Eyelid skin as a potential site for drug delivery to conjunctiva and ocular tissues

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ABSTRACT

The feasibility of topical application onto the (lower) eyelid skin to deliver hydrophilic and lipophilic compounds into the conjunctiva and ocular tissues was evaluated by comparing with conventional eye drop application. Skin permeation and the concentration of several model compounds, and skin impedance were determined utilizing eyelid skin from hairless rats, as well as abdominal skin in the same animals for comparison. *In vitro* static diffusion cells were used to assess the skin permeation in order to provide key insights into the relationship between the skin sites and drugs. The obtained results revealed that drug permeation through the eyelid skin was much higher than that through abdominal skin regardless of the drug lipophilicity. Specifically, diclofenac sodium salt and tranilast exhibited approximately 6-fold and 11-fold higher permeability coefficients, respectively, through eyelid skin compared with abdominal skin. Histomorphological evaluation and *in vivo* distribution of model fluorescent dyes were also examined in the conjunctiva and skin after eyelid administration by conventional microscope and confocal laser scanning microscope analyses. The result revealed that eyelid skin has a thinner stratum corneum, thereby showing lower impedance, which could be the reason for the higher drug permeation through eyelid skin. Comparative evaluation of lipophilic and hydrophilic model compounds administered via the eyelid skin over 8 h revealed stronger fluorescence intensity in the skin and surrounding tissues compared with eye drop administration. These results suggested that the (lower) eyelid skin is valuable as a prospective site for ophthalmic medicines.

Chemical compounds used in this article:

Aminopyrine (PubChem CID: 6009); Antipyrine (PubChem CID: 2206); Diclofenac sodium (PubChem CID: 5018304); Fluorescein sodium (PubChem CID: 9885981); Lidocaine

(PubChem CID: 3676); Pilocarpine hydrochloride (PubChem CID: 5909); Rhodamine B (PubChem CID: 6694); Tranilast (PubChem CID: 5282230)

Keywords:

ophthalmic transdermal drug delivery

eyelid skin

conjunctiva

skin permeation

eye drop

1. INTRODUCTION

For several decades, eye drops have remained one of the paramount and most extensively utilized pharmaceutical formulations for various ocular diseases (i.e., the eyeball and surrounding tissues) (Baranowski et al., 2014). Currently, eye drops account for about 90% of ophthalmic medicines, primarily due their to ease of administration and good patient compliance. However, several drawbacks are associated with the utilization of eye drops. Eye medications cannot be administered beyond the capacity of the conjunctival sac due to its limited volume. Most eye drops exhibit low bioavailability, poor targeting efficacy, and are virtually impossible to administer during sleep. Anatomical and physiological constrains such as tear turnover, nasolacrimal drainage, reflex blinking, and ocular static and dynamic barriers impede the bioavailability and controlled delivery of drugs administered as eye drops (Kimura et al., 2007; Gaudana et al., 2010; Isowaki et al., 2003; Gause et al., 2015). With these impediments, it is essential to search for an alternative approach to deliver ophthalmic drugs with high targeting ability while simultaneously improving drug absorption into the ocular tissues.

We paid attention to applying and delivering ophthalmic drugs onto the (lower) eyelid skin using various formulations. The lower eyelid skin exhibits less movement due to blinks compared with the upper eyelid skin, making it a good site for administration. Moreover, it is very interesting for skin researchers to explore the eyelids, which is the thinnest skin layer on the human body (< 1 mm) (Amirlak and Sahshabi, 2015). Generally, the thinner the stratum corneum, the greater the drug permeation. In addition, drug administration through the skin provides several advantages because it generally facilitates avoidance of premature metabolism, decreased toxicity, fewer side effects as well as greater patient compliance (Paudel et al., 2010).

A unique feature of the eyelids from the drug delivery standpoint is their proximity to the conjunctiva, which is between 2 and 30 times more permeable to drugs than the cornea (Davies, 2000). It should be noted that the conjunctiva has direct contact with the eyeball and its surrounding ocular tissues. With the presence of drugs in the conjunctiva, it is expected to distribute into the anterior and posterior ocular regions, which is the delivery target area in most ocular diseases. In addition, transdermal drug delivery could maintain a constant drug concentration in the dermal layers beneath the application site for a longer duration. This is the most beneficial point compared with eye drops application, which has many drawbacks, as described above. If drugs could be delivered to the conjunctival tissue via the eyelid skin, these drawbacks associated with the eye drops could be addressed. Moreover, once medications are prepared for eyelid administration, greater convenience is obtained for patients with ocular diseases (i.e. allergic conjunctivitis, eye infections, glaucoma) and health care professionals alike. Time for drug administration by patients or health care professionals is reduced while treatment may continue even when the patient is asleep, thereby increasing drug retention time and consequently improving patient comfort and quality of life.

With the limited number of studies investigating the delivery of drugs into the conjunctiva through eyelid skin and the paucity of data describing eyelid drug permeation, we were prompted to study hydrophilic and lipophilic model compounds and to compare permeability characteristics through the eyelid and the abdominal skin. In the present study, we selected hairless rats as a model animal, because it is more practical for conducting *in vivo* and *in vitro* experiments compared with other larger animal models. We believed that this is the first study on ophthalmic drug delivery utilizing rat eyelid skin. Pilocarpine hydrochloride, tranilast, antipyrine, diclofenac sodium, aminopyrine, and lidocaine were used to evaluate their skin permeation; the former two were used to evaluate the skin concentration, and two

fluorescent dyes (fluorescein sodium and rhodamine B) were used to determine the *in vivo* distribution of the drugs in the conjunctiva.

2. MATERIALS AND METHODS

2.1 Materials and Experimental Animals

Pilocarpine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lidocaine and tranilast were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Aminopyrine, antipyrine, diclofenac sodium, fluorescein sodium, and rhodamine B were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Table 1 lists the chemicals used as model permeants and their physicochemical properties.

Male hairless rats (WBM/ILA-Ht, 8 weeks of age, body weight of 220–260 g) were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratories (Fukaya, Saitama, Japan). All animal feeding and experiments were approved by Institutional Animal Care and Use Committee of Josai University.

2.2 Preparation of Skin Membranes

Whole abdominal and (lower) eyelid skin were freshly excised from hairless rats previously shaved while anesthetized using three types of anesthesia (0.375 mg/kg medetomidine, 2.5 mg/kg butorphanol, 2 mg/kg midazolam) administered intraperitoneally. The excised skin was cleaned with pH 7.4 phosphate-buffered saline (PBS).

2.3 Skin Permeation Experiments

Skin permeation experiments were carried out using Franz type vertical diffusion cells. Excess subcutaneous fat was trimmed off from the excised intact abdominal skin and no trimming was done for eyelid skin. The eyelid skin was placed on a stabilizing apparatus with an effective diffusion area of 0.0707 cm^2 before it was set in a vertical diffusion cell. The

abdominal skin sample was directly set in a vertical type diffusion cell with an effective diffusion area of 1.77 cm^2. One mL of PBS, which corresponded to the donor solution and contained no permeant, was applied to the epidermis side and 6.0 mL of PBS was applied to the dermis side of the skin to reach an equilibration state for about 1 hour. After an hour, the PBS of the epidermis side was replaced with the same volume of the donor solution (model compounds dissolved in PBS) to commence the permeation experiment. The receiver solution was stirred with a stirrer bar on a magnetic stirrer and maintained at 32°C using a thermostatically controlled heater throughout the experiments. An aliquot (500 μL) was withdrawn from the receiver chamber and the same volume of fresh PBS was added to the chamber to keep the volume constant. The penetrant concentration in the receiver chamber was determined by HPLC.

2.4 Determination of Skin Concentrations

The concentrations of the compounds in intact rat skin were measured at 8 h after the start of the experiments, which were performed separately from the experiments mentioned above. The donor solution was removed, the stratum corneum side was rinsed three times with 1.0 mL of PBS, and the compound-applied area was cut out. When the concentrations in viable epidermis and dermis were measured, the stratum corneum was removed by tape-stripping 20 times before clipping out the application area. The piece of skin was reduced in size using scissors, and 0.5 mL of PBS was added prior to homogenization at 12,000 rpm and 4°C for 5 min using a homogenizer (Polytron PT 1200 E, Kinematica AG, Littaue-Lucerne, Switzerland). For deproteinization, 0.5 mL of 16% trichloroacetic acid in PBS was added to the skin homogenate, followed by agitation at 32°C for 15 min, and then the mixture was centrifuged at 15,000 rpm and 4°C for 5 min. The compound concentration in the resulting supernatant was determined by high-performance liquid chromatography (HPLC).

2.5 HPLC Analysis

Samples were mixed with the same volume of acetonitrile containing the internal standard and centrifuged at 15,000 rpm and 4°C for 5 min. The obtained supernatant (20 μL) was injected into an HPLC system. The HPLC system (Shimadzu Co., Kyoto, Japan) consisted of a system controller (SCL-10A), pump (LC-20AD), degasser (DGU-20A$_3$), auto-injector (SIL-20A), column oven (CTO-20A), UV detector (SPD-20A) and analysis software (LC Solution). The column used was Inertsil® ODS-3 4.6 mm × 150 mm, 5 μm (GL Sciences Inc., Tokyo, Japan). The column was maintained at 40°C and the flow rate of the mobile phase was adjusted to 1.0 mL/min. Refer to Table 2 for the details of the HPLC conditions.

2.6 Data Analysis

The cumulative amount of model drugs permeated through the eyelid and the abdominal skin was calculated and expressed as the mean ± S.E. The permeation rate or flux (J, μg/cm^2/h), 6–8 h after starting the experiment, was determined based on the slope of linear regression of the cumulative amount of the model drugs against permeation time.

2.7 *In Vivo* Application Study

In this study, three hairless rats were used. The left eye was designated for the model compound to be delivered through a tube simulating transdermal drug delivery, whereas the right eye was designated for the compound to be administered using drops simulating an eye drop. The rats were anesthetized with three types of anesthesia (0.375 mg/kg medetomidine, 2.5 mg/kg butorphanol, 2 mg/kg midazolam) administered intraperitoneally for the duration of the sampling period. A Teflon tube (internal diameter 3 mm) was glued onto the left eyelid skin using cyanoacrylate bond (Aron Alpha, Konishi Co. Ltd., Osaka, Japan), but nothing was attached on the other side (Fig. 1). Ten microliters of the model compound (0.5 mM rhodamine B or 0.5 mM fluorescein sodium in PBS) was administered to each eye of the rat and rats were individually euthanized with pentobarbital sodium (100 mg/kg) intraperitoneally at each time point (10 min, 2 h, 8 h). After which, the (lower) eyelid skin

was excised from rats by making an incision in the skin of the cranium, peeled away towards the eyelids. At the palpebra, the skin was cut along the palpebral edge. The skin-free palpebra was then removed by cutting along the orbital arc (Maistrello et al., 1973). Thereafter, the excised rat lower eyelid skin was washed three times with 1.0 mL PBS on each side of the dermis and epidermis and kept at -30°C until further processing (skin sectioning).

2.8 Measurement of Skin Impedance

Excised hairless rat abdominal skin or eyelid skin (either for full-thickness or stripped skin) was placed between Franz diffusion cells. PBS was added to the stratum corneum and dermis sides. Skin impedance was determined using an impedance meter (10 Hz AC, Asahi Technolab, Tokyo, Japan).

2.9 Histomorphological Observation of Under Eyelid and Abdominal Skin

The skin samples, from hairless rat eyelid skin and abdominal skin, were cut vertically using a razor blade, embedded in supercryoembedding medium, and were allowed to freeze using isopentane, and cooled with dry ice. Skin slices (10 µm thickness) were made using a cryostat (CM3050, LEICA, Wetzler, Hessen, Germany).

Hematoxylin and Eosin Staining. The obtained skin sections were stained with hematoxylin and eosin (HE). The prepared specimens were observed for their morphology using an optical microscope (DMWBI-223 Digital Biological Microscope, Shimadzu Corporation).

Nile Red Staining. Cryosectioned skin samples were treated with Nile red in acetone solution (500 mg/mL) with 4% potassium hydroxide solution (Sheu et al., 2003). After preparing the specimen, it was observed using a confocal laser scanning microscope (CLSM) (Fluoview FV1000 and software: FV10-ASW, Olympus, Tokyo, Japan) for the observation of neutral fat in the stratum corneum. The CLSM conditions were as follows: wavelength,

473 nm; scan speed, 200μ/pix; laser power, 1%; high voltage, 236 V; gain, 1.125×; and offset, 0.

3.0 Statistical Analysis

All experimental data were tested for statistical significance ($p = 0.05$) using Student's *t*-test.

3. RESULTS

3.1 *In Vitro* Skin Permeation Parameters

In vitro skin permeation experiments were conducted, and the time courses of cumulative amount of drug permeation were determined in order to compare the permeation of six model compounds (antipyrine, diclofenac sodium, pilocarpine hydrochloride, aminopyrine, tranilast, and lidocaine) with different lipophilicities (log $K_{o/w}$) (Table 1) through eyelid and abdominal skin. Figure 2 shows the results. Typical lag time and following steady state permeation were found to be independent of the drug and skin site. In addition, drug permeation through the eyelid skin was higher than through the abdominal skin independent of the lipophilicity of the applied drugs. Table 3 summarizes the permeation fluxes and permeability coefficients that were calculated from the skin permeation profiles. These permeation parameters were much higher for all model compounds administered via the eyelid skin compared with the abdominal skin. In addition, significant differences in the fluxes and permeability coefficients were observed for all model compounds when comparing eyelid and abdominal skin. Furthermore, 11-fold higher tranilast permeation was observed in the eyelid skin than in the abdominal skin.

3.2 Drug Concentration in Skin

Among the model compounds, drug concentrations in skin were evaluated with tranilast and pilocarpine hydrochloride, which are important ophthalmic medications for

patients with allergic disease and glaucoma, respectively. Figure 3 shows the result of drug concentrations in the whole skin and viable epidermis and dermis (VED) skin after topical application of these drugs on the eyelid and abdominal skins. Drug concentrations of tranilast and pilocarpine in the whole and VED skins in the eyelid skin were higher than those in the abdominal skin. Significant differences in pilocarpine were observed in the whole and VED skins between the eyelid and abdominal skins, whereas for tranilast concentration, the VED showed a significant difference. An approximately 3.7-fold higher pilocarpine concentration was observed in whole skin and VED, whereas approximately 1.4- and 2.8-fold higher tranilast concentrations were confirmed in whole and VED skins, respectively.

3.3 Histomorphological Evaluation of Under Eyelid and Abdominal Skin

With interesting results showing high *in vitro* permeation profiles of hydrophilic and lipophilic drugs through the eyelid skin, further evaluation was conducted with impedance, morphology, and neutral fat content/distribution of the eyelid and abdominal skins in hairless rats. Skin impedances of the intact eyelid and abdominal skins were much higher than those of stratum corneum-stripped skins. Intact abdominal skin had impedance values of 0.94 ± 0.20, 0.88 ± 0.19, 0.85 ± 0.16, 0.85 ± 0.15 at 0 min, 15 mins, 30 mins, and 1 h, respectively, from the start of the experiment. On the other hand, intact eyelid skin has impedance values of 0.49 ± 0.18, 0.47 ± 0.17, 0.45 ± 0.16, 0.39 ± 0.17 at 0 min, 15 mins, 30 mins, and 1 h, respectively. The values of skin impedance in the intact skins decreased at the beginning of the application period, and then the values became constant about 1 h thereafter. Furthermore, the abdominal skin had approximately 2-fold higher impedance over 8 h compared with the eyelid skin.

HE staining revealed that the stratum corneum in the abdominal skin was denser compared with the eyelid skin (Fig. 4). Moreover, Nile red staining images of the stratum corneum (the area surrounded by a white line in Fig. 5) indicated that neutral fat in the

stratum corneum of the eyelid skin was evidently thinner and less compact compared with the abdominal skin.

3.4 *In Vivo* Eyelid Percutaneous Absorption Studies

With the concept of administering drugs to the conjunctiva through the eyelid skin, *in vivo* absorption studies were conducted with rhodamine B and fluorescein sodium salt as a lipophilic and a hydrophilic model dye compound, respectively. Figure 6 shows the drug distribution in skin after application of its solution in eye drops or through the (lower) eyelid skin. The presence of rhodamine B and fluorescein sodium administered as eye drops was markedly observed in the conjunctiva, as indicated by the fluorescence in the images 10 min after administration, whereas these were absent at 2 and 8 h. For topical administration onto the eyelid skin, a stronger intensity of the fluorescence given off by the model compounds was observed over time, which indicated greater amounts of the dye compound in the conjunctiva.

4. DISCUSSION

The delivery of ophthalmic medications still remains a challenge. Bioavailability for eye drops is poor, amounting to at best 10% of the applied dose, because the eye is protected by a series of complex defense mechanisms that hinder the achievement of an effective drug concentration within the target tissue of the eye. Frequent instillations of eye drops are often required to achieve the expected therapeutic benefit, which is inconvenient and often associated with adverse effects. (Li *et al*., 2008; Gan *et al*., 2010; Wang *et al*., 2011; Kumar and Sinha, 2016; Akhter et al., 2016). On the other hand, transdermal drug delivery systems offers greater patient comfort while allowing a constant supply of drug at the application site and thus provide superior pharmacokinetic profiles. A few studies have reported the usefulness of drug permeation through the eyelid skin (Kimura et al., 2007, Isowaki et al.,

2003) and evaluated drug concentrations in the conjunctiva in *in vivo* experiments. However, no reports have been published on the influence of drug lipophilicity on (lower) eyelid skin permeation and its comparison between eyelid and abdominal skin permeation. Thus, in the present study, drug delivery within the ocular region through the eyelid skin was investigated with several drugs that have a wide range of lipophilicity.

In order to demonstrate the proposed idea of administering medications through the eyelid skin, *in vitro* skin permeation and drug concentration in the eyelid VED skin were evaluated. Considering that sufficient human skin for a large number of skin permeation studies was usually unavailable because of ethical issues, hairless rat skin was used in this study. Moreover, hairless rats are easy to handle and manipulate (i.e. docile behavior), hence this was the most feasible animal to test the concept of delivering drugs to the conjunctiva via the eyelid skin. Several reports have been published that hairless rat skin is a good alternative for human skin membrane to evaluate skin permeation profiles and permeability coefficients of drugs from aqueous solutions (Watanabe et al., 2011; Morimoto et al., 1992; Abd et al., 2016; and Jung et al., 2014). Skin permeation profiles of several model drugs through the excised rat skin were measured to determine their fluxes and permeability coefficients in this study. Among various skin regions of the hairless rat, abdominal skin has often been used to evaluate skin permeation of drugs. Thus, skin permeation of model compounds was compared with hairless rat eyelid and abdominal skins to elucidate features in the skin permeation of drugs through (lower) eyelid skin.

Because topically applied drugs are mostly absorbed into the systemic circulation through the cutaneous vessels, prolonged retention of the drugs might be difficult below the application site. In normal (healthy) skin, 97–98% of drugs that penetrate through the stratum corneum are taken into the microcirculation located beneath the basal layer of the epidermis (Kimura et al., 2007). However, the possibility of direct transport of drugs to the conjunctiva

after topical skin application should be evaluated using *in vitro* permeation experiments, because skin permeation and concentration could be calculated with Fick's second law of diffusion model (Sugibayashi et al., 2010).

The ratio or fraction of topically applied drugs that penetrate into the systemic circulation or the deeper cutaneous or subcutaneous tissue using an *in situ* experimental model in hairless rats were determined in our previous study (Sugibayashi et al., 1999; Hasegawa et al., 2007). Most of the drug fraction that penetrated the skin after topical application was taken up by the cutaneous blood flow, whereas some directly migrated into deeper tissues such as the subcutis and muscle. In our previous study, topical application resulted in high drug concentrations in the subcutis and surrounding tissues. Thus, high drug concentrations in the conjunctiva can be obtained by topical application through the eyelid skin.

Percutaneous absorption of drug molecules is of particular importance in the case of transdermal drug delivery systems, and they should be able to cross the permeability barrier of the skin, namely the stratum corneum. Moreover, the lipids of the stratum corneum are important regulators against skin permeability of topically applied drugs (Feingold, 2009; Pappas, 2009). Morphological differences in the various anatomic sites of skin exist. Ya-Xian Z et al. (1999) reported that variations in the number of cell layers of the stratum corneum was observed according to the anatomical location of human skin. The number of stratum corneum layers in the eyelid was 8 ± 2 (n=16, mean ± SD), but the abdomen had 14 ± 4 (n=44, mean ± SD), and a significantly higher transepidermal water loss (TEWL) value was observed in the eyelid compared with the abdominal skin. In addition, Pratchyapruit et al. (2007) reported that eyelid showed a high TEWL value similar to other facial skin. The skin surface hydration was as high as that of adjacent skin regions even though the eyelid skin has extremely low amounts of surface lipids, unlike its neighboring skin regions. The present

results obtained from skin impedance and HE staining also corresponded with these reports. Moreover, based on our experimental results, the stratum corneum of eyelid skin had a lower neutral fat content. These results supported our findings, in which a significant difference was observed between the permeability coefficient of the model compounds through the eyelid skin compared with the abdominal skin. In terms of the relationship between molecular weight and log*P*, a strong positive relationship exists. Many reports have been published on the relationship of *MW* and log $K_{o/w}$ on skin permeation through abdominal skin (Morimoto et al., 1992; Potts and Guy, 1992; Chen et al., 2013). Although there is a regional difference in the skin permeation of drugs, skin permeation of drugs through eyelid skin would be expressed with a function of MW and log $K_{o/w}$. In the present experiment, only several drugs were used to compare the skin permeation differences between abdominal and lower eyelid skins. Thus, it was difficult to explain the effect of these parameters on the skin permeation of drugs through eyelid skin.

To further confirm drug delivery via the eyelid skin into the conjunctiva, *in vivo* experiments were performed with the fluorescent markers rhodamine B and fluorescein sodium salt. The disposition of these model compounds in the conjunctiva increased with the passage of time. The intensity of fluorescence at 8 h after administration via the eyelid skin was markedly higher than that of eye drop administration. There was no fluorescence observed at 2 h after eye drop administration, indicating the absence or very low distribution of the model compounds in the conjunctiva. The present results correspond with a study utilizing conventional eye drops, which resulted to lower bioavailability (Lux et al., 2003). Rapid and extensive loss caused by drainage and high tear fluid turnover of the instilled eye drops may be the main reason for the disappearance of the fluorescence within 2 h after administration. Moreover, the contact time of the model compound with ocular tissues was relatively short because of the constant production of lacrimal fluid. In contrast, transdermal

drug delivery through eyelid offers a steady concentration of the model compound for an extended period of time. This further supports the findings of our *in vivo* absorption studies wherein fluorescence were evident even after 8 h while being unnoticeable for eye drops. We have confirmed the migration of the model drugs from the eyelid skin into the conjunctiva and eyeball. Although drug concentration and its distribution profile into the eyeball is not reported in this study, a preliminary study revealed that administration of model compounds via the eyelid skin were detected in eyeball homogenates, suggesting the possibility of drug delivery into the conjunctiva and eyeball. The mechanism of drug migration from eyelid skin to the eyeball has not yet been determined. Currently, we assume that drug partitioning from eyelid skin to lacrimal fluid was the primal route for drug migration into the eyeball.

There is a need to address the bioavailability and the delivery of drugs applied on the eyelid skin and as eye drops. The bioavailability resulting from eye drop administration is presumed to be very low. In this study, the permeability coefficient of drugs applied onto eyelid skin was much higher than that of abdominal skin. The obtained permeability coefficients from the lowest ($3.66 \pm 1.25 \times 10^{-7}$ cm/s for tranilast) to the highest ($151 \pm 11.8 \times 10^{-7}$ cm/s for lidocaine) (Table 3) are similar or higher than those drugs that are already marketed as transdermal delivery systems. The targeting ability of eye drops to the conjunctiva is high because of its direct contact with the conjunctiva. However, its elimination is high, and at the same time the drug concentration in the conjunctiva may not be sustained (see Fig. 6). Despite lacking direct contact with the conjunctiva, eyelid administration as a delivery method exhibited an advantage of having controlled drug delivery, as shown in Fig. 6.

The eyelid skin is known to be thinner than the conventional skin administration sites, and it offers higher drug permeation, as demonstrated by the results of this study. Developing formulations dedicated to eyelid administration such as an eyelid patch is recommended. In

fact, ointments are often used for night time application but are associated with discomfort due to greasiness, tear film instability, and uncertain drug disposition after eye shutting while asleep (Desai and Lee, 2007). Thus, eyelid skin application is seen to be beneficial even during sleep when the use of liquid and semi-solid eye preparations is deemed impossible.

5. CONCLUSION

The drug concentration in rat conjunctiva after application on the eyelid skin was higher than that of conventional eye drops. In addition, drug permeation through eyelid skin was higher than that through abdominal skin regardless of lipophilicity. Diclofenac sodium salt and tranilast significantly showed higher permeation coefficients than abdominal skin of about 6-fold and 11-fold, respectively. With these preliminary findings, eyelid skin is considered to be a promising site for the administration of ophthalmic drugs. Further *in vivo* studies will be needed to establish the distribution of drugs in various parts of the ocular regions after eyelid administration.

CONFLICT OF INTEREST

The authors declare no known conflict of interest.

Table 1. Physicochemical properties of chemicals used in the study

Chemical	Molecular weight	Log $K_{o/w}$
Antipyrine	188.23	-1.51
Diclofenac sodium salt	318.13	-0.96
Fluorescein sodium salt	376.28	-0.61
Pilocarpine	208.26	0.17
Aminopyrine	231.29	1.07
Tranilast	327.33	1.80
Rhodamine B	479.02	2.28
Lidocaine	234.33	2.37

Table 2. HPLC conditions for analysis of chemicals used in the study

Chemical	Mobile phase	Detection wavelength (nm)	Internal standard
Aminopyrine	Acetonitrile:0.1% phosphoric acid containing 5 mM sodium dodecylsulphonate = 40:60	245	*p*-hydroxybenzoic acid methyl ester
Antipyrine	Acetonitrile:water = 20:80	254	---[a]
Diclofenac Na	Methanol:0.1% phosphoric acid = 80:20	286	*p*-hydroxybenzoic acid ethyl ester
Lidocaine	Acetonitrile:0.1% phosphoric acid containing 5 mM 1-heptanesulphonate = 35:65	245	*p*-hydroxybenzoic acid n-propyl ester
Pilocarpine	Acetonitrile:0.1% phosphoric acid = 5:95	212	---[a]
Tranilast	Acetonitrile:0.1% phosphoric acid = 40:60	230	*p*-hydroxybenzoic acid ethyl ester

[a] *Absolute calibration curve method was used*

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Table 3. Flux and permeability coefficient of model compounds

	Eyelid		Abdominal		
Chemical	Flux ($\mu g/cm^2/h$)	Permeability coefficient ($\times 10^{-7} cm/s$)	Flux ($\mu g/cm^2/h$)	Permeability coefficient ($\times 10^{-7} cm/s$)	Enhancement ratio ($P_{eyelid}/P_{abdominal}$)
Antipyrine[#, -]	150 ± 13.9	8.36 ± 0.77	66.5 ± 7.23	3.69 ± 0.40	2.3
Diclofenac sodium salt [#, -]	72.2 ± 21.7	4.01 ± 1.20	12.0 ± 3.23	0.67 ± 0.18	6.0
Pilocarpine[#, -]	24.3 ± 4.95	49.1 ± 7.28	8.27 ± 1.63	18.8 ± 3.70	2.6
Aminopyrine[#, -]	745 ± 146	69.0 ± 13.6	335 ± 59.7	31.0 ± 5.53	2.2
Tranilast[#,-]	0.64 ± 0.23	3.66 ± 1.25	0.05 ± 0.02	0.34 ± 0.13	11
Lidocaine[#, -]	179 ± 14.0	151 ± 11.8	40.3 ± 10.6	34.2 ± 8.94	4.4

Significant difference ($P<0.05$) between eyelid and abdominal for fluxes [(#)] and for permeability coefficients[(-)]

LIST OF FIGURES

Figure 1

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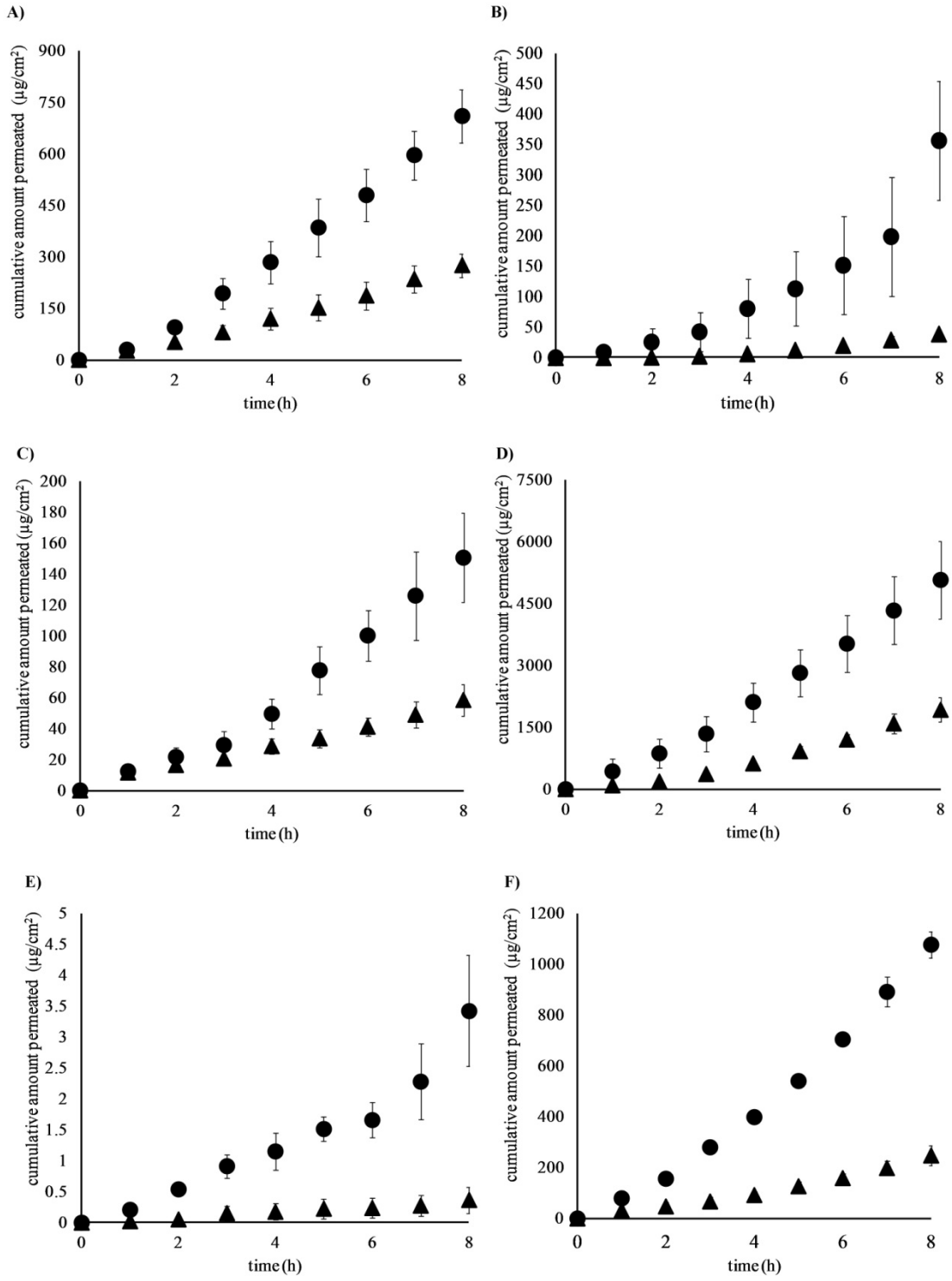
Figure 2


A)
cumulative amount permeated (µg/cm²)
900
750
600
450
300
150
0
0
2
4
6
8
time (h)
B)
cumulative amount permeated (µg/cm²)
500
450
400
350
300
250
200
150
100
50
0
time (h)
C)
cumulative amount permeated (µg/cm²)
200
180
160
140
120
100
80
60
40
20
0
time (h)
D)
cumulative amount permeated (µg/cm²)
7500
6000
4500
3000
1500
0
time (h)
E)
cumulative amount permeated (µg/cm²)
5
4.5
4
3.5
3
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2
1.5
1
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1200
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800
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400
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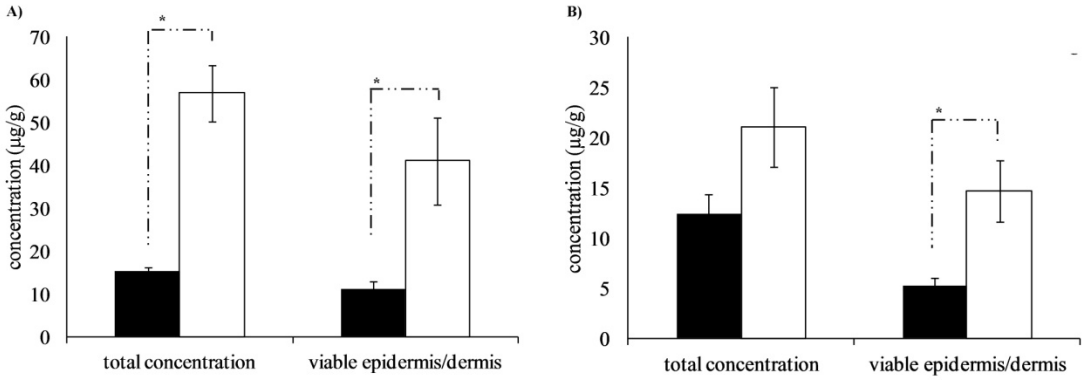
430 Figure 3


A)
70
60
50
40
30
20
10
0
concentration (µg/g)
*
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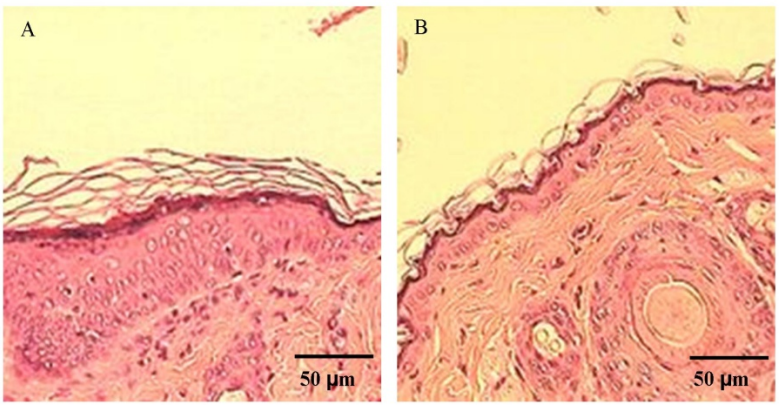
total concentration
viable epidermis/dermis
B)
30
25
20
15
10
5
0
concentration (µg/g)
*
total concentration
viable epidermis/dermis

Figure 4



435

Figure 5