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P-Glycoprotein in skin contributes to transdermal absorption of topical corticosteroids

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ABSTRACT

ATP binding cassette transporters, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), are expressed in skin, but their involvement in transdermal absorption of clinically used drugs remains unknown. Here, we examined their role in transdermal absorption of corticosteroids. Skin and plasma concentrations of dexamethasone after dermal application were reduced in P-gp and BCRP triple-knockout (*Mdr1a/1b/Bcrp*$^{-/-}$) mice. The skin concentration in *Mdr1a/1b/Bcrp*$^{-/-}$ mice was reduced in the dermis, but not in the epidermis, indicating that functional expression of these transporters in skin is compartmentalized. Involvement of these transporters in dermal transport of dexamethasone was also supported by the observation of a higher epidermal concentration in *Mdr1a/1b/Bcrp*$^{-/-}$ than wild-type mice during intravenous infusion. Transdermal absorption after dermal application of prednisolone, but not methylprednisolone or ethinyl estradiol, was also lower in *Mdr1a/1b/Bcrp*$^{-/-}$ than in wild-type mice. Transport studies in epithelial cell lines transfected with P-gp or BCRP showed that dexamethasone and prednisolone are substrates of P-gp, but are minimally transported by BCRP. Thus, our findings suggest that P-gp is involved in transdermal absorption of at least some corticosteroids *in vivo*. P-gp might be available as a target for inhibition in order to deliver topically applied drugs and cosmetics in a manner that minimizes systemic exposure.

1. Introduction

Transdermal drug delivery offers several advantages over conventional oral administration or injection, including the avoidance of first-pass metabolism, the minimization of pain and the possibility of controlling drug release (Prausnitz and Langer, 2008; Schoellhammer et al., 2014). However, skin serves as a physical and biological barrier between the body and the environment to prevent unregulated water loss from the body and percutaneous absorption of xenobiotics (Proksch et al., 2008). The physical barrier is mainly localized in the stratum corneum (SC), which is the outermost layer of skin and consists of anucleate corneocytes and intercellular lipids (Madison, 2003). Various techniques have been proposed to increase the skin permeability by disrupting the physical barrier in the SC in order to promote transdermal drug delivery (Prausnitz and Langer, 2008; Schoellhammer et al., 2014). Skin also functions as a biological barrier containing various metabolic enzymes and drug transporters that mediate detoxification and efflux of xenobiotics (Baron et al., 2001; Schiffer et al., 2003; Ahmad and Mukhtar, 2004; Li et al., 2005; Ito et al., 2008; Svensson 2009; Heise et al., 2010; Hewitt et al., 2013). Therefore, this barrier could also have an important influence on transdermal absorption of therapeutic agents, though the mechanisms involved remain to be fully clarified.

Abbreviations: P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; SC, stratum corneum; ABC, ATP-binding cassette; MDR1, multidrug resistance 1; MDCK II, Madin-Darby canine kidney II; BL, basal; AP, apical; ER, efflux ratio; P_{app}, apparent permeability coefficient; LC/MS/MS, high-performance liquid chromatography/tandem mass spectrometry; ESI, electrospray ionization; *m/z*, mass-to-charge ratios.

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Among drug transporters, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), which are encoded by *MDR1* (or *ABCB1*) and *ABCG2* genes, respectively, in humans, and belong to the ATP binding cassette (ABC) superfamily have been identified in epidermal keratinocytes of both human and mouse (Sleeman et al., 2000; Baron et al., 2001; Triel et al., 2004). Skazik et al. (2011) reported the expression of P-gp in basal epidermis and skin appendages including blood vessels in human. Hashimoto et al. (2013) have also shown that P-gp and BCRP are expressed in the basal layer of epidermis in human, and in dermal endothelial cells of both human and mouse. These two ABC transporters may play a central role in the transdermal absorption of their typical substrate rhodamine 123 (2-[6-amino-3-imino-3H-xanthen-9-yl] benzoic acid methyl ester), at least in rodents, since the concentrations of rhodamine 123 in the plasma and dermis after dermal application were greatly reduced in *mdr1a/1b* and *bcrp* gene knockout (*Mdr1a/1b/Bcrp*$^{-/-}$) mice, compared with wild-type mice (Hashimoto et al., 2013). Since these transporters have extremely broad substrate specificity, P-gp and/or BCRP might contribute to the transdermal absorption of a variety of therapeutic drugs and cosmetic components.

Various therapeutic agents that are topically applied to the skin or dermally administered for systemic delivery are known to be substrates of P-gp. These include immunosuppressive drugs, antibiotics, antiviral drugs and corticosteroids such as tacrolimus, erythromycin, acyclovir, dexamethasone, prednisolone and betamethasone (Ueda et al., 1992; Saeki et al., 1993; Kim et al., 1999; Yates et al., 2003; Palmberger et al., 2008) for topical application, and opioid analgesics, psychotropic drug, beta-blockers and estrogens such as fentanyl, buprenorphine, methylphenidate, bisoprolol and estradiol (Dagenais et al., 2004; Suzuki et al., 2007; Kim and Benet, 2004; Tahara et al., 2008) for transdermal delivery. P-gp mediates active secretion of its substrate drugs in liver and kidney (Koziolek et al., 2001; Hoffmaster et al., 2004), and is responsible for active efflux of its substrate drugs across brain endothelial cells and small intestinal epithelial cells (Greiner et al., 1999; Sasongko et al., 2005). Although information on the pharmacokinetic roles of BCRP is relatively limited, permeation of some therapeutic agents across the blood-brain barrier is hindered by BCRP-mediated efflux (Agarwal et al., 2011). In the case of skin, however, transdermal permeation of most therapeutic agents applied to the dermal surface is believed to occur mainly by passive diffusion, and the contribution of xenobiotic transporters to overall dermal disposition is mostly unknown and believed to be minor (Mitragotri et al., 2011). In addition, ABC transporters other than P-gp or BCRP are also expressed in the skin (Schiffer et al., 2003; Li et al., 2005; Markova et al., 2009; Heise et al., 2010; Kudo et al., 2016), and exhibit wide range of substrate specificity, so that it is difficult to estimate the contribution ratio of each transporter to overall dermal drug transport. Thus, it is challenging to demonstrate the involvement of these two ABC transporters in transdermal absorption of therapeutic agents *in vivo*.

The purpose of the present study is to clarify the role of P-gp and BCRP in transdermal absorption of therapeutic agents. Since P-gp and BCRP accept therapeutic agents with a variety of molecular sizes as substrates, and passive diffusion of solutes through the skin is hindered by larger molecular size, we focused on corticosteroids as transdermally administered P-gp substrate drugs with relatively large molecular weight in the present study. To examine the involvement of P-gp and/or BCRP in dermal absorption, dexamethasone and other corticosteroids were transdermally administered, and their concentration profiles in plasma and skin were compared in wild-type and *Mdr1a/1b/Bcrp*$^{-/-}$ mice. We also conducted a transcellular transport study in epithelial cell lines stably transfected with genes encoding P-gp and BCRP to confirm transporter-mediated permeation of those drugs.

2. Materials and methods

2.1. Materials

Dexamethasone was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Prednisolone, ethinyl estradiol and elacridar were obtained from Sigma Chemical Co. Ltd. (St. Louis, MO). Itraconazole, Ko143 and methylprednisolone were obtained from LKT Laboratories, Inc. (St. Paul, MN). Prednisone was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were commercial products of analytical grade.

2.2. Animals

Seven- to nine-week-old male FVB (wild-type) mice and *Mdr1a/1b/Bcrp*$^{-/-}$ mice were purchased from CLEA Japan Inc. (Tokyo, Japan) and Taconic Biosciences, Inc. (Hudson, NY), respectively. The mice were kept in a temperature- and light-controlled environment with standard food and tap water provided ad libitum. Animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals in Kanazawa University. At 72 h before transdermal absorption experiments, mice were anesthetized with a subcutaneous injection of sodium pentobarbital, and the fur over the abdominal skin was removed using an electric hair clipper and depilatory cream. The chemical components of the cream are thioglycolic acid, cetearyl alcohol, polyoxyethylene lauryl ether, polyoxyethylene cetyl ether, butylene glycol, ethanol, squalene, paraffin, sodium guaiazulene sulfonate, calcium hydroxide and sodium hydroxide. There is no report of their interaction with P-gp or BCRP.

2.3. Transdermal absorption study

Experiments were performed as previously reported with minor modifications (Hashimoto et al., 2013). Briefly, the test drug was dissolved in dimethyl sulfoxide (dexamethasone, prednisolone and methylprednisolone: 50 mg/mL, ethinyl estradiol: 10 mg/mL), and the drug solution (30 μL) was applied to a patch (9 mm diameter, Torii Pharmaceutical Co. Ltd., Tokyo, Japan). Under ether anesthesia, the abdominal skin was stripped with vinyl tape (Nichiban Co. Ltd., Tokyo, Japan), and two patches were applied to abdominal sites. Thus, the dose of dexamethasone, prednisolone and methylprednisolone was 2.4 mg/cm^2, and that of ethinyl estradiol was 0.47 mg/cm^2. At various intervals after dermal application of drug, blood was collected from the tail vein and centrifuged to obtain plasma. After the last blood sampling, mice were sacrificed under ether anesthesia, and the abdominal skin at the location of the patch and brain were collected. In some experiments, the skin was divided into epidermis and dermis as described previously (Surber et al., 1990). Briefly, the skin was wrapped in aluminum foil, put between two glass plates and warmed at 55 °C for 1 min, and then the epidermis was stripped off with the tip of an injection syringe (18G, Terumo, Tokyo, Japan).

2.4. Intravenous infusion study

Dexamethasone dissolved in saline (50 μg/mL) was injected into the jugular vein at a rate of 100 ng/min. This dose was set to reach the plasma concentration comparable with that observed after the topical administration. At various intervals during intravenous infusion, blood was collected from the tail vein and centrifuged to obtain plasma. At 6 h, several tissues including skin, ear, foot and brain were also excised. The distribution of dexamethasone was represented as the tissue-to-plasma concentration ratio (μL/mg tissue), which was calculated by dividing the distributed amount of dexamethasone in the tissues (ng/mg tissue)