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

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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)

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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.

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 to their 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 and Tojo, 2007; Gaudana et al., 2010; Isowaki et al., 2003; Gause et al., 2016). 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.

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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).

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

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 h. 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.