ELSEVIER

European Journal of Pharmaceutics and Biopharmaceutics 47 (1999) 99–104

European Journal of Pharmaceutics and Biopharmaceutics

Research paper

A pharmacokinetic model of intravitreal delivery of ganciclovir

Kakuji Tojo*, Katsunori Nakagawa, Yasushi Morita, Akira Ohtori

Kyushu Institute of Technology, Fukuoka, Japan

Received 30 September 1997; accepted 27 August 1998

Abstract

A pharmacokinetic model of intravitreal drug delivery was developed for describing the elimination and distribution of ganciclovir in the eye following intravitreous polymeric delivery. The model was based on Fick's second law of diffusion and assumed a cylindrical vitreous body. The model parameters such as the diffusion coefficient and the partition coefficient of the drug in the vitreous body and its surrounding tissues were determined from in vitro experiments using rabbit tissues. The time course of in vivo mean concentration of ganciclovir in the rabbit vitreous body agreed well with the profile calculated from the present pharmacokinetic model for both membrane-controlled polymeric devices and biodegradable rod-matrix systems. The clinical vitreous concentration following implantation of the membrane-controlled delivery system was the same order of magnitude but approximately four times lower than that predicted from the present model. This may indicate the metabolism of ganciclovir and/or the facilitated transport across the retina/choroid membrane in the human eye.

Keywords: Intravitreal delivery; Biodegradable polymer; Ocular pharmacokinetics; Ganciclovir; Vitreous body

1. Introduction

It is well known that only a small fraction of drugs applied topically may reach internal ocular tissues due to tear flow drainage, dilution by blinking, corneal diffusion resistance and aqueous humor washout [1]. Therefore, delivering drug molecules directly to the vitreous body using biodegradable or biocompatible polymeric implants [2] may treat vitroretinous diseases better. Recently, intravitreal injection or polymeric delivery systems for ganciclovir have been used for the treatment of cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome [3–5]. Since not only the efficacy but the toxicity is also influenced by the concentration at the site of action, the local concentration following intravitreal delivery of the drug must be carefully examined. The pharmacokinetics of intravitreal drug injection has been widely analyzed by a compartment model, which assumes the drug molecules are uniformly distributed throughout the vitreous body [6]. However, this assumption may not be applied to long-lasting delivery systems because of a constant rate of release and efficient elimination across the surrounding tissues. A significant concentration difference may be formed between the surface of the delivery device and the boundary on the elimination pathways such as the retina/choroid/sclera (RCS) membrane and the posterior aqueous chamber. The concentration distribution in the vitreous body is obviously influenced by the rate of elimination through these surrounding tissues.

The elimination profile of lomefloxacin in the rabbit eye following intravitreal solution injection was previously described by a cylindrical vitreous body model [7]. In this model, the diffusion coefficient, the partition coefficient and the metabolic reaction rate constant in the vitreous body

* Corresponding author. Laboratories of Therapeutic Systems Research, College of Computer Science and Biosystems Engineering, Kyushu Institute of Technology, Iizuka Campus, Fukuoka 820-8502, Japan. Tel.:+81-948-29-7816; fax: +81-948-29-7801; e-mail: tojo@bse.kyutech.ac.jp

0939-6411/99/$ - see front matter
PII: S0939-6411(98)00073-3

were evaluated from the in vitro experiment designed independently from the in vivo experiment [8]. In the present study, we have extended this cylindrical diffusion/partitioning model for predicting the performance of implantable delivery systems of ganciclovir. A series of in vitro experimental procedures for determining the model parameters for solving the pharmacokinetic model are also established using isolated rabbit tissues. The in vivo concentration profiles of ganciclovir in the rabbit vitreous body are then compared with the present model for both a biodegradable polymer-rod delivery system [9] and a membrane-controlled implantable device [10]. The clinical data for the membrane-controlled implantable device [11] are also compared with the profiles predicted from the present pharmacokinetic model using the model parameters determined from in vitro rabbit experiments.

2. Methods

2.1. Mathematical model

The present pharmacokinetic model of intravitreal drug delivery assumes a cylindrical vitreous body in contact with the retina/choroid membrane, the lens posterior capsule and the posterior aqueous humor (Fig. 1). The drug molecules released from a delivery system distribute throughout the vitreous body by diffusion and then move into the surrounding tissues. The rate of elimination of the drug molecules from the vitreous body depends upon the diffusion and partition characteristics of the surrounding tissues. In spite of high water content (more than 99%) in the vitreous body, a uniform distribution or single compartment model may not be assumed as indicated by Kinsey [12] because the local concentration gradient is influenced by the rate of elimination through each surrounding tissue.

The concentration of ganciclovir in the cylindrical vitreous body model following intravitreal drug delivery can be described by:

$$\frac{\partial C}{\partial t}=\frac{1}{x}\frac{\partial}{\partial x}\left(xD\frac{\partial D}{\partial x}\right)+\frac{\partial}{\partial y}\left(D\frac{\partial C}{\partial y}\right)-R(x,y,t)+S(x,y,t) \tag{1}$$

where D is the diffusion coefficient in the vitreous body, $R(x,y,t)$ is the metabolism and degradation rate of ganciclovir, and $S(x,y,t)$ is the source term for drug molecules released from the intravitreal delivery system. In this mathematical model, we assume that the drug is released in the small volume elements with 1.8% of the total vitreous body located along the central axis (the shaded area in Fig. 1). Beyond this volume elements, therefore, no source term is considered ($S = 0$). If the delivery system is a simple matrix device in which the drug molecules are uniformly distributed throughout the polymer matrix, the release rate decreases with increasing the time for release. On the other hand, if the device is a reservoir-type drug delivery system, the release rate is constant because of the rate-controlling membrane. Smith et al. measured the ganciclovir concentration both in the rabbit and in the human vitreous bodies following the implantation of a reservoir-type intravitreal delivery system [10,11]. Kunou et al. measured the in vivo release profile and the in vivo vitreous concentration in the rabbit eye following a biodegradable rod-shaped delivery system for ganciclovir [9]. The present pharmacokinetic model has been applied to correlate the release profile and the mean vitreous concentration in the rabbit eye for the intravitreal drug delivery systems. The appropriate initial and boundary conditions are described by:

$$t>0, x=0; \qquad \frac{dC}{dx}=0 \tag{2}$$

$$t>0, x=R; \qquad D\frac{dC}{dx}=-\frac{D_r K_r C}{\delta_r} \tag{3}$$

$$t>0, y=0; \qquad D\frac{dC}{dy}=\frac{D_r K_r C}{\delta_r} \tag{4}$$

$$t>0, y=H; \qquad D\frac{dC}{dy}=-\frac{D_l K_l C}{\delta_l} \qquad (0\le\chi\le\beta_0) \tag{5}$$

$$t>0, y=H; \qquad D\frac{dC}{dy}=-\frac{D_p K_p C}{\delta_p} \qquad (b_0\le\chi\le R) \tag{6}$$

where b_0, K, R and H are the effective radius of the lens, the partition coefficient, the effective radius of the vitreous body, and the effective height of the vitreous body, respectively (Fig. 1). D_r and δ_r in Eqs. (3) and (4)) are the diffusion coefficient through the RCS membrane and its effective thickness, respectively. D_l and δ_l in Eq. (5) are

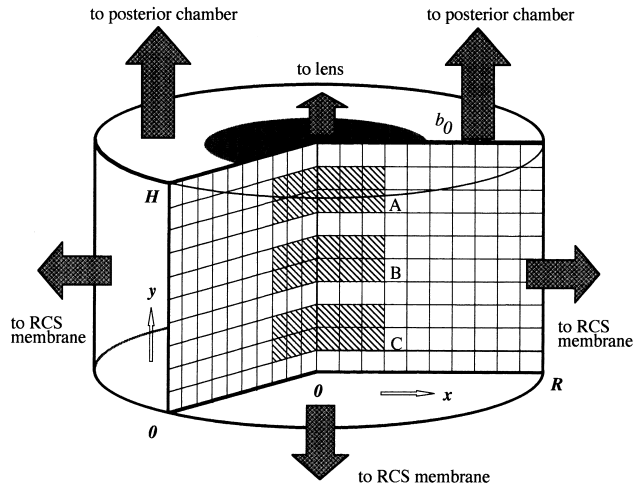

Fig. 1. Cylindrical vitreous body model for analyzing the pharmacokinetics of intravitreal delivery of ganciclovir. The surface of the vitreous body is in contact with three different tissues as elimination pathways: the posterior chamber, retina/choroid membrane and posterior lens capsule. Key (site of implantation) A, anterior; B, center; C, posterior.

the diffusion coefficient in the lens tissue and the effective thickness of the lens, respectively. We previously found that the thickness of the intact retina/choroid membrane is 0.082 cm for the rabbit. However the drug molecules may be eliminated through the central retinal capillary layer which is located in the retinal membrane. In the present model, therefore the distance from the vitreous surface to the front of the capillary layer is assumed to be 40 μm. D_p and δ_p are the aqueous diffusion coefficient and the thickness in a diffusion boundary layer formed in the vitreous body/aqueous humor boundary. The diffusion boundary layer in the posterior chamber is not known at this stage of research either in the human eye or in the rabbit eye. From mathematical simulation, however, the effect of the diffusion boundary layer on the drug transport from the vitreous body to the posterior chamber is almost negligible when the boundary layer is thinner than about 200 μm. Under mild mixing conditions in in vitro diffusion chamber experiments, the thickness of the diffusion boundary layer was found to be the order of 40–50 μm [7]. In the present study, therefore, we assume that the thickness of the boundary layer in the posterior aqueous chamber is 100 μm. The method of lines [13] is employed to solve Eq. (1). The details of the solution method were previously described [14]. All calculation in this study was carried out on an IBM ThinkPad personal computer. A Microsoft Fortran optimizing compiler was used for running the computer program, written in FORTRAN77. The cumulative amount of the drug eliminated was then calculated by integrating the concentration distribution profiles in the vitreous body.

2.2. In vitro experiment for determining the model parameters

The model parameters for solving the present pharmacokinetic model together with the initial and boundary conditions were determined from the in vitro membrane diffusion and partitioning experiment using isolated rabbit tissues.

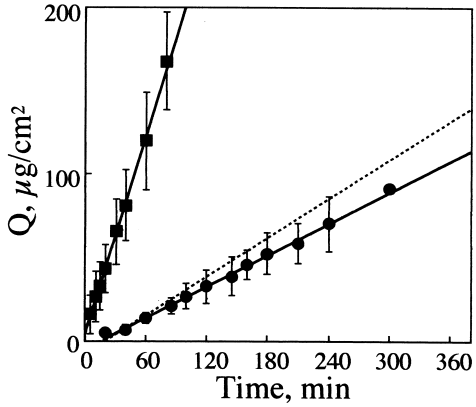

Fig. 2. The cumulative amount of ganciclovir which penetrated the vitreous gel membrane of rabbit. (●) Vitreous body; (■) membrane filters. Data presented as mean ± SD (n = 6). The dashed line is the intrinsic penetration profile after correcting the effect of supporting filter membranes [8].

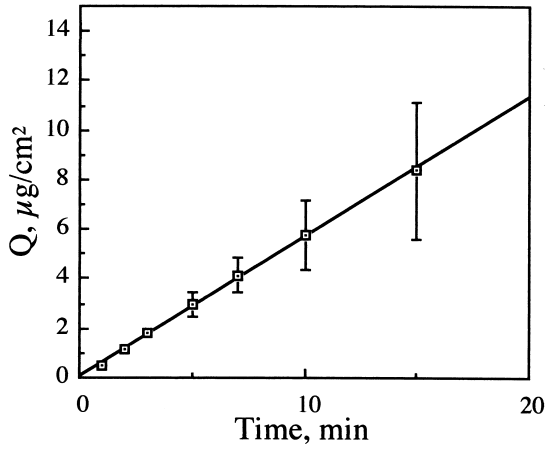

Fig. 3. The cumulative amount of ganciclovir which permeated the posterior lens capsule. Data presented as mean ± SD (n = 6).

The vitreous humor carefully removed from the rabbit eyes was inserted into a glass ring of 3.0 mm thick and 9.0 mm i.d. After supporting it with two membrane filters (Millipore: pore size 0.45 μm) to avoid the leakage of vitreous humor, the ring was mounted in the side-by-side diffusion cell system [8]. A solution of ganciclovir (250 μg/ml) was charged in the donor cell, while the fresh phosphate buffer solution (pH 7.2) was charged in the receptor cell. The temperature of the in vitro system was maintained at 37°C. At predetermined time intervals, 100 μl samples were taken from the receptor solution. The concentration of the drug in the receptor solution was then assayed by HPLC [10]. The concentration of the drug in the donor solution was approximately constant during the entire period of the experiment since only a small fraction of the drug molecules penetrated the membrane. The diffusion coefficient in the vitreous body and the partition coefficient between the vitreous body and the donor solution were then determined by the time lag and the steady state penetration rate [16]. For the posterior lens capsule, however, it is difficult to obtain the time lag from penetration experiments because of the thickness of the membrane (approximately 15 μm). Therefore, the partition coefficient of ganciclovir across the lens tissues (posterior capsule) was determined by the in vitro partitioning experiment. The diffusion coefficient was then evaluated from the steady state rate of penetration based on the linear portion of the penetration-time profile.

To determine the rate of elimination through the retina/choroid/sclera membrane, the permeability of ganciclovir was measured in an in vitro side-by-side diffusion cell system designed specially to adjust the curvature of the RCS membrane of rabbits. The same experimental procedure as in the vitreous diffusion experiment was employed in the RCS membrane permeability study. Either intact RCS membrane or sclera with the retina/choroid removed was mounted to the diffusion cell system. The cumulative amount of the drug permeation was then measured by sampling from the receptor solution at predetermined time intervals.

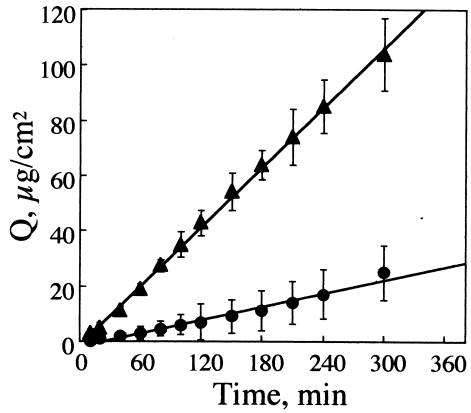

Fig. 4. The cumulative amount of ganciclovir which penetrated the retina/choroid/sclera membrane. (●) Intact RCS membrane; (▲) sclera (retina/choroid was removed). Data presented as mean ± SD (n = 6). The major resistance to ganciclovir transport resides in the retina/choroid membrane; less resistance was found in the scleral transport.

The elimination of the drug in the vitreous body due to metabolic reaction or degradation was studied by means of an incubation experiment in a light-resistant vial (5 ml). Vitreous humor (0.5 ml) freshly removed from the rabbit eye was quickly placed in the vial. After injecting 100 μl of the drug solution (25 μg/ml), the vitreous gel was incubated at 37°C for 0, 1, 3, 6 and 24 h. The drug concentration at each time interval was determined by HPLC. We found that the drug was stable in the vitreous humor for both rabbits and bovines [17].

The ocular membrane/donor solution partition coefficient was also determined by measuring the ratio of membrane concentration to elution medium concentration after 24-h incubation. The drug concentration in the ocular tissues was determined after complete extraction using methanol.

All the in vitro studies were performed following the guiding principles for the care and use of laboratory animals approved by the Japanese Pharmacological Society.

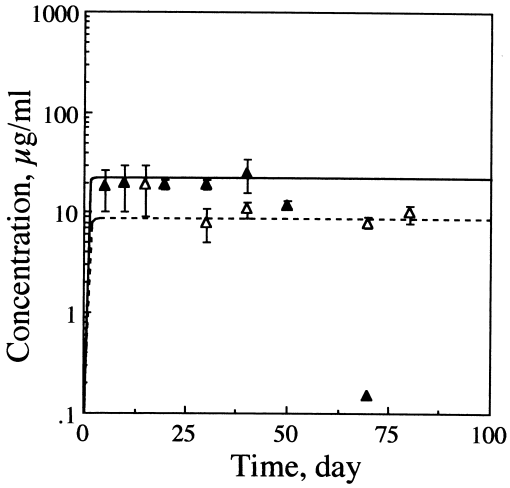

Fig. 5. Comparison of mean vitreous concentration following membrane-controlled ganciclovir delivery system in rabbit eye [10] with the present calculation. (△) dQ/dt = 2 μg/h; (▲) dQ/dt = 5 μg/h. The lines are calculated from the present model.

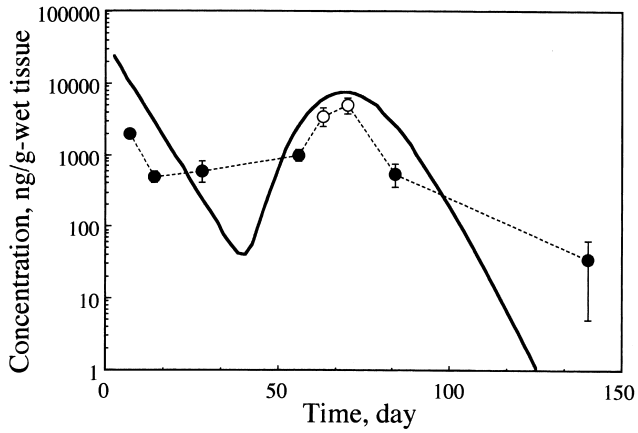

Fig. 6. Comparison of mean vitreous concentration following biodegradable ganciclovir delivery system in rabbit eye with the present calculation. (●) Kunou et al. [9], (○) Hashizoe et al. [18]; the solid line was calculated from the present mathematical model.

3. Results and discussion

The penetration profiles of ganciclovir across the vitreous humor, the posterior lens capsule and the retina/choroid/sclera membrane are shown in Figs. 2–4, respectively. The diffusion coefficient and the partition coefficient in the ocular tissues were then determined from the in vitro penetration profiles and summarized in Table 1. The effective radius and height of the cylindrical vitreous body model were evaluated after cutting frozen rabbit eyes. The effective dimensions of the human eye are also summarized in Table 1. Once the model parameters have been determined, the governing equation Eq. (1) subject to the boundary conditions Eqs. (2)–(6)) can be solved numerically for the rabbit eye as well as for the human eye.

3.1. Comparison with rabbit in vivo data

The mean vitreous concentration of ganciclovir following a membrane-controlled polymeric delivery in the rabbit vitreous body [10] was compared with the concentration calculated from the present pharmacokinetic model (Fig. 5). The calculated profiles of the mean concentration agreed well with the in vivo data of Smith et al. [10]. From integration of the concentration gradient on the surface of vitreous body, the fraction of elimination of ganciclovir from three elimination pathways could be evaluated. Table 2 summarizes the mean vitreous concentration and the fraction of elimination from each pathway as a function of the position of the delivery system placed following the constant release of ganciclovir at 2 μg/h. The mean concentration of ganciclovir in the vitreous body is higher in the anterior implantation than in the posterior implantation. This is due to the surface area of the retina/choroid membrane which is much greater than that of the posterior aqueous humor. The present mathematical simulation indicates that the elimination of ganciclovir from the vitreous body in rabbits, and presumably in humans, occurs mainly across

Table 1

Model parameters obtained in the in vitro experiment and the dimensions of the vitreous body used in the pharmacokinetic model

Diffusion coefficient in the vitreous body, D (cm^2/s)	9.89×10^{-6}
Diffusion coefficient in the retina/choroid membrane, D_r (cm^2/s)	5.51×10^{-7}
Diffusion coefficient in the lens (capsule), D_l (cm^2/s)	1.83×10^{-6}
Diffusion coefficient in the aqueous humor, D_p (cm^2/s)	1.00×10^{-5}
Partition coefficient of retina/vitreous humor, K_r	0.74
Partition coefficient of aqueous humor/vitreous humor, K_p	1.11
Partition coefficient of lens (capsule)/vitreous humor, K_l	0.324
Thickness of retina, δ_r (cm)	0.0040
Thickness of the diffusion boundary layer in the posterior aqueous chamber, δ_p (cm)	0.0100
Effective thickness of the lens, δ_l (cm)	0.38
Effective volume of the vitreous body, V (ml)	
Rabbit	1.2
Human	3.9
Radius of the vitreous body, R (cm)	
Rabbit	0.72
Human	0.94
Height of the vitreous body, H (cm)	
Rabbit	0.72
Human	1.41
Mean Radius of the lens, b_0 (cm)	
Rabbit	0.36
Human	0.40
Metabolic reaction rate constant in the vitreous body, $R(x,y,C,t)$	0
Release rate of ganciclovir $S(x,y,t)$	
Reservoir system (μg/h)	2.0, 5.0
Biodegradable implant (Fig. 7)	$f(t)$

the retinal surface. Because of a large surface area of the retina/choroid membrane, the anterior implantation caused the mean vitreous concentration to be appreciably higher than that for the posterior implantation. The elimination through the lens is insignificant because of a small effective area.

The in vivo rabbit data following the biodegradable rod matrix delivery system reported by Kunou et al.[9] and Hashizoe et al.[18] are also compared with the calculated profile from the present pharmacokinetic model (Fig. 6). The in vivo release rate which is a function of time as shown in Fig. 7 is substituted to $S(x,y,t)$ in Eq. (1). As can be seen from Fig. 6, the experimental time course of the drug concentration in the vitreous body is similar to the calculated profiles although the deviation is observed around 30–40 days and beyond 100 days. This deviation is probably due to back diffusion of ganciclovir from the surrounding tissues most likely the retina and choroid to the vitreous body since the in vivo release of ganciclovir during 30–40 days and beyond 100 days after implantation is almost negligible as shown in Fig. 7. The back diffusion was not considered in the present pharmacokinetic model. The deviation in the low concentration as shown in Fig. 6 may not cause any significant misunderstanding on pharmacological or toxicological effects. The highest concentration appeared both in the in vivo and calculated profiles around 70 days after implantation is caused by the late stage bursting release due to bulk erosion of the polymer as can be expected from the in vivo release profile (Fig. 7). This phenomenon was not clearly observed in the original in vivo experiment by Kunou et al. [9]. However, the same authors reported new data recently, as shown in Fig. 6, indicating clearly that the rabbit in vivo data agreed very well with the present mathematical model (Hashizoe et al. [18]). A high plasma concentration similar to the present analysis was also observed at the late period in the PLGA microcapsule formulations (Sanders et al. [15]). This late period bursting release due to bulk erosion of the polymer must be carefully investigated in order to avoid the toxic levels in the vitreous body and its surrounding tissues.

3.2. Comparison with clinical data

The present pharmacokinetic model of intravitreal drug delivery is used to predict the clinical performance of the reservoir-type ganciclovir delivery system reported in the literature [11]. We assume that the physicochemical proper-

Table 2

Mean vitreous concentration and fraction of elimination across three elimination pathways as a function of the site of implantation of reservoir-type delivery system

Site of implantation (Fig. 1)	Mean vitreous concentration (μg/ml)	Fraction of elimination		
		Posterior chamber	Lens	Retina/choroid
Anterior	8.61	0.380	0.096	0.524
Center	8.26	0.275	0.042	0.683
Posterior	5.12	0.137	0.019	0.844

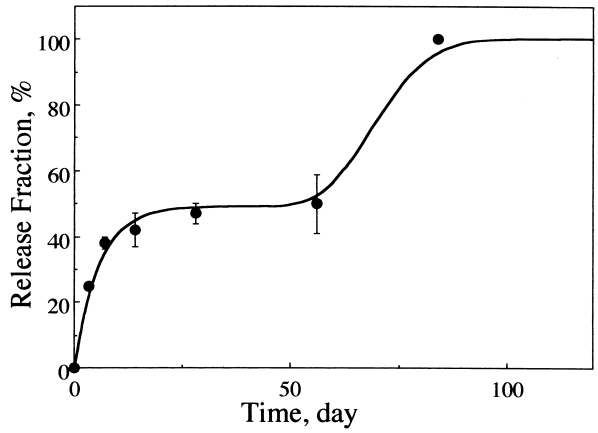

Fig. 7. The fraction of cumulative amount of ganciclovir released from biodegradable PLGA polymer rod device. The line was used in the present calculation. The data were obtained by Kunou et al. [9].

ties such as the diffusion coefficient and the partition coefficient across the human ocular tissues equal to the ones of rabbit tissues. In this analysis, therefore, only the shape difference between human and rabbit eyes is taken into account as summarized in Table 1. The clinical data of the ganciclovir delivery system was compared with the predicted profile based on the present pharmacokinetic model together with the model parameters obtained from the in vitro rabbit experiments. The calculated mean vitreous concentration (8.74 μg/ml) of ganciclovir following 2 μg/h release system was approximately four times greater than the clinical data (0.69 ± 4.62 μg/ml, C_{av} = 2.1 μg/ml). This difference is probably not caused by the difference in the human physicochemical parameters predicted from the rabbit data because the vitreous diffusion coefficient is very close to the aqueous diffusion coefficient and not so much influenced by the animal model. Furthermore, our simulation indicates that if elimination across the surrounding tissues occurs under perfect sink conditions, the mean vitreous concentration decreases to 4.35 μg/ml. Therefore, the clinical data suggest that the enhanced transport process may involve elimination across the retina/choroid membrane or otherwise, ganciclovir may be metabolized in the human vitreous body although this is not likely in the rabbit vitreous body. Smith et al. suggested the active transport of ganciclovir across the retina/choroid membrane in spite of a lack of clear evidence.

The present pharmacokinetic model for the invtravitreal delivery of ganciclovir can be used not only to optimize the delivery system design but predict the toxicity of the drug due to high local concentrations in the ocular tissues. The in vivo and in vitro animal experiments can also be designed to minimize the number of animals used by the help of computer simulation based on the present pharmacokinetic model of intravitreal drug delivery.

Acknowledgements

This work was supported in part by a Grant-In-Aid for General Scientific Research (No. 03454416) from the Ministry of Education, Science and Culture of Japan.

References

[1] A. Merkli, C. Tabatabay, R. Gurny, Use of insoluble biodegradable polymers in ophthalmic systems for the sustained release of drugs, Eur. J. Pharm. Biopharm. 41 (1995) 271–283.
[2] N. Worakal, J.R. Robinson, Ocular pharmacokinetics/pharmacodynamics, Eur. J. Pharm. Biopharm. 44 (1997) 71–81.
[3] H.L. Cantrill, K. Henry, N.H. Melroe, W.H. Knobloch, R.C. Ramsay, N.H. Balfour, Treatment of cytomegalovirus retinitis with intravitreal ganciclovir, Ophthalmology 96 (1989) 367–374.
[4] F.M. Ussery, S.R. Gibson, R.H. Conklin et al., Intravitreal ganciclovir in the treatment of AIDS-associated cytomegalovirus retinitis, Ophthalmology 95 (1988) 640–648.
[5] I. Cochereau-Massin, P. Lehoang, M. Lautier-Frau et al., Efficacy and tolerance of intravitreal ganciclovir in cytomegalovirus retinitis in acquired immune deficiency syndrome, Ophthalmology 98 (1991) 1348–1355.
[6] J. Ben-Nun, D.A. Joyce, R.L. Cooper, S.J. Cringle, I.J. Constable, Pharmacokinetics of intravitreal injection, Invest. Ophthal. Vis. Sci. 30 (1989) 1055–1061.
[7] A. Ohtori, K. Tojo, In vivo/in vitro correlation of intravitreal delivery of drugs with the help to computer simulation, Biol. Pharm. Bull. 17 (1994) 283–290.
[8] K. Uno, A. Ohtori, K. Tojo, Drug transport in the vitreous body of rabbits in vitro, J. Eye (Atarashii Ganka) 11 (1994) 607–609.
[9] N. Kunou, Y. Ogura, M. Hashizoe, Y. Honda, S.H. Hyon, Y. Ikada, Controlled intraocular delivery of ganciclovir with use of biodegradable scleral implant in rabbits, J. Cont. Rel. 37 (1995) 143–150.
[10] T.J. Smith, P. Pearson, D.L. Blandford et al., Intravitreal sustained-release ganciclovir, Arch. Ophthalmol. 110 (1992) 255–258.
[11] G.E. Sanborn, R. Anand, R.E. Torti et al., Sustained-release ganciclovir therapy for treatment of cytomegalovirus retinitis, Arch. Ophthalmol. 110 (1992) 188–195.
[12] V.E. Kinsey, Ion movement in the eye, Circulation 21 (1960) 968–987.
[13] P.J.D. Mayes, Method of lines, Biotech. Prog. 1 (1985) 269–273.
[14] K. Tojo, L.T. Fan, Membrane moderated controlled release, Math. Biosci. 57 (1981) 279–300.
[15] L.M. Sanders, G.I. McRae, K.M. Vitale, B.A. Kell, Controlled delivery of an LHRH analogue from biodegradable injectable microspheres, J. Cont. Rel. 2 (1985) 187–195.
[16] K. Tojo, D.S. Desai,, J. Eye (Atarashii Ganka) 8 (1991) 945–948.
[17] K. Nakagawa, Movement of drugs in the eye, MS Thesis, Kyushu Institute of Technology, 1995.
[18] M. Hashizoe, Y. Ogura, T. Kunou, Y. Honda, Y. Ikada, Biodegradable polymer device for sustained intravitreal release of ganciclovir in rabbits, Curr. Eye Res. 16 (1977) 633–639.