Development of a Stick-Type Transdermal Eyelid Delivery System of Ketotifen Fumarate for Ophthalmic Diseases

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A transdermal eyelid delivery system for treating ocular diseases (eye-stick) has been developed. Ketotifen fumarate (KT) was used as a model drug. An *in vivo* study using rabbits showed that the eye-stick device maintained a constant conjunctival concentration of the drug for an extended period of time, which was equivalent or higher than the therapeutic level following eye drop administration. Moreover, the conjunctival concentration after eye-stick application was well predicted using the physicochemical parameters, diffusion coefficient and partition coefficient, obtained from *in vitro* hairless mouse skin permeation experiments.

Key words eye-stick; transdermal delivery system; ketotifen fumarate; eyelid; eye drop

Drug molecules applied to the eye normally undergo quick diluting and enzymatic degradation in the tear fluid and the diffusion barrier of the cornea and excretion by aqueous humor flow. It is extremely difficult to deliver drugs especially to the posterior eye tissues. As a result, the bioavailability of ocular drugs is very low.[1] To improve the low bioavailability of ocular drugs, several ophthalmic drug delivery systems have been developed.[2] One of these approaches is transdermal delivery system for the eye,[3] which can maintain a constant blood concentration for a long duration.

In this study, we developed a stick-type transdermal delivery system applied easily on the eyelid skin. To test this system, the skin permeability of ketotifen fumarate (KT) was measured by *in vitro* skin permeation experiments using hairless mouse abdominal skin. *In vivo* rabbit experiment was also carried out and compared with conventional eye drop administration.

Experimental

Materials Female Hr/Kud strain hairless mice (Kyudo Co., Ltd.,) and male JW rabbits (2.0—2.49 kg KITAYAMA LABES Co., Ltd.) were used. KT was purchased from Sigma Chemical Co. Materials used for the base of the stick were yellow beeswax and isopropyl myristate (IPM) (Wako Pure Chemical Industries, Ltd.). Polyoxyethylene oleyl ether (POE) (NOF Corporation) was used as penetration enhancer. Other reagents used in the experiment were of special HPLC grade.

Preparation of Eye-Stick of KT After KT was weighed in a beaker, IPM and POE were added and mixed thoroughly with a stirrer. Yellow beeswax was then added and mixed at 70—80 °C. After the yellow beeswax was completely dissolved, the mixture was poured into the stick-type container (rip tube, 5 ml H68.0 mm×16.0 mm, PINOA) and stored at room temperature. The device was then used as the eye-stick of KT. The weight fraction of KT, IPM, POE, and yellow beeswax in the device was 8%, 45%, 5%, and 42%, respectively.

***In Vivo* Experiment of Eye-Stick Using Rabbits** This experiment was conducted in accordance with the appropriate experimental animal guidelines. On the day before the experiment, the hair around the eye of rabbits was removed by a hair clipper and an electric shaver. Eye-stick was applied 10 times to the lower eyelid of the rabbit (area 3.5—4 cm^2). At 4, 8, and 24 h, rabbits were sacrificed and their conjunctivas were collected in a test tube to measure the *in vivo* drug concentration.

One milliliter of sodium dihydrogen phosphate buffer (10 mM, pH 7.0) was added into the test tube, then the conjunctival samples were finely sectioned. After 4 ml of acetonitrile was added and mixed at 300 rpm for 10 min, the samples were centrifuged at 3000 rpm for 10 min. The supernatant (4 ml) was collected and evaporated to dryness under reduced pressure for 18 h. The residue was dissolved in 300 μl of the mobile phase and collected into the sample tube. Collected sample was centrifuged at 14000 rpm for 5 min and the supernatant was filtered. KT concentrations in the samples were determined by HPLC. There were three samples per sampling time, but those samples were triplicated and measured in view of the detection limit of the method of analysis.

***In Vitro* Experiment for Drug Release** A modified Franz diffusion cell[4] was used for the *in vitro* release experiment of KT from the eye-stick. The receptor compartment was filled with 10 ml phosphate buffer (pH 7.4). The membrane filter (0.45 μm, Millipore) was placed to support the eye-stick. The temperature was controlled at 37 °C, and 200 μl of the receptor solution was sampled at predetermined time points. Thereafter, the same amount of fresh phosphate buffer was added to the receptor cell.

***In Vitro* Skin Permeation Experiment Using Hairless Mice** The receptor compartment of the diffusion cell[4] was filled with 10 ml phosphate buffer (pH 7.4). The excised abdominal skin of hairless mice was used as intact skin or stripped skin, from which the stratum corneum was completely removed by tape stripping 20 times consecutively. The eye-stick of KT was then applied 10 times to the skin with a force of 2.5±0.5 N. After that, the skin was mounted on the diffusion cell quickly. The temperature was controlled at 37 °C. Two hundred microliters of the receptor solution was sampled at predetermined time points. Thereafter, the same amount of fresh phosphate buffer was added to the receptor cell. This experiment was also conducted in accordance with the appropriate experimental animal guidelines.

HPLC Assay of KT The concentration of KT in the *in vivo* and *in vitro* experiments was determined under the following HPLC (Shimadzu Corporation) conditions. The assay system comprised a liquid chromatograph (LC-10AS), column oven (CTO-10A), UV–VIS detector (SPD-10A), system controller (SCL-10A), and auto injector (SIL-10A). The column was Capcell pak C18 MG S5 μm 4.5×250 mm (Shiseido) and its temperature was 40 °C. The measuring wavelength was 300 nm. The mobile phase was a mixture of 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 9) : acetonitrile = 30 : 70.

Simulation of Conjunctival Concentration after Eye-Stick Application Device Design Parameters: The device design parameter was calculated on the basis of drug release tests from the eye-stick. The cumulative amount of KT released per unit area was plotted against square root of time. The steady-state rate of release was obtained from the slope. The diffusion coefficient (D) of KT in the eye-stick was calculated using Eq. 1,[5]

$$Q = 2C_0\sqrt{D \cdot t / \pi} \quad (1)$$

where Q is the cumulative amount of drug released, C_0 is the initial drug concentration in the device, and t is time.

Skin Permeation Parameters: Skin permeation data were analyzed by bilayer diffusion/partition model.[6] The cumulative amount of KT permeated per unit area was plotted against time. Lag time (t_d) was determined as the intercept between the linear portion of each curve and the time axis. The steady-state rate of penetration (dQ/dt) was then calculated from the slope. The diffusion coefficient in the stratum corneum (D_{SC}) or in viable skin (D_{VS}), the stratum corneum/viable skin partition coefficient (K_{SC}/K_{VS}), and the skin surface concentration (C_S) were calculated from dQ/dt and t_d.[6,7]

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Table 1. Tear Fluid Pharmacokinetic Parameters in Rabbits

Volume of distribution [ml]	8.00×10^{-3}
Elimination rate constant [1/s]	3.85×10^{-3}

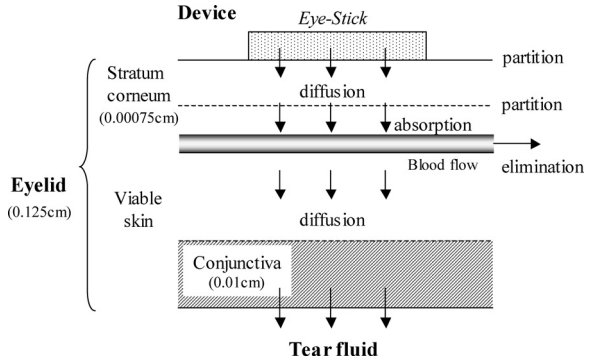

Fig. 1. Eyelid Skin-Tear Fluid Kinetics Model (Rabbit)

Table 2. Thickness of Hairless Mouse Skin and Rabbit Eyelid

	Haireless mouse skin	Rabbit eyelid
Thickness of stratum corneum [cm]	0.0010	0.00075
Thickness of whole skin/eyelid [cm]	0.0370	0.125

Pharmacokinetic Parameters in Tear Fluid of Rabbits: Pharmacokinetic data in tear fluid were analyzed using one-compartment model. The distribution volume and elimination rate constant of the tear fluid of rabbits were calculated from values reported in the literature[8] (Table 1).

Simulation of Conjunctival Concentration of Rabbits: The conjunctival concentration of KT after application of eye-stick was simulated using commercially available simulation software (SKIN-CAD®).[9] In this simulation, an eyelid skin-tear fluid kinetics model (Fig. 1) was used. This model consists of the device diffusion model, the skin penetration model (diffusion/partitioning model), and the tear fluid distribution/elimination model (one-compartment model). We assumed that the conjunctiva corresponded to the layer of 100 μm from the bottom of the viable skin. The device design parameters, skin permeation parameters, and tear fluid pharmacokinetic parameters were then used in SKIN-CAD®. The thickness of the hairless mouse skin was used the value cited in the literature.[7] The thickness of the whole eyelid and the stratum corneum of the rabbit eyelid skin were measured to be 0.125 and 0.00075 cm, respectively (Table 2).

Results and Discussion

***In Vivo* Conjunctival Concentration of Rabbits** Figure 2 compares the KT conjunctival concentration in rabbit after eye-stick application *versus* eye drop administration.[10] For eye drop, the drug concentration reached the maximum value of 2.68 μg/g at 15 min and quickly decreased over $\leq$2 h then gradually decreased. On the other hand, the eye-stick had a lag time of about 4 h after application and a constant concentration level of drug was maintained by the end of experiment. Furthermore, the concentration was approximately equal to that at 30 min after eye drop administration. The lag time of about 4 h appearing in the first application of the eye-stick may not occur with multiple applications of the stick.

The minimum effective concentration of KT may be estimated >0.028 μg/g (the value at 6 h after eye drop administration) because commercial eye drops are normally administered four times per day. The application dose of the eye-stick (16 mg as KT) was much higher than that of the eye drop (0.0345 mg as KT). However, the application dose of the stick can be greatly reduced because a constant conjunctival concentration after eye-stick application is >10 fold the minimum effective concentration. This may justify the efficacy of the eye-stick as a the transdermal therapeutic system.

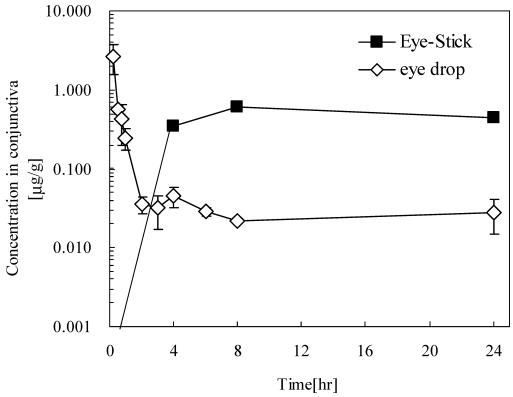

Fig. 2. Conjunctival Concentration of KT after Eye-Stick Administration or Eye Drop Administration in Rabbits

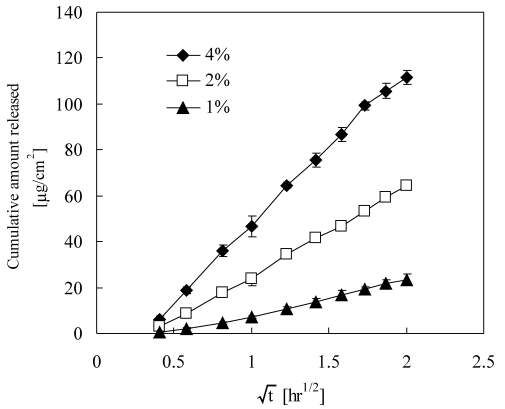

Fig. 3. Release Profiles of KT from Eye-Stick with Different Drug Loading (Mean±S.D., n=3)

In a precise sense, the experimental release data do not agree with Higuchi's equation; the time lag occurs in the Q *vs.* $\sqrt{t}$ profile. This phenomenon was observed in the presence of the diffusion boundary layer. The intrinsic release rates evaluated from the method in the literature [14] agree approximately with the apparent release rate.

Table 3. Diffusion Coefficient of KT in Eye-Stick

Drug concentration [%(w/w)]	Release flux [μg/cm^2/h$^{1/2}$]	Diffusion coefficient [$\times10^{-10}$ cm^2/s]
1	14.87±1.23	4.83
2	38.76±1.01	8.19
4	67.24±3.67	6.16

Release flux represents the mean±S.D. (n=3). Diffusion coefficient was analyzed using Eq. 1.

***In Vitro* Parameters** The release profiles of KT from the eye-stick and the diffusion coefficient evaluated are shown in Fig. 3 and Table 3, respectively. The weight fraction of IPM and POE in the device for this study was 45% and 5%, respectively. The concentration of KT was 1, 2, or 4% and the total volume of the device was balanced by the amount of yellow beeswax. The diffusion coefficient in the device was calculated 6.39×10^{-10} cm^2/s, which was the average value of release data obtained from the eye-stick with different concentrations of KT. The skin permeation profile *in vitro* and the permeation parameters determined are summarized in

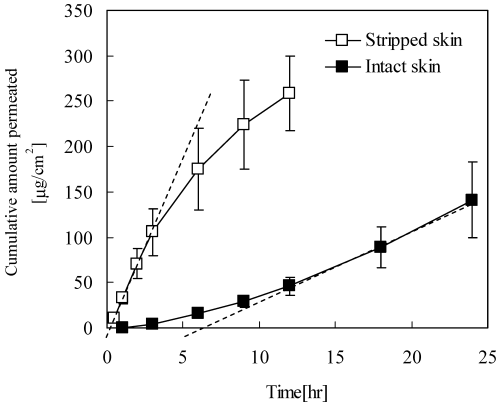

Fig. 4. Permeation Profile of KT across Hairless Mouse Skin for Eye-Stick Containing 8% KT, 45% IPM, 5% POE, and 42% Yellow Beeswax (Mean±S.D. $n=3$)

Table 4. Skin Permeation Parameters of KT

	Intact skin	Stripped skin
dQ/dt [μg/cm^2/h]	7.93±2.60	37.91±10.15
t_d [h]	6.29±0.71	0.16±0.09
D_{SC} [cm^2/s]	1.12×10^{-11}	
D_{VS} [cm^2/s]	3.75×10^{-7}	
K_{SC}/K_{VS} [—]	2.47×10^{2}	
C_S [μg/ml]	2.49×10^{5}	

Each value represents the mean±S.D. ($n=3$), Skin thickness: stratum corneum=0.0010 [cm], whole skin=0.0370 [cm].

Fig. 4 and Table 4, respectively.

Simulation of Rabbit Conjunctival Concentration The conjunctival concentration of KT in rabbits was simulated by a skin permeation pharmacokinetic model, SKIN-CAD®, on the basis of the diffusion coefficient in the device, skin permeation parameters (diffusion coefficient and partition coefficient in the skin), and tear fluid pharmacokinetic parameters reported in the literature. At first, the effect of the absorption rate in the capillary layer on the conjunctival concentration was simulated as shown in Fig. 5. In normal skin, 97—98% of the drug penetrated through the stratum corneum is taken into the microcirculation located beneath the basal layer of the epidermis.[11] As seen from this figure, the simulated profile well agreed with the *in vivo* data for the value of the blood absorption rate constant K_b (0.07 s^{-1}) at which almost 99% of the drug is absorbed into the microcirculation. In this simulation, we assumed that the blood vessels in the eyelids were located at the dermal layer; the distance to the blood vessels was about 200 μm[11] from the surface of the skin. Under *in vivo* conditions, however, blood vessels in the eyelids are also located at the orbicularis oculi muscle and the conjunctival layer.[12,13] Drug penetrating through the eyelid skin may be absorbed into each blood vessel with a different absorption rate. In the *in vivo* experiment, eye-stick was applied ten times to the lower eyelid of rabbits. In clinical use, however, less application frequency is preferable. The effects of the application frequency of eye-stick on the conjunctival concentration are shown in Fig. 6, where we assume a device thickness of 0.05 cm, which corresponds to ten times of application. The device thickness of 0.005 cm therefore corresponds to one application by eye-stick device.

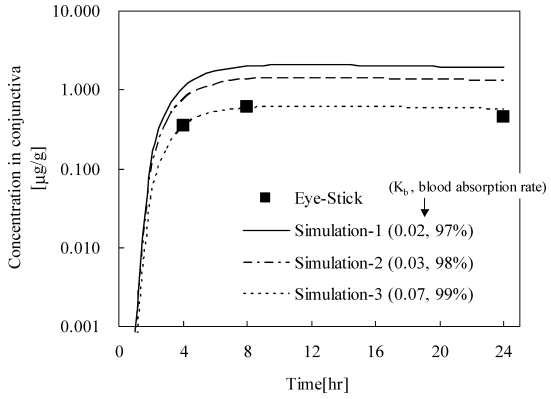

Fig. 5. Comparison of KT Conjunctival Concentration of Calculated and *in Vivo* Experimental Data

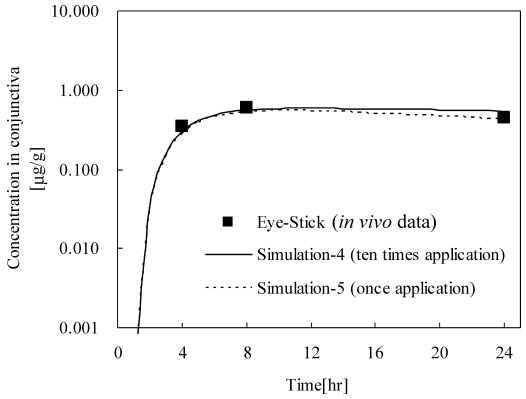

Fig. 6. Effect of Application Frequency of Eye-Stick for Conjunctival Concentration of KT

We found little difference between ten-time application and one application.

Conclusion

The conjunctival concentration in rabbits following eye-stick application is higher than that after conventional eye drop administration. Although eye drops need to be administered four times per day to maintain effective concentrations of ketotifen, eye-stick can provide a constant conjunctival concentration for an extended duration by once-daily application. The conjunctival concentration of KT in rabbits following eye-stick application could be predicted using parameters obtained from *in vitro* skin permeation experiments and the drug release experiments. When the absorption rate into the blood is 99%, the simulated profiles well agreed with the *in vivo* data. The predicted profiles indicate that application frequency have little influence on the conjunctival concentration. The eye-stick can provide a constant concentration with low application frequency, even once a day, and the present transdermal therapeutic system may be clinically effective as a new treatment device for ocular diseases.

References

1) Davies N. M., *Clin. Exp. Pharmacol. Physiol.*, **27**, 558—562 (2000).
2) Bartlett J. D., "Clinical Ocular Pharmacology," Butterworth Heinemann, Boston, 2001, pp. 47—71.
3) Isowaki A., Ohtori A., Matsuo Y., Tojo K., *Biol. Pharm. Bull.*, **26**, 69—72 (2003).
4) Tojo K., "Design and Calibration of In Vitro Permeation Apparatus in

Transdermal Controlled Systematic Medications," ed. by Chien Y. W., Marcel Dekker, New York, 1987, pp. 127—158.
5) Paul D. R., McSpadden S. K., *J. Membr. Sci.*, **1**, 33—48 (1976).
6) Martin A., "Physical Pharmacy," 4th ed., Lea & Febiger, Philadelphia, 1993, pp. 538—545.
7) Tojo K., Chiang C. C., Chien Y. W., *J. Pharm. Sci.*, **76**, 123—126 (1987).
8) Maurice D. M., Mishima S., "Pharmacology of the Eye," ed. by Sears M. L., Springer Verlag, New York, 1984, pp. 19—116.
9) Mori D., Tojo K., *Pharm. Tech. Jpn.*, **17**, 771—778 (2001).
10) Ohta S., Yanagida Y., Higaki Y., Uda H., Hujino A., *Rinshoiyaku*, **4**, 2183—2191 (1988) in Japanese.
11) Tojo K., "Mathematical Models of Transdermal and Topical Drug Delivery," 2nd ed., Biocom Systems, Fukuoka, 2005, pp. 26—27.
12) Ishihara S., "Nihongankazensyo," (in Japanese), Vol. 14, Japan Medical Publishers, Tokyo, 1953, pp. 48—69.
13) Callahan M., Beard C., "Gankenkasui," (in Japanese), Medical-Aoi Publications, Tokyo, 1998, pp. 15—33.
14) Tojo K., *J. Pharm. Sci.*, **74**, 685—687 (1985).