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What is the discrepancy between drug permeation into/across intact and diseased skins? Atopic dermatitis as a model

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ABSTRACT

The discrepancy in drug absorption between healthy and diseased skins is an issue that needs to be elucidated. The present study attempted to explore the percutaneous absorption of drugs via lesional skin by using atopic dermatitis (AD) as a model. Tape-stripping and ovalbumin (OVA) sensitization induced AD-like skin. The lesions were evaluated by physiological parameters, histology, cytokines, and differentiation proteins. The permeants of tacrolimus, 8-methoxypsoralen, methotrexate, and dextran were used to examine in vitro and in vivo cutaneous permeation. Transepidermal water loss (TEWL) increased from 5.2 to 27.4 $g/m^2/h$ by OVA treatment. AD-like lesions were characterized by hyperplasia, skin redness, desquamation, and infiltration of inflammatory cells. Repeated OVA challenge produced a T-helper 2 (Th2) hypersensitivity accompanied by downregulation of filaggrin, involucrin, and integrin β. Tacrolimus, the most lipophilic permeant, revealed an increase of cutaneous deposition by 2.7-fold in AD-like skin compared to intact skin. The transdermal flux of methotrexate and dextran, the hydrophilic permeants, across AD-like skin increased about 18 times compared to the control skin. Surprisingly, AD-like skin showed less skin deposition of 8-methoxypsoralen than intact skin. This may be because the deficient lipids in the atopic-affected stratum corneum (SC) diminished drug partitioning into the superficial skin layer. The fluorescence and confocal microscopic images demonstrated a broad and deep passage of small-molecular and macromolecular dyes into AD-like skin. The results obtained from this report were advantageous for showing how the lesional skin influenced percutaneous absorption.

1. Introduction

Skin diseases are the most common human disorders, affecting 30–70% of populations (Hay et al., 2014). The epidemiologic data report an increased incidence of skin diseases over the decades (Andersen and Davis, 2013). The barrier function is usually compromised in diseased conditions. Atopic dermatitis (AD) is a relapsing chronic inflammatory skin disorder with signs of eczema, erythema, pruritus, and lichenification. The T-helper 2 (Th2) immune response plays a key role in the pathogenesis of AD (Schneider et al., 2013). The prevalence of AD is measured at 15–30% for children and 2–10% for adults (Bieber, 2010). The increase of AD incidence by 2–3-fold during the last 3 decades results from increased exposure to environmental allergens, life-style change, and reduced immune tolerance.

Skin-barrier deterioration is always accompanied by the occurrence of AD. Previous studies (Wollenberg et al., 2003; Miyagaki and Sugaya, 2015) demonstrated the facile entrance of viruses and bacteria into AD skin due to the defective barrier. There is no information available pertaining to the impact of AD on cutaneous absorption of drugs and actives. It is expected that drug permeation via disrupted skin is greater as compared to that

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viaintact skin. However, the level of this discrepancy between AD and healthy skins is not known. Whether the overabsorption of the drugs induces the adverse risk is also unrevealed. The aim of this study was to evaluate the cutaneous penetration of drugs and macromolecules into/across AD skin in contrast to normal skin. Balb/c mice were used as the animal model by repeated epicutaneous sensitization with ovalalbumin (OVA) to induce AD-like skin. This method has proven to be successful for eliciting the Th2 cell-infiltrating immune response (Oyoshi et al., 2012).

Three anti-AD drugs were selected as the permeants in this work for comparing the permeation between diseased and intact skins. These included tacrolimus, 8-methoxypsoralen, and methotrexate. These drugs have various molecular sizes and polarities that are beneficial to exploring the penetration mechanisms of AD-like skin. A macromolecule of fluorescein isothiocyanate (FITC)-conjugated dextran with a molecular weight (MW) of 4 kDa (FD4) was also used as a permeant. The skin after OVA treatment was examined by macroscopic and microscopic observations, physiological parameters, and levels of Th2 cytokines. The proteins involved in barrier function such as filaggrin, involucrin, and integrin β were determined by quantitative real-time polymerase chain reaction (qRT-PCR). The Franz diffusion cell was the in vitro skin permeation platform. The in vivo permeant distribution in the skin was monitored by fluorescence and confocal microscopies for vertical and horizontal views, respectively. The present study's findings may have critical implications for dissecting how the cutaneous diseases influenced skin permeability and the subsequent drug absorption.

2. Materials and methods

2.1. Materials

8-Methoxypsoralen, methotrexate, FITC, FD4, polyethylene glycol 400 (PEG400), and OVA from chicken egg white were purchased from Sigma–Aldrich (St. Louis, MO, USA). Tacrolimus was supplied by United States Pharmacopeia (Rockville, MD, USA).

2.2. Animals

Seven-week-old Balb/c mice were supplied by National Laboratory Animal Center (Taipei, Taiwan). This study was carried out in strict accordance with the recommendation in the Guidelines for the Care and Use of Laboratory Animals of Chang Gung University of Science and Technology. All efforts were made to minimize suffering.

2.3. OVA sensitization

The procedures of epicutaneous sensitization for the mice were modified from previous studies (Kim et al., 2012; Yanaba et al., 2013). At first, the mice were sensitized with 1 mg/ml OVA (0.1 ml) by intraperitoneal administration every other day for 10 days. At day 8, the dorsal hair of the mice was shaved using an electric clipper and tape-stripped 6 times, mimicking cutaneous injury produced by scratching in AD patients. Next, 100 μg of OVA in 100 μl normal saline was pipetted onto a 1 × 1 cm sterile gauze, which was topically applied to the dorsal skin. Normal saline without OVA was employed as the vehicle control. The gauze was fixed with Tegaderm adhesive dressing (3M, Maplewood, MN, USA) and a Silkypore stretch bandage (Alcare, Tokyo, Japan). After 24 h, the gauze was removed and new gauze was applied. This procedure was repeated for 7 consecutive days.

2.4. Macroscopic visualization

After OVA challenge or vehicle treatment, the macroscopic images of mouse skin were observed using a digital camera (Nikon, Tokyo, Japan) and a handheld digital magnifier (Mini Scope-V, M&T Optics, Taipei, Taiwan). A magnification of 100× was used to capture the images for the magnifier.

2.5. Physiological parameters

After OVA or vehicle application, the mouse skin was examined for physiological parameters, including transepidermal water loss (TEWL), erythema (a*), and cutaneous surface pH. A Tewameter (TM300, Courage and Khazaka, Köln, Germany) was employed for estimating TEWL (g/m^2/h). A spectrocolorimeter (CD100, Yokogawa, Tokyo, Japan) was used to quantify cutaneous erythema. The pH was determined by Skin-pH-Meter PH905 (Courage and Khazaka).

2.6. Microscopic visualization

The dorsal skin was excised from the mouse after sacrifice. The excised skin was immersed in a 10% buffered formaldehyde using ethanol, embedded in paraffin wax, and sliced at a thickness of 5 μm. The specimens were stained with hemoxylin and eosin (H&E) and viewed under light microscopy (IX81, Olympus, Tokyo, Japan).

2.7. Cytokine measurements

The mouse skin was removed 24 h after the last challenge of OVA. The excised skin was suspended in PBS and homogenized by MagNA Lyser (Roche, Indianapolis, IN, USA). After centrifugation, levels of interleukin (IL)-4, IL-5, IL-10, and IL-13 in the tissue supernatant were measured using an enzyme immunoassay kit according to the manufacturer's instructions (BioLegend).

2.8. Quantitative real-time PCR (qRT-PCR)

The levels of filaggrin, involucrin, and integrin β were determined for their mRNA expression. Total RNA was extracted with Trizol (Invitrogen), and cDNA was synthesized by reverse transcription using an iScript cDNA Synthesis Kit (Bio-Rad). The qRT-PCR was performed using iQ SYBR Green Supermix (Bio-Rad) based on the manufacturer's instructions. The housekeeping gene GAPDH was used as an internal control, and gene expression was calculated using the comparative threshold cycle method.

2.9. In vitro cutaneous permeation

The in vitro drug permeation into/across the skin was conducted with the Franz cell. The excised OVA- or vehicle-treated skin was mounted between the donor and receptor compartments with the stratum corneum (SC) facing upward toward the donor. The receptor medium was 30% ethanol in pH 7.4 buffer for tacrolimus, 8-methoxypsoralen, and methotrexate to maintain the sink condition. The pH 7.4 buffer was the receptor medium for FD4. The donor (0.5 ml) was loaded with tacrolimus (4% w/v in 30% PEG400/water), 8-methoxypsoralen (0.8% in 40% PEG400/pH 7.4 buffer), methotrexate (1% in water), or FD4 (0.56% in pH 7.4 buffer). The effective diffusion region for the permeants was 0.785 cm^2. The receptor temperature and stirring rate of the stirrer were kept at 37 °C and 600 rpm, respectively. A 300-μl medium was withdrawn from the receptor at determined intervals. After a 24-h application, the skin was removed from the Franz cell. The skin was washed by double-distilled water for three times to