Drug Penetration of the Posterior Eye Tissues after Topical Instillation: In Vivo and in Silico Simulation

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The purpose of this study was to analyze drug pharmacokinetics in the posterior eye tissues after topical instillation. For the in vivo study, the concentrations of ofloxacin in rabbit ocular tissues were analyzed by high performance liquid chromatography at 1, 2, and 3 h after instillation. For the in silico simulation, the concentration distribution of ofloxacin in the eye was calculated by the ocular pharmacokinetic model based on the diffusion/partition model. The simulated profiles were then compared with the in vivo experimental findings. In the in vivo study, the drug concentration in the posterior vitreous body initially decreased with time after topical instillation, and thereafter, the concentration increased. The in silico simulation of ocular pharmacokinetics indicated that the drug penetration of the posterior vitreous body was determined by three major pathways: (1) the initial transscleral penetration, (2) the intermediate transcorneal penetration, and (3) the late transretinal penetration. The in vivo findings were well described by a series of contributions by these three pathways. In conclusion, the present in vivo and in silico studies suggest that the instilled drugs initially reached the posterior vitreous body by diffusion through the sclera and then later by corneal penetration and systemic circulation.

Key words ocular pharmacokinetics; topical; vitreous body; ofloxacin; diffusion

There is great interest in the ocular pharmacokinetics of the anterior segments of the eye because many drugs are targeted to the anterior eye tissues. However, new drugs have recently been developed for posterior-segment diseases, such as age-related macular degeneration and diabetic retinopathy. In case of conventional eye drops, most drugs used as age-related macular degeneration and diabetic retinopathy are targeted to the anterior eye tissues with great difficulty. Therefore, novel drug delivery systems, such as systemic administration, intravitreal, periocular and subconjunctival injections, implants, and iontophoresis, have been developed to improve ocular absorption.

Generally, a drug penetrates in the eye by diffusion and distributes in each ocular tissue. The ocular drug is not distributed homogeneously but disseminates in a complicated manner throughout the ocular tissue. The pharmacokinetics and pharmacodynamics of the posterior eye have been analyzed by assuming a compartment model. There has been limited research on the local concentration distribution of drugs in the eye. Some studies have indicated that drugs may effectively reach the retina after topical administration. It is important to investigate the detailed pharmacokinetics of the posterior eye tissues with respect to the concentration distribution of drugs.

Pharmacokinetic/pharmacodynamic modeling and simulation can be used to examine the efficacy and safety of new drugs. In the ophthamlic field, in silico studies of pharmacokinetics have been carried out. With an in silico approach, the pathways of drug penetration into the eye can be described in more detail.

The present study has investigated the drug pharmacokinetics in the eye tissues after topical instillation. Ofloxacin ophthalmic solution was administered to rabbits, and the concentration of ofloxacin in the eye was analyzed. Pharmacokinetic simulation model, which was based on the diffusion/partition model, was applied to determine the drug concentration distribution in each eye tissue.

Experimental

Materials Ofloxacin was purchased from LKT Laboratories, Inc. (St. Paul, MN, U.S.A.). Sodium pentobarbital was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sodium chloride and hydrochloric acid were purchased from Manac Inc. (Hiroshima, Japan) and Nacalai Tesque, Inc. (Kyoto, Japan), respectively.

Preparation of Drug Solution The 0.3% (w/v) ofloxacin ophthalmic solution was prepared by dissolving 0.3% ofloxacin in aqueous solution (pH 6.5) containing sodium chloride (0.85%) and hydrochloric acid.

In Vivo Pharmacokinetic Study Japanese albino rabbits (Kitayama Labes Co., Ltd., Nagano, Japan) weighing 2 to 3 kg were used. The study adhered to the Association for Research in Vision and Ophthalmology (ARVO) declaration for the use of laboratory animals in research. The rabbits were topically administered with 50 μl of drug solution using a micropipette in three groups of rabbits (n = 3). After 1, 2, and 3 h, the rabbits were sacrificed by intravenous injection of 5% sodium pentobarbital solution. The eyes were rinsed with an isotonic saline solution. The aqueous humor was removed from the eye using a 1-ml syringe attached to a 27-gauge needle. The conjunctiva was dissected and the eyes were then emulsified and immediately frozen at −80 °C. The corneas were obtained from the frozen eyes. Finally, the frozen eyes were divided into three sections, as shown in Fig. 1. The anterior, central, and posterior sections of both the sclera, retina/choroid, and vitreous body were obtained using a microtome.

Quantitative Determination of Ofloxacin The aqueous humor samples were filtrated using filters with 0.22-μm pores and analyzed by HPLC. The other ocular tissues, including the conjunctiva, cornea, sclera, retina/choroid, and vitreous body, were added to 5 ml of acetonitrile and homogenized using

Fig. 1. The Three Sections of the Posterior Eye Tissues (Sclera, Retina/Choroid, and Vitreous Body) Examined in Order to Measure Ofloxacin Concentration in Vivo

A: Anterior section, C: central section, P: posterior section.
The drug was assumed to be eliminated across three different diffusion pathways: the anterior chamber surface, the posterior chamber surface, and the vitreous body surface. In addition, this pharmacokinetic eye model was modified in order to examine the more elaborate transocular pathway. In the present model, the top of the anterior chamber surface was assumed to be the tear fluid, cornea, and conjunctiva (Fig. 2).

The drug concentration in each eye tissue was described by the following equation (Eq. 1).

\[ C_{\text{whole}} = C_{\text{ant}} W_{\text{ant}} + C_{\text{cent}} W_{\text{cent}} + C_{\text{post}} W_{\text{post}} \]

where \( C_{\text{whole}} \), \( C_{\text{ant}} \), \( C_{\text{cent}} \), and \( C_{\text{post}} \) are the drug concentrations in the whole ocular tissue, anterior ocular tissue, central ocular tissue, and posterior ocular tissue, respectively (\( \mu g/g \)). \( W_{\text{ant}} \), \( W_{\text{cent}} \), and \( W_{\text{post}} \) are the weights of the anterior ocular tissue, central ocular tissue, and posterior ocular tissue, respectively (g).

Theoretical

Ocular Pharmacokinetic Model

In the ocular pharmacokinetic model based on Fick’s second law of diffusion, a modified cylindrical eye model was applied. The drug was assumed to be eliminated across three different diffusion pathways: the anterior chamber surface, the posterior chamber surface, and the vitreous body surface. In addition, this pharmacokinetic eye model was modified in order to examine the more elaborate transocular pathway. In the present model, the top of the anterior chamber surface was assumed to be the tear fluid, cornea, and conjunctiva (Fig. 2).

The drug concentration in each eye tissue was described by the following Eq. 2, which assumed neither binding nor metabolism in the eye.

\[ \frac{dC}{dt} = \frac{1}{x} \left( \frac{\partial D}{\partial x} \frac{\partial C}{\partial x} + \frac{\partial}{\partial y} \left( D \frac{\partial C}{\partial y} \right) \right) \]

The diffusion coefficient \( D \) may vary in the ocular tissues as summarized in Table 1.

Table 1. Diffusion Coefficients in Various Ocular Tissues

<table>
<thead>
<tr>
<th>Tissue in the model</th>
<th>(cm²/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitreous body</td>
<td>( D_v \times 10^{-3 \pm 1,14} )</td>
</tr>
<tr>
<td>Tear fluid</td>
<td>( D_t \times 1000 )</td>
</tr>
<tr>
<td>Cornea</td>
<td>( D_c /10 )</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>( D_c /10 )</td>
</tr>
<tr>
<td>Iris body</td>
<td>( D_i /10 )</td>
</tr>
<tr>
<td>Anterior chamber</td>
<td>( D_a \times 10^{7 \pm 1,13} )</td>
</tr>
<tr>
<td>Posterior chamber</td>
<td>( D_p \times 10^{7 \pm 1,13} )</td>
</tr>
<tr>
<td>Lens capsule</td>
<td>( D_L /10^7 \pm 1,13 )</td>
</tr>
<tr>
<td>Lens cortex</td>
<td>( D_L /10^7 \pm 1,13 )</td>
</tr>
<tr>
<td>Lens nucleus</td>
<td>( D_L /250 \pm 1,13 )</td>
</tr>
<tr>
<td>Outside of inscribed sphere</td>
<td>( D_L \times 10^{10} )</td>
</tr>
</tbody>
</table>

\( D_v \) is the diffusion coefficient in the vitreous fluid (cm²/s).

\( R \) and \( H \) are the effective radius and height of the eyeball, \( k_i \) is the elimination rate constant of the drug in the tear fluid (s⁻¹), \( D \) is the diffusion coefficient in the cylindrical eye (cm²/s), \( K \) is the membrane partition coefficient, \( L \) is the membrane thickness, and the subscripts, a, p, and r, refer to the anterior chamber membrane, the posterior chamber membrane, and the retina/choroid/sclera (RCS) membrane, respectively.

The rates of drug movements across the surrounding barrier membranes are influenced on the drug lipophilicity. These rates are characterized by the Sherwood number \( SH \), which is defined as follows:

\[ SH = \frac{D_m \times D_K}{R L} \]

where \( D_m \) is the diffusion coefficient in the barrier membrane (cm²/s).

In Silico Pharmacokinetic Study

Ofloxacin has a molecular weight of 361.37(14) and an octanol/buffer partition coefficient of 0.33(15). Model parameters, such as the coefficient of elimination rate and the Sherwood numbers, were determined as follows. The initial tear concentration on the surface of the cornea was 45% of the formulation concentration due to the instantaneous dilution by tear fluid. Thus, the concentration in the tear fluid was assumed to be 1350 mg/ml(15). Model parameters for evaluating Eq. 10, such as the diffusion coefficient and the partition coefficient in the eye tissue, were determined from the membrane penetration experiments. The value of the membrane thickness of the eye tissue was calculated from the dimensionless ratio of the actual membrane thickness to the spherical radius (12 mm). Accordingly, the Sherwood numbers of the vitreous body and the posterior chamber were defined as 6.17×10⁻⁴ and 7.24, respectively.

Several previous studies on the elimination of ofloxacin in the tear fluid were reported in the literature. In our study, the concentration–time profiles of ofloxacin in the rabbit tear fluid were analyzed after instillation. When the drug was instilled in the eyes 16 times every 30 min in healthy adult volunteers, the blood concentration of ofloxacin 30 min after the last instillation was
The appropriate blood concentration is described in Eq. 11.

\[ C = C_0(e^{-kt} - e^{-kt_1}) \]  

(11)

where \( k_1 \) and \( k_2 \) are the elimination rate constant and the absorption rate constant in the blood, respectively.

The absorption rate constant and the elimination rate constant in the systemic blood were assumed to be \( 2 \times 10^{-4} \text{s}^{-1} \) and \( 8 \times 10^{-5} \text{s}^{-1} \) respectively. The initial ofloxacin concentration, which penetrated from the tear fluid to the blood after single instillation, was calculated using Eq. 11. As a result, the initial penetrated concentration to the blood was approximately 0.15 \( \mu \text{g/ml} \) (0.01% of the initial ofloxacin concentration in the tear fluid). Accordingly, about 0.01% of the initial ofloxacin in the tear fluid was assumed to be absorbed in the systemic blood using Eq. 11. And then, about 35% of the ofloxacin in the systemic blood penetrated into the vitreous body with about 1 h as lag time.23)

As mentioned earlier, the model parameters were independently obtained from in vitro and in vivo experiments. If experiment data were not available, the model parameters were obtained from the literature.23–25)

Results and Discussion

**In Vivo Pharmacokinetics** Table 2 shows the experimental ofloxacin concentrations in various eye tissues of the rabbit after eye drop instillation. The concentrations of the anterior tissues (cornea, aqueous humor, and conjunctiva) were higher than those of the posterior tissues (sclera, retina/choroid, and vitreous body), and the vitreous body showed the lowest concentration. Figure 3 shows the elimination profiles of ofloxacin in the three sections of the posterior ocular tissues. In the sclera, the drug concentrations of the anterior (A), central (C), and posterior (P) sections decreased with time (Fig. 3a). In contrast, the drug concentration of the P section in the retina/choroid remained constant at 2 to 3 h (Fig. 3b). Furthermore, the drug concentration of the P section in the vitreous body decreased until 2 h and then increased at 2 to 3 h (Fig. 3c).

Table 2. The Experimental Ofloxacin Concentrations in Various Eye Tissues of the Rabbit

<table>
<thead>
<tr>
<th>Eye tissue</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornea</td>
<td>2.919±0.473</td>
<td>1.750±0.705</td>
<td>1.291±0.180</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>1.171±0.397</td>
<td>0.598±0.361</td>
<td>0.420±0.032</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>0.818±0.187</td>
<td>0.161±0.062</td>
<td>0.110±0.013</td>
</tr>
<tr>
<td>Whole sclera</td>
<td>0.404±0.085</td>
<td>0.125±0.064</td>
<td>0.082±0.004</td>
</tr>
<tr>
<td>Whole retina/choroid</td>
<td>0.335±0.036</td>
<td>0.068±0.045</td>
<td>0.046±0.020</td>
</tr>
<tr>
<td>Whole vitreous body</td>
<td>0.016±0.007</td>
<td>0.004±0.002</td>
<td>0.006±0.003</td>
</tr>
</tbody>
</table>

Each data represents the mean±S.D. (n=3).

The drug concentration in the posterior vitreous body showed a characteristic profile, where the drug concentration decreased and then increased. Given that drug molecules reached the vitreous body through anterior tissue such as the aqueous humor, the drug concentration in the vitreous body decreased with time. These findings suggest that the drug concentration of the posterior vitreous body was affected by the drug penetration of not only the anterior tissues, but also the other tissues as well.

**In Silico Pharmacokinetics** The pharmacokinetics after topical administration was simulated under the following two conditions: (I) transscleral and transcorneal penetration, and (II) transscleral, transcorneal, and transretinal (by the systemic circulation) penetration.

Figure 4a shows that the simulated profiles of the aqueous humor (anterior eye) and the whole vitreous body (posterior eye) in condition (I) were similar to condition (II). Furthermore, the simulated profiles were in good agreement with the experimental elimination findings. However, the simulated profile of the posterior vitreous body in condition (II) was different from condition (I) (Fig. 4b). Under condition (I), the drug concentration in the posterior vitreous body initially decreased and then remained constant 2 to 3 h after instillation. In contrast, the drug concentration in the posterior vitreous body in condition (II) initially decreased and thereafter increased. As a result, the simulated profiles in condition (II) were in agreement with the experimental elimination findings. Therefore, a tiny amount of drug could penetrate into the serum after a single instillation.

In our study, the simulated profiles were in agreement with
the experimental findings when taking into account not only the anterior penetration, but the systemic circulation as well. Accordingly, it can be presumed that the characteristic profiles of the posterior vitreous body were due to some influence of the systemic circulation. The penetration and distribution of a drug into the posterior tissues of the eye after topical administration can occur through the following pathways. First, the drug diffuses into the iris root and then into the posterior aqueous humor and the posterior tissues. Second, the drug enters directly through the pars plana without encountering the blood-retinal barrier. Third, the drug diffuses across the sclera by lateral diffusion, which is followed by penetration of Bruch’s membrane and the retinal pigment epithelium. Fourth and to a lesser extent, the drug can be absorbed into the systemic circulation either through the conjunctival vessels or through the nasolacrimal duct, and it then gains systemic access to the retinal vessels.

Figure 5 shows the simulated concentration profiles in the eye in condition (II), consisting of the transscleral, transcorneal, and systemic circulation. The instilled drug level was initially high in the tear fluid and anterior eye. In the posterior eye, a slight amount of drug reached the posterior section, even though the drug level was low (Fig. 5, 0.75 h). The arrow in Fig. 5 (1 h) shows that the drug, penetrated to the conjunctiva and sclera, reached the posterior vitreous body, and then the drug was eliminated in a short time through the surface of the vitreous body (RCS membrane). Accordingly, the instilled drug entered directly through the pars plana without encountering the blood-retinal barrier or diffusing across the sclera, and it reached the posterior eye in a short time after instillation. It can be presumed that this transscleral route (R1) is a major pathway for drug penetration to the posterior eye after ofloxacin instillation. Similarly, nepadilol, which has a similar molecular weight and partition coefficient as ofloxacin, reached the posterior eye from

Fig. 5. The Simulated Concentration Profiles in the Eye in Condition (II)

AC: Anterior chamber, VB: vitreous body, R1: Route 1 of drug movement through the sclera (transscleral), R2: Route 2 of drug movement through the cornea (transcorneal), R3: Route 3 of drug movement derived from the systemic circulation (transretinal). Arrows show the direction of the drug movement.

Fig. 6. Three Major Pathways of Drug Distribution into the Posterior Tissues of the Eye after Topical Administration

R1: Route 1 of drug movement through the sclera (transscleral), R2: Route 2 of drug movement through the cornea (transcorneal), R3: Route 3 of drug movement derived from the systemic circulation (transretinal). Arrows show the directions of drug movements.
the anterior eye through the surface of the vitreous body after instillation.\textsuperscript{26} The arrow in Fig. 5 (2 h) shows that the drug concentration decreased with time because the drug moved to the posterior from the anterior at 2 h. Accordingly, it can be suggested that the instilled drug can diffuse through the iris root and subsequently into the posterior aqueous humor and the posterior tissues at about 1—2 h (R2). Finally, the arrow in Fig. 5 (3 h) shows that the drug penetrated to the vitreous body by the systemic circulation. It seems natural to presume that the instilled drug can be absorbed into the systemic circulation and thereby gain systemic access to the retinal vessels so that the drug can penetrate the posterior eye a few hours after ofloxacin instillation (R3).

It should be noted that the drug distribution profile of the posterior vitreous body showed unique behavior. Figure 6 shows the distribution pathways of a drug into the posterior tissues of the eye after topical administration. Our results indicate that drug penetration to the posterior vitreous body was determined by three major pathways after topical instillation: (R1) initial transscleral penetration, (R2) intermediate transcorneal penetration, and (R3) late transretinal penetration by the systemic circulation.

Thus, the instilled drug had different delivery times depending on the diffusion pathways. The drug seemed to take a long time to reach the posterior eye for the drug absorbed into the systemic circulation. Instilled drug was absorbed into the systemic blood either through the conjunctival vessels or through the nasolacrimal duct.\textsuperscript{20} And then, the drug was absorbed in the systemic blood needed to diffuse across static anatomic permeability barriers such as Bruch’s membrane and the retinal pigment epithelium.\textsuperscript{5} Ofloxacin, a small molecular and hydrophilic drug, easily penetrates to the posterior vitreous body from the surrounding tissues (retinal choriocapillaris, and anterior and central vitreous body). Therefore, it hardly occurred that the drug in the surrounding tissues take about 3 h to reach the posterior vitreous body. Thus, there was a longer lag time for the drug absorbed into the serum to reach the posterior eye. The findings of a biphasic distribution profile of the posterior vitreous body were very interesting for this reason.

Tojo and Ueda developed a mathematical model of eye pharmacokinetics and showed the drug concentration distribution \textit{in silico}.\textsuperscript{7,13} The pharmacokinetics of a drug in the eyeball was experimentally verified more in detail in the present study. It was clear that the concentration distributes in a complicated manner in the posterior tissues, such as the sclera, retina, choroid, and vitreous body. Furthermore, the drug concentration distribution of the posterior vitreous body had the characteristic bimodal profile. Accordingly, the present mathematical model of the ocular pharmacokinetics described the drug pathways and \textit{in vivo} drug movements in each tissue in detail in order to minimize experimental animals. The drug concentration distributions in the anterior and posterior eye were well described by the present ocular pharmacokinetic model. In addition, this \textit{in silico} approach is useful for simulating the effects of various factors in the ocular delivery systems that are designed to target not only the anterior eye, but also the posterior eye.

### Conclusion

The instilled drugs reached the posterior eye through three major pathways: (1) transscleral penetration, (2) transcorneal penetration, and (3) transretinal penetration by the systemic circulation. The transscleral penetration of ofloxacin had a shorter duration than the transcorneal penetration. The transretinal penetration by the systemic circulation had the longest lag time in reaching the posterior eye due to the length of time required to diffuse through the various barriers. The drug distribution profile of the posterior vitreous body showed a unique behavior because of the difference in the lag times of each pathway. The mathematical model of the ocular pharmacokinetics described the drug pathways and \textit{in vivo} drug movements in the posterior segments of the eye in order to minimize experimental animals.

### References