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An insight into the role of barrier related skin proteins

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ABSTRACT

It is well-known that intercellular lipids in the stratum corneum (SC) of the skin play an important role in maintaining barrier function, and many types of penetration enhancers affecting lipids are used in topical products to improve transdermal drug permeability. Recently, it was reported that functional proteins in tight junctions of the epidermis are important for barrier function. In this study, the effects of penetration enhancers such as fatty esters, amines/amides, and alcohols on the barrier function of the skin were evaluated in rat skin and normal human-derived epidermal keratinocytes (NHEK). All penetration enhancers decreased the electrical impedance (EI), however, the potencies of some penetration enhancers were not equal between rat skin and NHEK. The differences were clarified by immunohistochemical studies: some fatty esters decreased the immunoreactivity of involucrin and keratin 10 in the upper layer of the epidermis, while alcohols decreased the immunoreactivity of desmoglein-1, claudin-1, and E-cadherin located in the lower layer of the epidermis. From these results, it is suggested that penetration enhancers show new action mechanisms disturbing barrier-related proteins in epidermis, which are classified into two categories depending on their action sites.

1. Introduction

Mammalian skin composed of epidermis, dermis and subcutaneous tissue plays an important role in the environmental barrier function due to its unique multilayer structure. Epidermis is mostly consisted of keratinocytes, which in basal layer differentiate into and form spinous, granular layers and stratum corneum (SC) toward the surface. The SC is the most important structure for the maintenance of skin barrier function and consists of lamellar layers of a continuous sheath of cornified cells and extracellular matrix enriched in lipids and ceramides (Hamanaka, 2007). Keratinocytes also perform important barrier functions, deriving strength from their cytoskeletons and intercellular adhesion molecules such as desmosome, tight and gap junctions (Kitajima, 2007).

There are 2 drug penetration pathways through the skin: a paracellular route and a transcellular route. These routes are recognized as important targets of penetration enhancers for improving drug permeability (Kondoh, 2006), and structural changes in lipids such as fluidization and extraction have often been observed as main action mechanisms of penetration enhancers in both pathways (Obata et al., 2006; Williams and Barry, 2004).

Recently, claudin-1, a functional protein present in tight junctions, was reported to affect transdermal drug penetration through a size-selective molecular sieve (Furuse et al., 2002). Kondoh (2006) reported the enhancement of drug absorption in rat jejunum by using a cluadin-4 modulator. Uchida et al. (2011) reported that tight junction modulation peptides accelerated the transdermal delivery of small interfering RNA through the paracellular route. Furthermore, Lee et al. (2008) reported that transdermal penetration of model drugs was greatly enhanced by calcium thioglycolate, which alters intercellular lipids and intracellular keratin matrix. Accordingly, barrier-related proteins such as involucrin, a component of the cornified cell envelope, and several types of adherent molecules are important targets for drug delivery systems.

In the present paper, the potencies of penetration enhancers first were evaluated evaluating in rat skin with SC structures and normal human-derived epidermal keratinocytes (NHEK) without SC structures by measuring electrical impedance (EI), which has been widely used to estimate skin permeability (Karande et al., 2005, 2006; Rachakonda et al., 2008). Furthermore, detailed effects on barrier-related proteins were examined using immunohistochemical techniques on rat skin. In this paper, we report the new action mechanisms of penetration enhancers with a focus on the structural changes of barrier-related proteins.

Abbreviations: NHEK, normal human-derived epidermal keratinocytes; SC, stratum corneum; EI, electrical impedance; ER, enhancement ratio; IP, irritation potential; DID, diisopropyl adipate; DS, diethyl sebacate; IS, diisopropyl sebacate; IM, isopropyl myristate; NMP, n-methylpyrrolidone; CT, crotamiton; UR, urea; OA, oleyl alcohol; LA, lauryl alcohol; PG, propylene glycol; S80, sorbitan monooleate; LM, lauromacrogol; T20, polysorbate 20; SLS, sodium lauryl sulfate; TEWL, transepidermal water loss.

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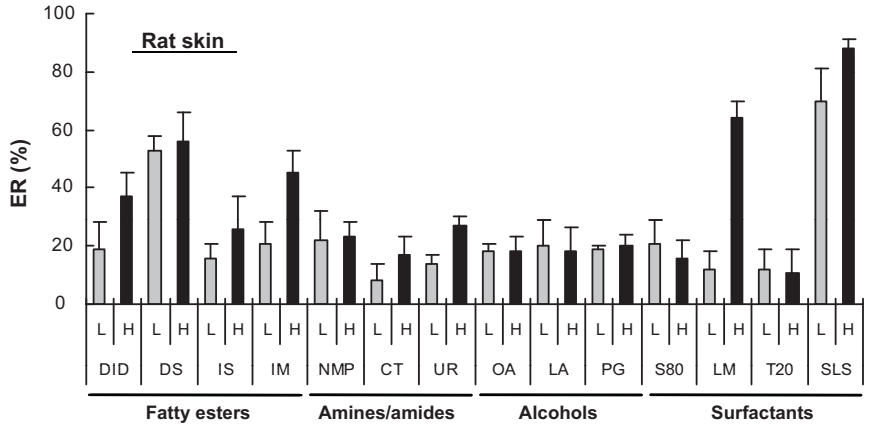

Fig. 1. Effects of penetration enhancers on ER of rat skin. "L" and "H" indicate the low and high doses in Table 1, respectively, of each penetration enhancer. ERs of amines/amides and alcohols were less than those of fatty esters and surfactants. Each value represents the mean ± S.E. of quadruplicate experiments.

2. Materials and methods

2.1. Animals and materials

Male Wistar rats aged 8 weeks were purchased from Japan SLC, Inc. (Shizuoka, Japan). Animals were housed in a controlled 12-h light/12-h dark cycle, and food and water were provided *ad libitum*. Animal experiments were undertaken according to the guidelines for the care and use of experimental animals provided by the Japanese Association for Laboratory Animal Science (1987), and all experiments were approved by the Experimental Animal Ethics Committee of Fuji Research Laboratories, Kowa Co., Inc. (Shizuoka, Japan).

NHEK and keratinocyte growth medium (KGM) were purchased from Lonza Walkersville, Inc. (Walkersville, MD, USA). Antibodies and other reagents were purchased from the following suppliers: diisopropyl adipate (DID), diethyl sebacate (DS), diisopropyl sebacate (IS), isopropyl myristate (IM), sorbitan monooleate (S80), and lauromacrogol (LM) from Nikko chemicals Co., Ltd. (Tokyo, Japan); oleyl alcohol (OA) from Croda Japan KK (Tokyo, Japan); lauryl alcohol (LA) from NOF Corporation (Tokyo, Japan); propylene glycol (PG) from ADEKA Corporation (Tokyo, Japan); n-methylpyrrolidone (NMP) from ISP Japan Ltd. (Tokyo, Japan); crotamiton (CT) from Sumitomo Chemical Co., Ltd. (Tokyo, Japan); urea (UR) from Takasugi Pharmaceutical Co., Ltd. (Fukuoka, Japan); polysorbate 20 (T20) from Sigma–Aldrich Co. (St. Louis, MO, USA); sodium lauryl sulfate (SLS) from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); mouse monoclonal anti-keratin 10 antibody, mouse monoclonal anti-involucrin antibody, and rabbit anti-claudin-1 antibody from Thermo Fisher Scientific Inc. (Waltham, MA, USA); mouse anti-E-cadherin antibody and mouse anti-desmoglein-1 antibody from BD Biosciences (San Jose, CA, USA); goat anti-mouse IgG fluorescence conjugated antibody and goat anti-rabbit IgG fluorescence conjugated antibody from Chemicon International, Inc. (Temecula, CA, USA); normal goat serum from Covance Research Product Inc. (Princeton, NJ, USA); and Vectashield with DAPI from Vector Laboratories Inc. (Burlingame, CA, USA).

2.2. Measurements of enhancement ratio (ER) on NHEK

EI was used to estimate the potency, or ER, of each penetration enhancer.

NHEK were used after differentiation by high Ca^{2+} concentration to increase epidermal barrier molecules such as keratin 10, involucrin, desmoglein-1, claudin-1, and E-cadherin (Kim et al., 2006), i.e., cells were seeded in 24-well collagen-coated transwells (Corning Inc., Corning, NY, USA) at a density of 2.0×10^5 cells/cm^2. Two days after seeding, cells were differentiated by changing the medium (KGM) to one containing 1.5 mM Ca^{2+} and cultured for 4 additional days in 5% CO_2 at 37 °C. EI (pre) was measured in each well with a Millicell-ERS (Millipore Corporation, Billerica, MA, USA). Test samples (200 μL) of the concentrations indicated in Table 1 were then added to the upper layer of each transwell. After an 8-h incubation, wells were washed with PBS(−), and EI (post) was measured. ER was calculated using following equation (1):

$$\mathrm{ER}\ (\%) = 100 - \frac{\mathrm{EI\ (post)}}{\mathrm{EI\ (pre)}} \times 100 \quad (1)$$

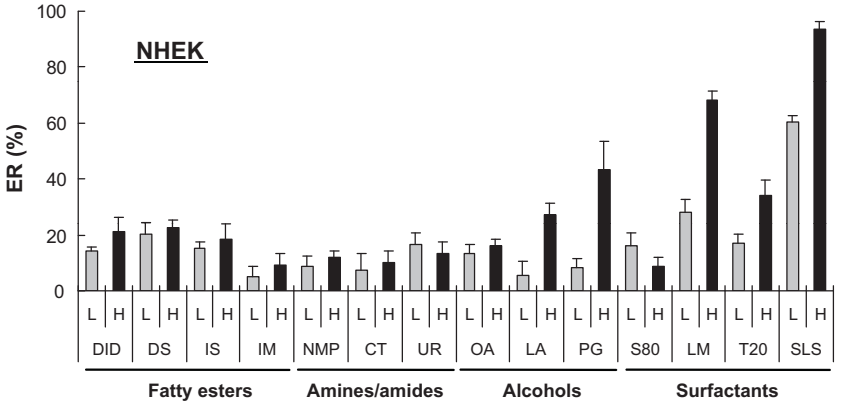

Fig. 2. Effects of penetration enhancers on ER of NHEK. "L" and "H" indicate the low and high doses in Table 1, respectively, of each penetration enhancer. ERs of fatty esters and amines/amides were less than those of alcohols and surfactants. Each value represents the mean ± S.E. of quadruplicate experiments.