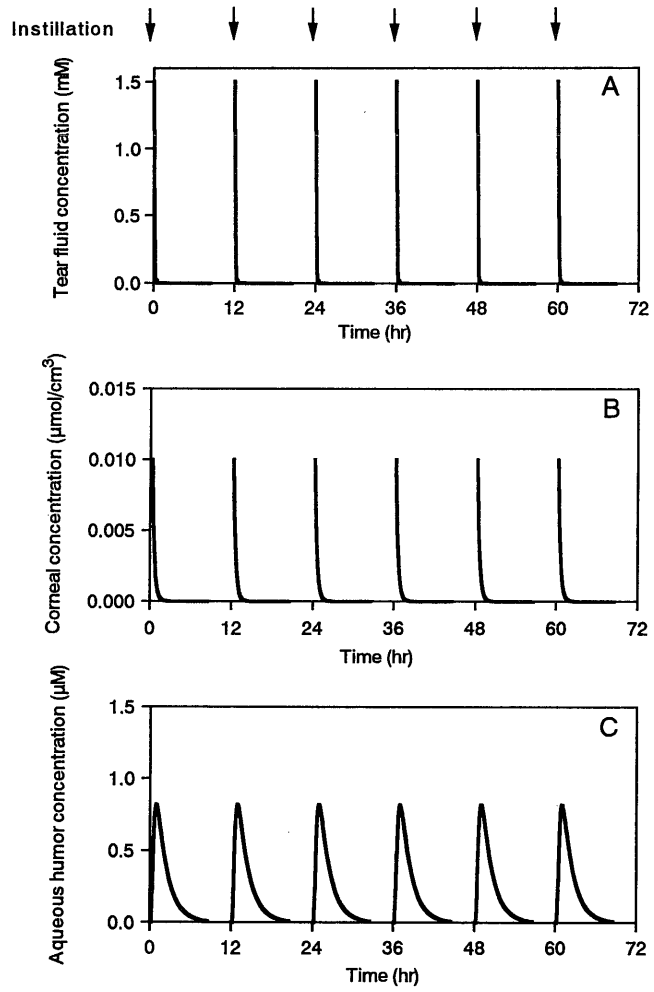

Fig. 4. Simulation curves of timolol in the tear fluid (A), cornea (B), and aqueous humor (C) after multiple instillations into human eyes twice a day for 3 days. (−) simulation line.

Drug Disposition in Tear Fluid

Fig. 2 shows the concentration-time profiles of timolol and fluorescein in the tear fluid after instillation into the eyes of human volunteers. These profiles almost showed a mono-exponential curve. Elimination rate constant and apparent distribution volume were estimated according to a one-compartment model and are listed in Table 1. These pharmacokinetic parameters of timolol were not significantly different from those of fluorescein. The parameters calculated from an average concentration-time profile were used for the estimation of in vivo corneal penetration parameters and pharmacokinetic parameters for timolol in the aqueous humor.

Estimation of in vivo penetration parameters and pharmacokinetic parameters

The concentrations of timolol in the aqueous humor after instillation into human eyes were obtained from the data reported previously[2,3] and are shown in Fig. 3C. The estimation of in vivo penetration parameters of timolol were performed by using these data, the parameters in the tear fluid (Table 1), and the in vivo pharmacokinetic model (Fig. 1). Table 2 shows the estimated parameters for human with those for rabbits reported previously[1]. The parameters for human did not show a big difference from those for rabbits. The in vivo diffusion parameter and partition parameter for human were $0.32hr^{-1}$ and 0.006 cm^3, respectively. The fitting curve was well consistent with the aqueous humor concentration of timolol after instillation as shown in Fig. 3C. The simulation curves of drug concentrations in the tear and cornea were also calculated from the pharmacokinetic model and parameters (Table 1 and 2) and are shown in Figs. 3A and 3B.

Discussion

Compartment models frequently have applied to describe the pharmacokinetics of ophthalmic drugs in the eye[15,16,23-26]. However, the application of this model to the corneal penetration of drugs may be limited in use. A main defect of the compartment model is lack of physical meaning of the model parameters in the drug penetration. An elimination of drug in the precorneal area is also progress under a non-steady state and the drug penetration across the cornea is a combined process of diffusion and partition. This behavior results in a phenomena called 'back diffusion' of drug in the precorneal area and in the phenomenon of 'flip-flop' of drug in the aqueous humor. Corneal penetration process of drugs may be better described by the diffusion model based on Fick's second low. In fact, the in vitro penetration profiles of beta-blockers through an isolated cornea have been sufficiently analyzed by Fick's diffusion equation[27-29]. Therefore, we developed an in vivo kinetic model including a corneal diffusion process as shown in Fig. 1. In the previous report, this model and in vivo parameters well described the concentrations of timolol in target sites such as tear, cornea, and aqueous humor after an instillation into rabbit eyes[1].

The bioavailability and pharmacokinetics of instilled drugs in the anterior segment of the eye are mainly controlled by three factors: disposition of drugs in the precorneal area (tear), penetration of drugs in the cornea, and elimination and distribution of drugs in the aqueous chamber[5,16]. The physiological and anatomical similarity has been demonstrated between the eyes of human and rabbits[4]. The human eye is very close to rabbit eye in lacrimal volume, aqueous humor volume, corneal thickness, and number of epithelial layers. However, there is a big difference in blinking rate, tear turnover, and presence of nictitating membrane. These indicate the importance of disposition in the precorneal area for carrying out animal scale up from rabbits to human. Therefore, the dispositions of timolol and fluorescein in the tear fluid were examined after instillation into human eyes as shown in Fig. 2. The in vivo parameters in the tear fluid were estimated from these profiles and are shown in Table 1.

Fluorescein is a hydrophilic dye, that was often used as a probe to measure the physiological volume and turnover of the tear fluid and aqueous humor because of its impermeability on biological membranes. The elimination rate constant of fluorescein in the tear fluid was almost equal to a physiologic turnover rate[6,30]. Timolol is a nonselective beta-blocker widely used in the treatment of open-angle glaucoma[19]. It is a lipophilic molecule. In the tear fluid, the elimination rate constant of timolol was not significantly different from that of fluorescein, indicating that the tear turnover mainly contributes to the drug disposition in the precorneal area.

The estimation of other in vivo pharmacokinetic parameters for human were performed by using the aqueous humor concentrations of timolol reported by Phillips et al.[2] and Ellis et al.[3] after the data were standardized by dose. Their data were adequate for the estimation of parameters because many points in the aqueous humor were determined at various times after instillation. The estimated parameters for human are almost close to those for rabbits (Table 2). This similarity of human to rabbits could indicate the usefulness of rabbits as an animal model for human in ophthalmic chemotherapy. On the other hand, the clearance of timolol ($Ke_{AH} \times V_{AHc}$) in the aqueous humor of human was lower than that in rabbit. It might be explained by the species differences in elimination process, distribution to melanin tissue, and pharmacological response of beta-blocker[31,32].

The mathematical model using in vivo pharmacokinetic parameters well described the concentrations of timolol in the tear and aqueous humor after instillation as shown in Figs. 3A and 3C. The model can calculate not only the drug concentrations in whole cornea (Fig. 3B) but also that in a portion of cornea principally. Fig. 4 shows the simulation profiles of timolol in the aqueous humor after multiple instillations into human eyes twice a day for 3 days based on the model and parameters.

A better understanding about the behavior of instilled drugs and a thorough knowledge of the physiology of the eye will result in improvement in chemotherapy. Therefore, it is important to develop the in vivo pharmacokinetic model and in vivo parameters for predicting the drug concentration in the tear, cornea and aqueous humor. We established the in vivo mathematical model and parameters for timolol to describe the ocular absorption in human. This kinetic model will be effective to estimate the adequate regimen for ophthalmic chemotherapy and develop the ocular drug delivery systems.

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