Drug Absorption Behavior after Periocular Injections

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The purpose of this study was to investigate the absorption behavior of an ophthalmic drug injected in rabbit periocular tissues. After intracapsular, retrobulbar and palpebral conjunctival injections of 150 μl and 50 μl fluorescein isothiocyanate dextran (FITC-dextran, average molecular weight 11000), leakage of the dye into the tear fluid was dependent on the injection route and volume. After periocular injections (50 μl) of tilisolol, as a model beta-blocker, the concentrations in the tear fluid, blood, aqueous humor and vitreous body were determined by HPLC. Slight drug leakage was observed in the tear fluid after injections. The periocular injections showed a faster absorption and a higher area under the concentration–time curve (AUC) in the plasma and a lower AUC in the aqueous humor than those observed in instillation. They also showed a higher ratio of AUC of tilisolol in the vitreous body to AUC in the aqueous humor than that observed in the instillation. Among the periocular injections, retrobulbar injection showed the highest concentrations in the plasma and the lowest in the aqueous humor and vitreous body, while intracapsular injection showed the lowest in the plasma and the highest in the aqueous humor and vitreous body. Although the periocular injections showed a rapid systemic absorption of drug by a rich topical vasculature, it might be an effective approach to deliver the drug to the periocular tissues and vitreous body.

Key words Drug delivery system; periocular injection; retrobulbar injection; palpebral conjunctiva; pharmacokinetics, tilisolol

Most drugs administered systemically have poor access to the aqueous humor and vitreous body because of the blood–aqueous barrier and the blood–retinal barrier which prevent drugs from entering the eye.1 2 In ophthalmology, instillation is most commonly used in chemotherapy with convenience and safety. However, the rapid elimination of drugs in the preocular area by turnover and drainage of tear fluid and the poor permeability of most drugs to the cornea results in low bioavailability and systemic side effects.3 4

Periocular injections of ophthalmic drugs have been available, and the injection technique in particular has been developed as a topical anesthetic for ophthalmic surgery.5 6 Retrobulbar injection is often used for surgery because of its good suppression of eye movement, although a variety of actions and the risk of additional damage have been reported.7 Periocular injection of drugs such as corticosteroids has commonly been used for an ocular inflammatory condition and after ocular surgery.8–10 Administration of antibiotics via the subconjunctival route is also an important mode of therapy for bacterial corneal infections.11 12 Possible advantages of this route include delivery of the drug with high topical concentration using a relatively small dose compared with the systemic dosing. However, there have been few systematic studies on the absorption behaviors of periocular injected drugs.

In the present study, drug absorption behavior was investigated after intracapsular, retrobulbar and palpebral conjunctival injections of tilisolol into rabbits (Fig. 1). Tilisolol was used as a model of an ophthalmic drug. It was synthesized as a non-selective and hydrophilic beta-blocker, and has been reported to reduce intraocular pressure after instillation in rabbit eyes.13 14

MATERIALS AND METHODS

Materials Tilisolol hydrochloride was supplied by Nisshin Flour Milling Co., Ltd. (Tokyo, Japan). Fluorescein isothiocyanate dextran (FITC-dextran, average molecular weight 11000) was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). O-Ethoxybenzamide and all other chemicals were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Salicyl tyrosine and salicyl methionine were prepared by the method reported previously.19 Phosphate-buffered saline (pH 7.4) was prepared by mixing an isotonic phosphate buffer with an equal volume of saline.

Animals Male Nippon albino rabbits weighing 2.0–3.0 kg were used throughout the study. The animals were individually housed in cages in an air-conditioned room and maintained on a standard laboratory diet (ORC4, Oriental Yeast Co., Ltd., Tokyo). The rabbits were starved for 24 h before use but had free access to water. All experiments in the present study conformed to the “Principles of Laboratory Animal Care” (NIH publication #85-23, revised 1985).

In Vivo Experiment Unanesthetized rabbits were kept in a prone position on a wooden plate. About 10 min before drug administration, the eyes were topically anesthetized by 0.4% oxybuprocaine hydrochloride solution. Low volume (50 μl) and high volume (150 μl) of tilisolol (100 mgs) or FITC-dextran (1 ms) in pH 7.4 phosphate-buffered saline were injected into periocular tissues by microliter syringe fitted with a 30-gauge needle. Periocular injections included intracapsular, retrobulbar and palpebral conjunctival injections as shown in Fig. 1. In intracapsular injection, drug solution was subconjunctivally administered into Tenon's capsule at 1–2 o'clock and a distance of 8 mm from the limbarportion. In retrobulbar injection, the drug solution was subconjunctivally administered into the retrobulbar portion at a distance of 8 mm from Tenon's capsule. In palpebral conjunctival injection, the drug solution was subconjunctivally administered into the center of the palpebral conjunctiva. The injection sites were confirmed by injections of dye. At appropriate time intervals after injection, tear fluid samples (0.5 μl) were
collected by glass capillary (EM minicaps®, Hirschmann Laborgerate, Germany) at lower conjunctival sac, and blood samples (1.5 ml) were withdrawn via marginal ear vein. In the same manner, at various times, the rabbits were killed by an overdose of sodium pentobarbitone solution. After thoroughly rinsing the corneal and conjunctival surfaces with normal saline and blotting them for dryness, the aqueous humor was aspirated from the anterior chamber using a 1.0 ml disposable syringe with a 27-gauge needle. These samples were subjected to HPLC.

Drug Determination Tear fluid sample (0.5 μl) was mixed with pH 7.4 phosphate-buffered saline (200 μl) and methanol (100 μl) including internal standard (300 μg ml⁻¹ o-ethoxybenzamide). The sample for the aqueous humor and vitreous body (200 μl) was mixed with 1 M HCl (20 μl) and methanol (100 μl) including internal standard (50 μg ml⁻¹ salicyl methionine). These mixtures were centrifuged at 12000g for 10 min and 50 μl of supernatant was injected into the HPLC system.

Blood samples were centrifuged at 15000g for 15 min to obtain plasma. Plasma (700 μl) was mixed with 2 M perchloric acid (300 μl) to remove the protein. After the 15 min centrifugation at 15000g, the supernatant (800 μl) was mixed with 2 M NaOH (200 μl) and chloroform (6 ml). After shaking and centrifugation at 15000g for another 15 min, the organic layer (5 ml) was mixed with methanol (100 μl) including internal standard (2 μg ml⁻¹ salicyl tyrosine). The mixture was evaporated to dryness and the residue was dissolved in 20% methanol of pH 7.4 phosphate-buffered saline. The sample was injected into a HPLC system.

The HPLC system (LC-10A, Shimadzu Co., Ltd., Kyoto) was used in the reverse phase mode for assay. The stationary phase used was a Cosmosil 5C₁₈-P packed column (4.6 mm i.d. x 150 mm length, Nacalai Tesque Inc.). A mixture of methanol and 50 mM Na₂HPO₄ (37: 63 v/v) was used as the mobile phase for tear fluid samples and a mixture of acetonitrile, methanol and 50 mM Na₂HPO₄ (12: 8: 80 v/v) as the mobile phase for aqueous humor, vitreous body and blood samples. Flow rate of the mobile phase was 1.0 ml min⁻¹. Retention of drug was monitored with a fluorescence HPLC monitor (RF-535, Shimadzu Co., Ltd.; excitation wavelength 315 nm and emission wavelength 420 nm).

FITC-dextran was determined with a spectrofluorophotometer (RF-510, Shimadzu Co., Ltd.; excitation wavelength 489 nm and emission wavelength 515 nm) in pH 7.4 phosphate-buffered saline.

Data Analyses The plasma concentration (Cp) profile of tilisolol after intravenous injection was fitted to a bi-exponential equation described as follows, by the nonlinear least-squares computer program, MULTI (weight = 1).

\[ C_p = \frac{\text{Dose}}{K_e} \left( e^{-\beta t} - e^{-\alpha t} \right) + \frac{Dose \cdot K_2 \cdot V_c}{\alpha \cdot \beta} \frac{\alpha}{\beta - \alpha} \]

Hybrid parameters α and β are defined as \( \alpha + \beta = K12 + K21 + Kc \) and \( \alpha \cdot \beta = K21 \cdot Kc \cdot Vc \) is the volume of the central compartments. Kc is the first-order elimination rate constant from the central compartment. K12 and K21 are the first-order transfer rate constants between the central and peripheral compartments.

In the same way, the plasma concentration profile of tilisolol after periocular injection was fitted in a two-compartment model incorporating first-order absorption. In this model, the equation for plasma concentration is given as follows:

\[ C_p = \frac{\text{F} \cdot \text{Dose} \cdot K_a \cdot V_c}{(K21 - Kc) \cdot (\alpha - Kc) \cdot (\beta - Kc) \cdot \exp(-Kc)} + \frac{(K21 - Kc) \cdot (\beta - Kc) \cdot \exp(-\alpha t)}{\alpha \cdot \beta} \frac{\alpha}{\beta - \alpha} \]

\( K_a \) is the first-order rate constant for absorption into the bloodstream from the injection site. F is the availability of tilisolol after periocular injection. In the case of curve-fitting for the plasma concentration profile of tilisolol after instillation and periocular injections, the pharmacokinetic parameters (α, β, K21, Vc) obtained from the average plasma con-
concentrations following intravenous administration were substituted into Eq. (2).

The area under the drug concentration–time curve (AUC) from time 0 to infinity in the tear fluid and plasma was calculated by the linear trapezoidal rule. AUC from time 0 to 120 min in aqueous humor and vitreous body was calculated from the average points at 30, 60 and 120 min.

All data were statistically evaluated with ANOVA followed by Scheffe’s F. The value of possible statistical probability (p) less than 0.05 was considered significant.

RESULTS

A leak of FITC-dextran to the tear fluid from the injection site was determined after periorcular injections (1 mm) at high volume (150 μl) and low volume (50 μl). FITC-dextran showed poor permeability to biological membrane because of its high molecular weight and hydrophilicity. Figure 2A shows the drug concentration in the tear fluid at high volume (1 mm, 150 μl). Retrobulbar injection showed a lower AUC (264.5±37.0 min·μM) in the tear fluid that observed in other injections (AUC for intracapsular injection: 626.5±39.4 min·μM, AUC for palpebral conjunctival injection: 670.7±15.3 min·μM).

Figure 2B shows the concentration of FITC-dextran in the tear fluid after periorcular injections (1 mm) at low volume (50 μl) and instillation of 1 μl (1 mm). The AUCs of FITC-dextran in the tear fluid were 104.5±31.7 min·μM for intracapsular injection, 77.1±21.9 min·μM for retrobulbar injection, and 163.2±56.4 min·μM for palpebral conjunctival injection, respectively. Among various volumes of drug instillation (1 mm), 2% (1 μl) of instillation showed almost equal AUC (141.2±51.9 min·μM). The leak of FITC-dextran after periorcular injections was approximately 1.1—2.3% based on the AUC of instillation.

Figure 3 shows the concentration of tilisisol in the tear fluid after periorcular injections (100 mm) at low volume (50 μl) and instillation of 1 μl (100 mm). The AUCs of tilisisol in the tear fluid were 16.3±3.1 min·μM for intracapsular injection, 8.3±3.3 min·μM for retrobulbar injection, and 21.3±7.9 min·μM for palpebral conjunctival injection, respectively. Among various volumes of drug instillation (1 mm), 2% (1 μl) showed almost equal AUC (18.0±6.4 min·μM). The leak of tilisisol after periorcular injections was approximately 0.9—2.4% based on the AUC of instillation.

Figure 4A shows the plasma profiles of tilisisol after instillation and intravenous injection of 50 μl (100 mm). The elimination of tilisisol after intravenous injection showed a bi-exponential curve. Therefore, the kinetic parameters were estimated according to two-compartment model from the profiles. The values of mean±standard error for at least 4 experiments are 0.540±0.084 min⁻¹ for K12, 0.070±0.007 min⁻¹ for K21, 0.258±0.041 min⁻¹ for Kel, 0.617±0.0761 for Fc, 4.304±0.2461 for distribution volume in the peripheral compartment (Vp), and 36.8±2.7 min·μM for AUC, respectively. Figure 4B shows the plasma profiles of tilisisol after various periorcular injections (100 mm, 50 μl). Periorcular injections showed high concentrations of tilisisol in plasma and the times for reaching maximum concentration were within 10 min. The absorption rate constants of tilisisol after periorcular injections were estimated from these profiles using
pharmacokinetic parameters of intravenous injection. The results are summarized in Table 1. In comparison to the absorption rate constant after instillation, periocular injections showed an 8.1-fold increase of the absorption rate constant for intracapsular injection, a 23.4-fold increase for retrobulbar injection and a 9.8-fold increase for palpebral conjunctival injection, respectively.

Figure 5 shows the concentration of tilisolol in the aqueous humor and vitreous body after periocular injections and instillation (100 μm, 50 μl). Tilisolol was undetectable in both areas after intravenous injection. The AUCs of tilisolol in the aqueous humor and vitreous body are listed in Table 2. Among periocular injections, intracapsular injection showed...
the highest AUC of tilisolol in the aqueous humor although the lowest AUC was observed in palpebral conjunctival injection. The ratios of AUC in the vitreous body to AUC in the aqueous humor after periocular injections were 8.5—11.6-fold higher than that observed after instillation.

DISCUSSION

Periocular injections of ophthalmic drugs have been used for delivery of drugs into the inside of the eye. Wine et al. found that the penetration of steroid into the anterior chamber from a subconjunctival depot was much greater when it was injected by a needle penetrating the conjunctiva in the conventional manner than by a needle penetrating the skin of the eyelid and passing underneath the conjunctiva to the injection site. They interpreted this finding as being the result of escape of the drug out of the injection hole in the conjunctiva. Therefore, the presence of drug in the tear fluid after periocular injections was examined.

The leak of FITC-dextran into the tear fluid from the injection site was dependent on injection route and volume. A slight leak of not only FITC-dextran but also tilisolol in the tear fluid suggests that tilisolol cannot penetrate through the periocular tissues. More lipophilic drug may penetrate through these tissues and enhance the drug concentration in the tear fluid. Among the periocular injections, the retrobulbar injection showed the lowest leak into the tear fluid. The leak of drug is important for determining the drug concentration in the eye and can be easily influenced by numerous factors such as tissue tension, size and depth of puncture, and distance from injection site to the tear fluid.

Tilisolol concentrations in plasma, aqueous humor, and vitreous body were examined after injection at low volume. After periocular injections, tilisolol was rapidly absorbed into the systemic circulation and was detected in the aqueous humor and vitreous body. In the aqueous humor, periocular injections showed a lower AUC than instillation. The drug in the tear fluid is mainly absorbed into the aqueous chamber through the cornea. Periocular injections showed comparable AUC of tilisolol in the vitreous body to AUC in the aqueous humor than that observed in the case of instillation. Thus, the periocular injections showed a higher ratio of AUC of tilisolol in the vitreous body to AUC in the aqueous humor than that observed in the case of instillation. The periocular injections are useful to deliver drug to the vitreous body. A portion of injected drug may be absorbed in the area through sclera or via topical circulation. Lipophilic drug might be more useful for delivery to the vitreous body after periocular injection than hydrophilic drug because of its penetrability of membrane.

The results in the present study are consistent with those previously described in in situ absorption experiments. The scleral and conjunctival application of drugs showed rapid and high concentrations in the plasma and a slight concentration in the aqueous humor and vitreous body. Among the periocular injections, the retrobulbar injection showed the highest concentrations in the plasma and the lowest concentrations in the aqueous humor and vitreous body while the intracapsular injection showed the lowest in the plasma and the highest in the aqueous humor and vitreous body. These results suggest that the clearance of drug in the periocular tissues by vasculature reduces the drug concentration in the eye. The rich topical vasculature in the periocular tissues must contribute to rapid absorption of injected drug into the blood circulation and also act as a barrier to drug penetration to the inside of the eye.

Thus, the drug absorption behavior after periocular injection was dependent on the leakage of drug into the tear fluid after periocular injections, which was influenced by the injection volume and injection site. Although the periocular injections showed rapid systemic absorption of drug by vasculature, it might be an effective approach to deliver the drug to the periocular tissues and vitreous body.

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