International Journal of Pharmaceutics 427 (2012) 293–298

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

An insight into the role of barrier related skin proteins

Kiyomi Wato[a], Takuya Hara[a], Kenjirou Yamana[a], Hiroshi Nakao[a,*], Toshio Inagi[a], Katsuhide Terada[b]

[a] Fuji Research Laboratories, Pharmaceutical Division, Kowa Company, Ltd., 332-1. Ohnoshinden, Fuji-City, Shizuoka 417-8650, Japan
[b] Faculty of Pharmaceutical Sciences, Toho University, 2–2–1 Miyama, Funabashi, Chiba 274–8510, Japan

ARTICLE INFO

Article history:
Received 11 August 2011
Received in revised form 17 January 2012
Accepted 9 February 2012
Available online 16 February 2012

Keywords:
Penetration enhancer
Cutaneous administration
Keratin 10
Involucrin
Claudin-1
E-cadherin

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.

* Corresponding author. Tel.: +81 545 33 1716; fax: +81 545 33 1805.
E-mail address: h-nakao@kowa.co.jp (H. Nakao).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2012.02.014

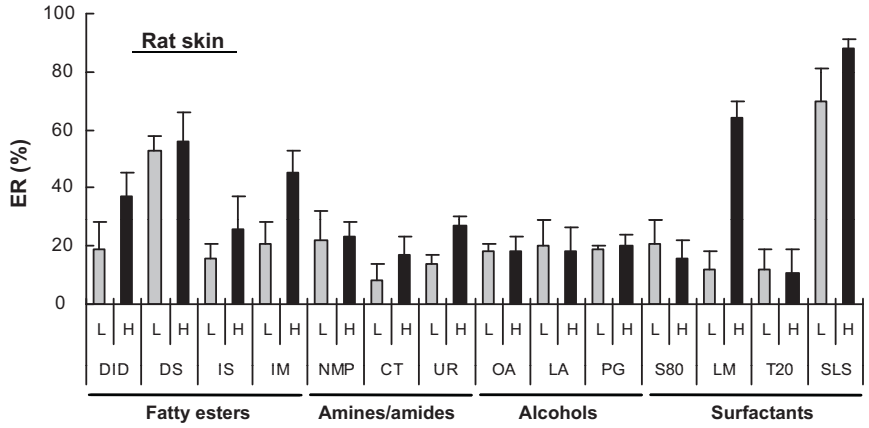

Fig. 1. Effects of penetration enhancers on ER of rat skin. "L" and "H" indicate the low and high doses in Table 1, respectively, of each penetration enhancer. ERs of amines/amides and alcohols were less than those of fatty esters and surfactants. Each value represents the mean ± S.E. of quadruplicate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

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

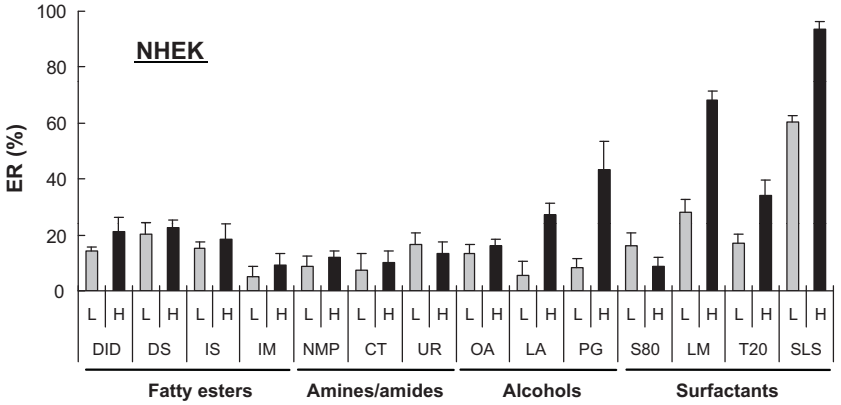

Fig. 2. Effects of penetration enhancers on ER of NHEK. "L" and "H" indicate the low and high doses in Table 1, respectively, of each penetration enhancer. ERs of fatty esters and amines/amides were less than those of alcohols and surfactants. Each value represents the mean ± S.E. of quadruplicate experiments.

Table 1
Summary of penetration enhancers.

		Maximum concentration[a]	Concentration tested (mM)[b]	
			NHEK	Rat skin
Fatty esters	DID	120 mg/g	0.1/1/0	2.0/20
	DS	400 mg/g	3.1/31	62/620
	IS	50 mg/g	0.35/3.5	7.0/70
	IM	450 mg/g	3.3/33	66/660
Amine/amides	NMP	80 mg/g	1.6/16	32/320
	CT	10 mg/g	0.098/0.98	2.0/20
	UR	50 mg/g	1.7/17	34/340
Alcohols	OA	100 mg/g	0.74/7.4	15/150
	LA	30 mg/g	0.32/3.2	6.4/64
	PG	0.72 mL/mL	2.0/20	40/400
Detergents	S80	100 mg/g	0.74/7.4	15/150
	LM	150 mg/g	0.12/1.2	2.3/23
	T20	25 mg/g	0.040/0.40	0.80/8.0
	SLS	20 mg/g	0.14/1.4	2.8/28

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.

[a] Maximum concentration for external application.

[b] Low/high concentration tested in normal human-derived epidermal keratinocytes (NHEK) and rat skin, respectively.

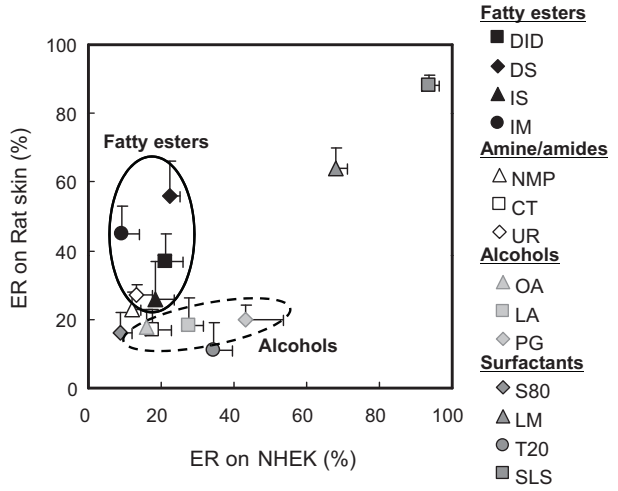

Fig. 3. Relation of ERs on rat skin and NHEK. X and Y axes represent ER in NHEK and rat skin, respectively. Fatty esters and alcohols showed relatively potent effects on rat skin and NHEK, respectively. Each value represents the mean ± S.E. of multiple high-dose treatment experiments shown in Figs. 1 and 2.

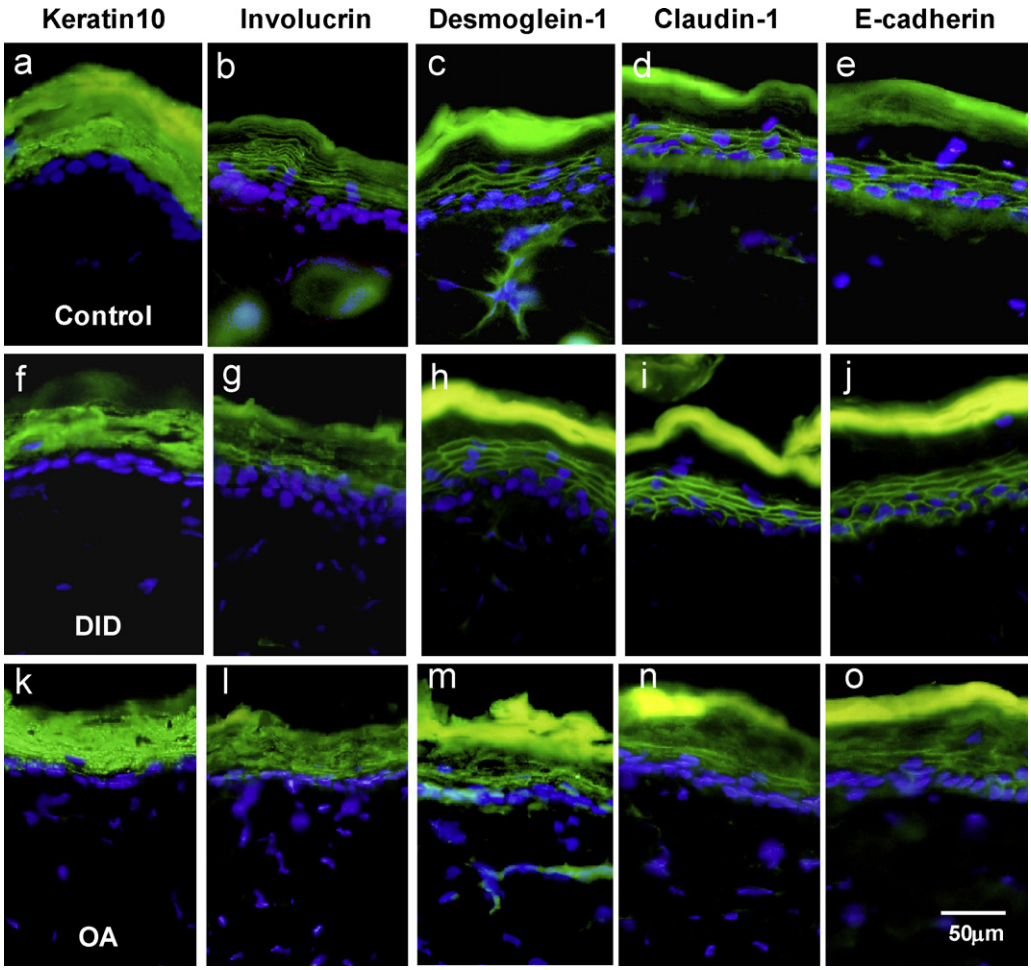

Fig. 4. Effects of penetration enhancers (DID and OA) on the expression of barrier-function molecules in rat skin. Green (FITC): each barrier functional molecule (a, f, k: keratin 10; b, g, l: involucrin; c, h, m: desmoglein-1; d, i, n: claudin-1; e, j, o: E-cadherin), blue (DAPI): cell nuclei. (a–e): In normal epidermis, keratin 10 distributed all over from SC to spinous layer, while involucrin did as meshes from SC to spinous layer. Desmoglein-1 and claudin-1 mainly distributed as meshes in granular and spinous layers, and E-cadherin did as meshes in basal and spinous layers. Cell nuclei distributed clearly in basal layer. (f–j): DID treatment shows clearly decrease on keratin 10 and involucrin. (k–o): OA treatment shows decrease on desmoglein-1, claudin-1, and E-cadherin.

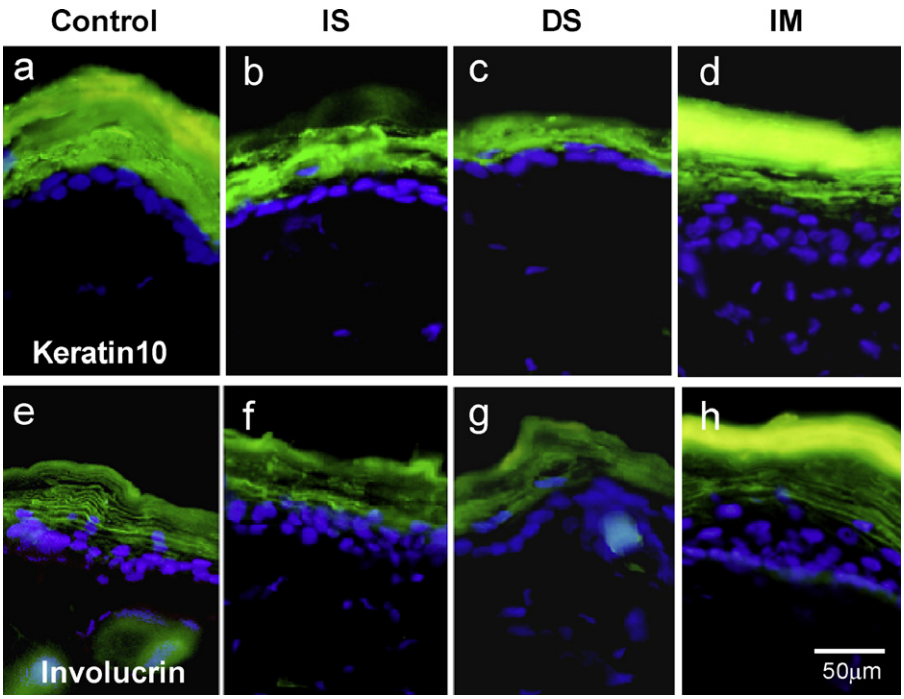

Fig. 5. Effects of other fatty esters on the expression of keratin 10 and involucrin of rat skin. Green (FITC): each barrier-function molecule (a–d: keratin 10, e–h: involucrin), blue (DAPI): cell nuclei. IS, DS, or IM treatment shows decrease on keratin 10 and involucrin compared with no treatment(control).

2.3. Measurements of ER on rat skin

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and sacrificed by carbon dioxide inhalation. An area of abdominal skin (3 cm × 7 cm) was isolated after clipping hair, and subcutaneous fat was removed. Skin samples were then mounted on a 0.5-mL multiwell equilibrium dialysis apparatus (Sanplatec Co., Ltd., Osaka, Japan) 10 mm in diameter, 700 μL of PBS(−) was added to both epidermal and dermal sides, and EI (pre) was measured. PBS(−) on the epidermal side was then replaced with a test solution of the concentration indicated in Table 1, and skin samples were incubated for 4 h at 25 °C. After several washes with PBS(−), EI (post) was measured. ER was calculated using Eq. (1).

2.4. Immunohistochemical analysis of rat skin

Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and the hair on the back was clipped. An area of back skin 3 cm^2 and at least 4 cm from the tail was challenged with 0.3 mL of a test solution of the high concentration indicated in Table 1. After a 2-h treatment using supplemental anesthesia, rats were sacrificed by carbon dioxide inhalation. Isolated skin samples were then embedded in OCT compound (Sakura Finetechnical Co., Ltd., Torrance, CA, USA) by cooling with liquid nitrogen, cut into 6-μm sections, air-dried for 1 h, and fixed with methanol. The specimens were blocked with normal goat serum; incubated with antibodies specific to keratin 10 (1 μg/mL), involucrin (2 μg/mL), desmoglein-1 (4 μg/mL), claudin-1 (1 μg/mL), or E-cadherin (2 μg/mL); stained with FITC-conjugated secondary antibody (5 μg/mL) for 30 min at room temperature, and then each specimen was mounted with Vectashield with DAPI to stain cell nuclei and observed with fluorescence microscopy.

3. Results

3.1. EI of each skin model

The mean EI of each skin model is shown in Table 2. The EI of NHEK and rat skin were 100 ± 20 Ω (mean ± S.E.) and 2415 ± 234 Ω, respectively. The applied concentration of each penetration enhancer was determined by multiplying the maximum concentration for external application (Table 1) (Japan Pharmaceutical Excipients Council, 2007) by the relative EI of each skin model. For example, because the mean EI of rat skin was almost 20 times higher than that of NHEK, the concentration of penetration enhancer used for rat skin was 20 times that used in NHEK.

3.2. ER on rat skin and NHEK

Results from rat skin are shown in Fig. 1. The ER of fatty esters and surfactants ranged from approximately 25–60% (20 mM DID: 37%, 620 mM DS: 56%, 70 mM IS: 26%, and 660 mM IM: 45%) and 10–90% (15 mM S80: 21%, 23 mM LM: 64%, 8.0 mM T20: 11%, and 28 mM SLS: 88%), respectively. In contrast, the ERs of amines/amides and alcohols were approximately 20% (320 mM NMP: 23%, 20 mM CT: 17%, 340 mM UR: 27%, 150 mM OA 18%, 8.4 mM LA 20%, and 400 mM PG 20%).

ER values in NHEK are shown in Fig. 2. The ER of alcohols and surfactants ranged from approximately 15–45% (7.4 mM OA: 16%, 3.2 mM LA: 27%, and 20 mM PG: 43%) and 15–95% (0.74 mM S80: 16%, 1.2 mM LM: 68%, 0.40 mM T20: 34%, and 1.4 mM SLS: 94%), respectively. However, the ER of fatty esters and amines/amides were approximately 10–25% (1.0 mM DID: 21%, 31 mM DS: 22%, 3.5 mM IS: 18%, 33 mM IM: 9%, 16 mM NMP: 12%, 0.98 mM CT: 10%, and 1.7 mM UR: 17%).

Table 2
The electrical impedance (EI) of each skin model and applied concentrations of penetration enhancers.

	NEHK	Rat skin (abdominal)
EI (Ω, Mean ± S.E.)	100 ± 20	2415 ± 234
Concentration tested[a]	1/50, 1/500	1/2.5, 1/25

Each value represents the mean ± S.E., $n = 10$. NHEK: normal human-derived epidermal keratinocytes.

[a] vs. maximum concentration for external application.

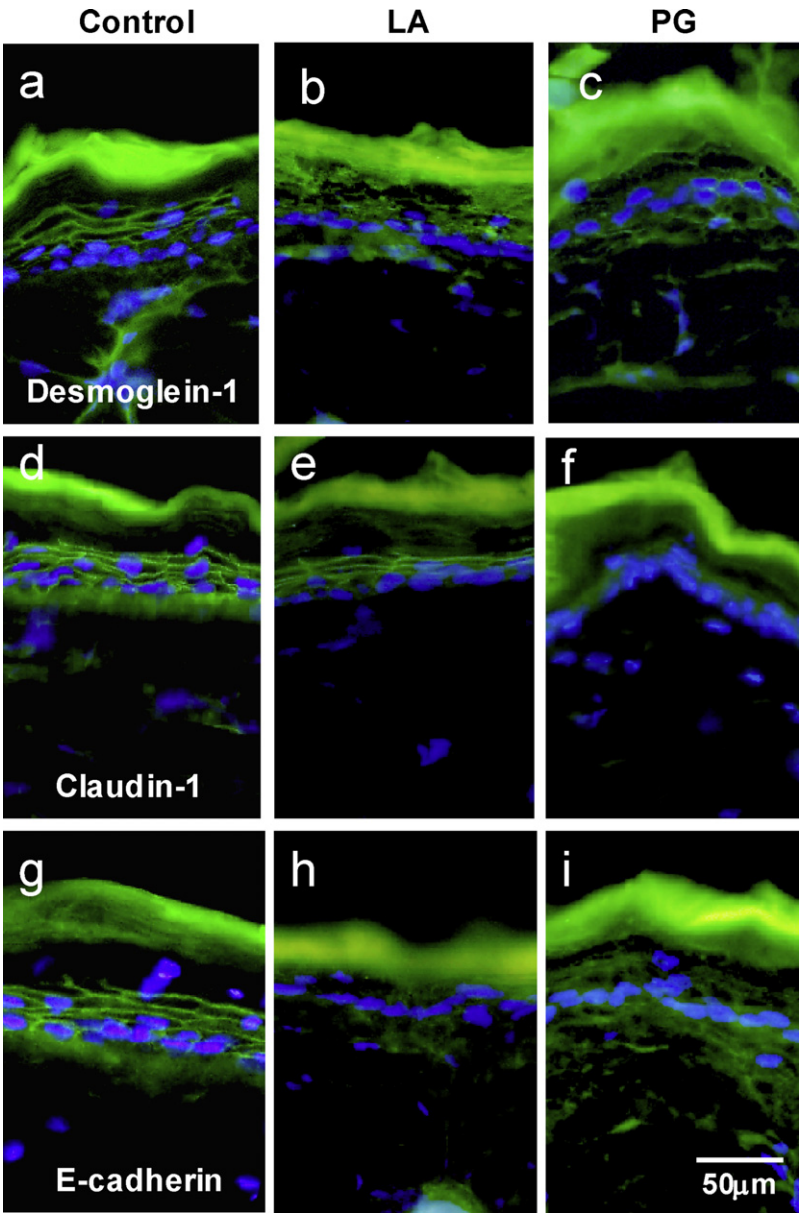

Fig. 6. Effects of other alcohols on the expression of the barrier functional molecules of rat skin. Green (FITC): each barrier-function molecule (a, b, c: desmoglein-1; d, e, f: claudin-1; g, h, i: E-cadherin), blue (DAPI): cell nuclei. LA or PG treatment shows decrease on desmoglein-1, claudin-1, and E-cadherin compared with no treatment(control).

Relation of ERs on rat skin and NHEK are shown in Fig. 3. Each value represents the mean of multiple high-dose treatment experiments shown in Figs. 1 and 2. These results suggested that fatty esters showed relatively potent effects on rat skin and alcohols showed relatively potent effects on NHEK.

3.3. *Effects of penetration enhancers on epidermal barrier-related proteins*

To investigate detailed effects of penetration enhancers on barrier-related proteins, we performed immunohistochemical studies on rat skin, which is more accessible than human skin (Figs. 4–6, green). Epidermis is consisted of SC, granular, spinous and basal layers in turn from the surface., and only in basal layer, cell nuclei are recognized clearly (blue). Fig. 4 (a–e) show the distribution of each barrier-related protein in normal epidermis. Keratin 10 distributed all over from SC to spinous layer, while involucrin was observed as meshes from SC to spinous layer. In contrast, desmoglein-1 and claudin-1 were mainly observed as meshes in granular and spinous layers, and E-cadherin was observed as meshes in basal and spinous layers. Fig. 4 (f–j) shows changes in the immunoreactivity of these barrier-related proteins after topical application of DID, a fatty acid ester, to rat skin for 2 h. No changes in desmoglein-1, claudin-1, or E-cadherin immunoreactivity were observed, but keratin 10 and involucrin immunoreactivity clearly decreased. We examined the effects of other fatty esters (IS, DS, and IM) on keratin 10 and involucrin and found that these enhancers also decreased the immunoreactivity of those proteins expressed in the upper layer of epidermis (Fig. 5). The effects of OA on rat skin are shown in Fig. 4 (k–o). No obvious changes in the immunoreactivity of keratin 10 or involucrin were observed, but desmoglein-1, claudin-1, and E-cadherin immunoreactivity decreased. Other alcohols (LA and PG) also decreased the immunoreactivity of desmoglein-1, claudin-1, and E-cadherin (Fig. 6). Surfactants did not induce any selective changes in barrier-related proteins but led to a nonspecific decrease in the immunoreactivity of all proteins (data not shown).

4. Discussion

The barrier function of the skin largely depends on tissue-specific structures, piled epidermis, dermis and subcutaneous tissue. The most critical seemed to be the SC structures in epidermis for a long period. Recently, several components of keratinocytes in epidermis, which was constructed with basal, spinous, granular layers and SC toward the surface, have also been reported to comprise important parts of the skin barrier (Kitajima, 2007), including intracellular proteins such as involucrin, keratin (Sevilla et al., 2007) and adherent structures such as desmoglein, claudin and cadherin (Furuse et al., 2002; Segre, 2003). In rat skin, keratin 10 and involucrin localized all over and as meshes from SC to spinous layer, respectively. Desmoglein-1 and claudin-1 mainly consisted as meshes in granular and spinous layers, and then E-cadherin as meshes in basal and spinous layers (Fig. 4, a–e). However, although barrier functions are essential to maintain life, they also work as limiting factors for transdermal drug delivery.

To improve transdermal drug permeability, many types of penetration enhancers that weaken the skin barrier by affecting the configuration of intercellular lipids in the SC have been used in topical products (Obata et al., 2006; Williams and Barry, 2004). Actually, as shown in Figs. 1 and 2, all penetration enhancers, containing fatty esters, alcohols and surfactants, decreased the EI in rat skin and NHEK. The EI is known to be well correlated with skin barrier function and drug permeability (Karande et al., 2005, 2006; Rachakonda et al., 2008). However, the potencies of some penetration enhancers were not equal between rat skin and NHEK. For example, fatty esters had relatively potent effects on rat skin in which SC structures were preserved (Figs. 1 and 3). In contrast, alcohols showed relatively potent effects on NHEK, which lacks SC structures (Figs. 2 and 3). These results suggest that fatty esters mainly interact with SC, while alcohols interact with the granular, spinous, and basal layers. Meanwhile, surfactants seemed to affect all layers of the epidermis, demonstrating almost equal effects on both rat skin and NHEK.

Many studies on penetration enhancers had been focused to lipids and protein of the SC. Calcium thioglycolate greatly enhanced transdermal penetration of model drugs by altering barrier-related proteins such as keratin and intercellular lipids in the SC (Lee et al., 2008). Furthermore, dimethylsulphoxide and 1-dodecyl-azacycloheptan-2-one induced the conformational change of SC keratin (Anigbogu et al., 1995; Zhou et al., 2005). However, recently, it was reported that the control of barrier-related proteins in epidermis is important for transdermal delivery of high-molecular-weight drugs. For example, increased drug permeability and a rise in transepidermal water loss (TEWL) were observed in claudin-1 knockout mice (Furuse et al., 2002). Claudin-4 modulator also enhanced model drug adsorption in rat jejunum (Kondoh, 2006).

Accordingly, the changes in intracellular proteins and adherent molecules by penetration enhancers must be considered as well as SC lipids when evaluating their enhancement activity. Actually, in immunohistochemical studies on rat skin, it was indicated that fatty esters interacted with proteins that appeared in the upper epidermal layer (keratin 10 and involucrin), while alcohols interacted with proteins in the lower epidermal layer (desmoglein-1, claudin-1, and E-cadherin) (Figs. 4–6).

From these results, penetration enhancers were classified into two categories depending on their action sites, the difference of penetration pathways and affected proteins. Therefore, it might be useful for the effective enhancement of drug absorption to combine with the different types such as fatty esters and alcohols. The permeation studies are in progress using hydrophobic/lipophilic drugs of different molecular sizes.

5. Conclusion

In this paper, we suggest new action mechanisms of penetration enhancers on barrier function of the skin. As new action sites, fatty esters interact with proteins appearing in the upper layer of epidermis (keratin 10 and involucrin) while alcohols interact with proteins appearing in the lower layer of epidermis (desmoglein-1, claudin-1, and E-cadherin). Keratin 10 and involucrin play important roles in regulating the permeability of drugs through the transcellular route. In contrast, claudin-1, desmoglein-1, and E-cadherin, which are components of adherent structures, are important for the paracellular route of drug delivery through a size-selective molecular sieve (Kondoh, 2006). A combination of penetration enhancers affecting different proteins might increase transdermal drug delivery synergistically.

References

Anigbogu, A.N.C., Williams, A.C., Barry, B.W., Edwards, H.G.M., 1995. Fourier transform Raman spectroscopy of interactions between the penetration enhancer dimethylsulphoxide and human stratum corneum. Int. J. Pharm. 125, 265–282.

Furuse, M., Hata, M., Furuse, K., Yoshida, Y., Haratake, A., Sugitani, Y., Noda, T., Kubo, A., Tsukita, S., 2002. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. J. Cell Biol. 156, 1099–1111.

Hamanaka, S., 2007. Intercellular lipid. Nippon Hifuka Gakkai Zasshi 117, 143–148.

Japan Pharmaceutical Excipients Council, 2007. Iyakuhintenkabutsujiten 2007. Yakuji Nippo Ltd., Tokyo.

Karande, P., Jain, A., Ergun, K., Kispersky, V., Mitragotri, S., 2005. Design principles of chemical penetration enhancers for transdermal drug delivery. Proc. Natl. Acad. Sci. U. S. A. 102, 4688–4693.

Karande, P., Jain, A., Mitragotri, S., 2006. Relationships between skin's electrical impedance and permeability in the presence of chemical enhancers. J. Control. Release 110, 307–313.

Kim, S., Hong, I., Hwang, J.S., Choi, J.K., Rho, H.S., Kim, D.H., Chang, I., Lee, S.H., Lee, M.O., Hwang, J.S., 2006. Phytosphingosine stimulates the differentiation of human keratinocytes and inhibits TPA-induced inflammatory epidermal hyperplasia in hairless mouse skin. Mol. Med. 12, 17–24.

Kitajima, Y., 2007. Skin barrier function and its regulation in terms of epidermal molecular structure. Drug Deliv. Syst. 22, 424–432.

Kondoh, M., 2006. Claudin as a novel target for drug delivery system. Yakugaku Zasshi 126, 711–721.

Lee, J.N., Jee, S.H., Chan, C.C., Lo, W., Dong, C.Y., Lin, S.J., 2008. The effects of depilatory agents as penetration enhancers on human stratum corneum structures. J. Invest. Dermatol. 128, 2240–2247.

Obata, Y., Hatta, I., Ohta, N., Kunizawa, N., Yagi, N., Takayama, K., 2006. Combined effects of ethanol and L-menthol on hairless rat stratum corneum, investigated by synchrotron X-ray diffraction. J. Control. Release 115, 275–279.

Rachakonda, V.K., Yerramsetty, K.M., Madihally, S.V., Robinson Jr., R.L., Gasem, K.A., 2008. Screening of chemical penetration enhancers for transdermal drug delivery using electrical resistance of skin. Pharm. Res. 25, 2697–2704.

Segre, J., 2003. Complex redundancy to build a simple epidermal permeability barrier. Curr. Opin. Cell Biol. 15, 776–782.

Sevilla, L.M., Nachat, R., Groot, K.R., Klement, J.F., Uitto, J., Dajian, P., Määttä, A., Watt, F.M., 2007. Mice deficient in involucrin, envoplakin, and periplakin have a defective epidermal barrier. J. Cell Biol. 179, 1599–1612.

Uchida, T., Kanazawa, T., Takashima, Y., Okada, H., 2011. Development of an efficient transdermal delivery system of small interfering RNA using functional peptides, tat and AT-1002. Chem. Pharm. Bull. 59, 196–201.

Williams, A.C., Barry, B.W., 2004. Penetration enhancers. Adv. Drug Deliv. Rev. 56, 603–618.

Zhou, X., Xu, J., Yao, K., Liu, D., Wang, L., Wang, X., Yang, X., Liu, Y., Fang, Y., 2005. Interaction of 1-dodecyl-azacycloheptan-2-one with mouse stratum corneum. J. Biomater. Sci. Polym. Ed. 16, 563–574.