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Effect of Topical Application of Intercellular Lipids on Sodium Lauryl Sulphate-Damaged Skin Barrier Function in Dogs

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Abstract : Ceramide, cholesterol and free fatty acids are the major intercellular lipids, maintaining the integrity of the skin barrier. However, the roles of these lipids in canine skin barrier function are little known. The aim of this study was to evaluate the repairing effects of 2% ceramide (CER), 2% cholesterol (CHO), 2% linoleic acid (LIN) and 2% intercellular lipid mixture (ILM) on damaged canine skin barrier by 1.25% sodium lauryl sulphate (SLS). Transepidermal water loss (TEWL), skin hydration, skin pH and skin thickness were assessed. Histological profiles and transmission electron microscopic (TEM) profiles were assessed on day 12. SLS effectively induced the canine skin barrier damage. TEWL was significantly decreased by topical application of CER and ILM in SLS and vehicle-treated skin on day 8 and 12, respectively ($p < 0.05$, $p < 0.01$). By end of the experiment all lipids significantly decreased the TEWL as compared with SLS and vehicle control, but CER and ILM more significantly decreased the TEWL than LIN and CHO, respectively ($p < 0.01$). Skin hydration was significantly increased by CER and ILM during experimental periods ($p < 0.01$). Skin pH was significantly decreased by CER, LIN and ILM. In histological profiles, the thickness of the stratum corneum (SC) was significantly increased by the SC lipids as compared with vehicle and SLS ($p < 0.01$). Especially, CER and ILM showed more prominent improvement of barrier recovery. In TEM of the SC, SLS induced exfoliations of corneodesmosomes in the SC, and CER and ILM effectively protected exfoliations of corneodesmosomes on SLS-damaged canine skin. These results indicated that topical application of CER and ILM dramatically improved damaged-skin barrier function by SLS. Also, it was considered that the use of CER or ILM was recommended for the management of skin barrier dysfunction by irritant and inflammatory skin disorders such as atopic dermatitis.

Key Words : cereamide, lipids, SLS, skin barrier, dog

INTRODUCTION

The skin plays a critical role in providing the first-line defense against exogenous irritants from the environment. It is a complex, multilayered organ, which produced several specialized derivative structures called appendages and consists of heterogeneous cell type and extracellular components (17). Three layers of the skin are epidermis, dermis and subcutis. The epidermis is a stratified, continually renewing epithelium that exhibits progressive differentiation (keratinization, cornification) in basale to superficial direction. It is comprised of multiple cell types such as keratinocytes, melanocytes, langerhans cells and merkel cells, and most of epidermal cells are keratinocytes. The keratinocytes are organized into basal, spinous, granular and cornified layers that correspond to progressive stages of differentiation. The process of differentiation in the epidermis, known as keratization or cornification, is genetically programmed, which involved synthesis of new lipids. Epidermal surface lipids originate from maturing corneocytes, which contain about six times the amounts of intracellular lipid as keratinocytes in the basal layer (17,27). The structure of an individual keratinocyte correlates with its position within the epidermis and its state of differentiation (13).

The stratum corneum (SC) of the skin is the most important structure for skin barrier function. Its role is predominately as a barrier to protect the body from environmental irritant and prevent excessive water loss from the body (17,27). The loose, basket weave SC was considered an unimportant organ as a process of desquamation and an impermeable plastic wrap. However, Elias suggested the 'brick and mortar' model of the SC, and one major difference has been noted concerning SC cohesion and desquamation, namely that the principal factors responsible for maintaining attachment between corneocytes are not lipids but desmosomes. The concepts of the SC are evolved as a living organ by its persistent metabolic activity (10,15,17). The SC is comprised of corneocytes and lipid-enriched intercellular space. In 'brick and mortar' model, corneocytes play a role of UV barrier, mechanical barrier and hydrating role as brick, and intercellular lipids play a role of anti-microbial barrier, anti-oxidant

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barrier and permeability barrier as mortar (17,27). The corneocytes are the largest and terminally differentiated stage of the keratinocytes, and are a flattened and polyhedral shape. The shape and features of the corneocytes are adapted to maintain the integrity of SC (17,27).

The lipid lamellae in the SC play a key role in barrier function of the skin. The major lipids are ceramide, cholesterol and free fatty acid, which are required for skin integrity (10, 15). Especially, the ceramide plays an important role to maintain a permeability of skin barrier. In previous studies, it was found an inverse correlation between the quantity of ceramide and transepidermal water loss (8). The ceramide is main polar lipid in the SC and synthesized *de novo* in epidermis from acetate. Six chromatographically separable fractions of ceramides have been isolated from porcine epidermis (30). It was demonstrated that ceramide 1, owing to its very long chain, ensures the connection between adjacent bilayer (4,31). The quantity of ceramide 1 and 3 were significantly correlated with TEWL impairment. Exogenous application of ceramides, especially ceramide 3 led accelerated barrier regeneration (8,30).

The skin barrier function is clearly linked with TEWL as a linear relationship could be shown between the total amounts of lipids removed from the SC (8,12,23). It is generally accepted that the rate of evaporation of water through the upper layers of skin reflects the skin barrier function. This is why TEWL measurements are frequently used to assess SC integrity and consequently skin barrier function. Therefore, TEWL measurements seem to be a useful tool to evaluate the effects of topically applied products on a damaged skin barrier (15,24,).

It is well known that topical application of SLS influences skin barrier function by extraction of the SC lipids in human (11,15,23,24,28). It is generally accepted that damage by acetone is a superficial skin damage model and damage by SLS is a deep skin damage model (24). Damages by acetone are limited to remove the skin surface lipids, whereas damages by SLS included disruption of the SC structure. SLS induces not only changes in the SC but also penetrations into the deeper parts of the viable epidermis (11).

Normal dogs have naturally less lipid content in the epidermis than human, and the lipid content also varies among breeds. The turnover time of canine epidermis is shorter than human (17,22). However, the role of intercellular lipids and SLS-induced damages in canine skin barrier function are little known, and TEWL as an index of skin barrier function has not been well documented in dogs.

The purpose of this study is to evaluate the effect of CER, CHO, LIN and ILM on SLS-damaged skin barrier function in dogs. Particularly, ceramide-3, which plays a key role in skin barrier function, was used to evaluate repair of damaged-skin barrier function.

MATERIALS AND METHODS

Experimental animals

Five healthy beagle dogs were used in this experiment. Each animal was housed separately in a different cage and fed commercial diet. The physical and dermatological examinations were performed. Dogs were kept in a room for at least 20 min before measuring.

Experimental agents

Polypropylene glycol was obtained from DC chemical Co., Ltd (Korea). Ethanol was obtained from Mallinckrodt Baker, Inc (Malaysia). Polyethylene glycol / ethanol (7/3 ; v/v) was used as a vehicle. CER was obtained from Doosan serdary research laboratories (Korea). CHO, LIN and SLS were obtained from Sigma-Aldrich (Germany). ILM (CER 3: CHO 1: LIN 1; ILM) was made with these lipids.

Experimental designs

The back of dogs was divided into seven areas, sized in 3×3 cm^2. The hairs of the back were clipped with clipper (No. 20 Clipper, Oyster, USA). All dogs were housed in standard condition. Base data were measured before experiment. SLS was applied on the back of dogs twice a day and CER, CHO, LIN and ILM were applied once a day for 4 days, and CER, CHO, LIN and ILM were applied without SLS application once a day for following 8 days. All data were assessed on day 4, 8 and 12. During experimental period, they were kept in the room with standard condition for acclimation (Room temperature was maintained at 21.5 ± 0.5°C. Relative humidity was constant at 45 ± 3%).

Non-invasive measurements

Transepidermal water loss; TEWL

TEWL was measured with a Vapometer® (Delfin Technologies Ltd, Finland). The values were expressed as the mean of three times to each site.

Skin hydration

Hydration was measured with a corneometry (CM 820 corneometer®, Courage & Khazaka, Germany). The values were expressed as the mean of three times to each site.

Skin surface pH

Canine skin pH levels were measured with the Skin-pH-Meter (pH900®, Courage & Khazaka, Germany). The values were expressed as the mean of three times to each site.

Double fold skin Thickness

Double fold skin Thickness was measured with an electronic digital caliper (Digimatic caliper®, Mitutoyo Coporation, Japan). The values were expressed as the mean of three times to each site.

Histopathology

Samples from skin biopsy were fixed in 4% buffered paraformaldehyde at least 24 hours, then embedded in paraffin, sectioned (2-3μm) and stained with Hematoxylin and Eosin, and after that the histopathological profiles of the epi-

dermis and upper parts of dermis on each samples were observed under light microscope (Zeiss, Germany)

On each prepared histological samples, the number of keratin layers in the SC, thickness of each keratin layers (KL) and from the stratum granulosum to basale (SGB) were measured using automated image analyzer (DMI CCD Image system; DMI, Korea) under magnification 400 of five individual microscopical fields (n=5), respectively. And the thickness of the SC (TSC) and the epidermis (TED) were calculated as follows:

Thickness of stratum corneum (TSC) = sum of each KL (μm)
Thickness of epidermis (TED) = TSC + SGB (μm)
% Changes compared with intact skin (%)
= [((Data of SLS or vehicle-Data of intact skin)/Data of intact skin)× 100]

% Changes compared with vehicle (%)
= [((Data of each applied agent-Data of vehicle)/Data of vehicle)× 100]

Transmission electron microscopy; TEM

Skin biopsy specimens from each area were fixed in buffered 4% paraformaldehyde, 1% glutaraldehyde, washed in a 0.1M cacodylate buffer, and post-fixed in 1% osmium tetroxide for 2 hours. The tissues were dehydrated with graded ethanol solutions, cleared in acetone, and infiltrated with and embedded in spurr's resin. The sections were examined by electron microscope (Hitachi 7100 FA, Hitachi, Japan) (20).

Statistical analysis

Statistical analyses were performed using ANOVA test (SPSS Inc, Ver 12.0). Normal distribution was tested before calculating the comparison. P-values of < 0.05 and < 0.01 were considered significant.

RESULTS

Transepidermal water loss; TEWL

During experimental period, TEWL of vehicle and SLS were significantly increased as compared with intact skin ($p < 0.05$, $p < 0.01$). However, no significant difference between vehicle and SLS was observed. It was considered that vehicle didn't affect on SLS induced damages.

Generally, TEWL was increased by skin barrier impairment. TEWL values were measured on day 0, 4, 8 and 12. The highest TEWL values were seen on 4th day after irritation by SLS in all 4 types of applied agents. On day 8, CER and ILM showed a significant decrease of TEWL ($p < 0.05$). On day 12, all 4 types of applied agents significantly decreased TEWL as compared with vehicle ($p < 0.01$), most of all CER and ILM significantly decreased TEWL more than CHO and LIN. (Fig 1 & 2)

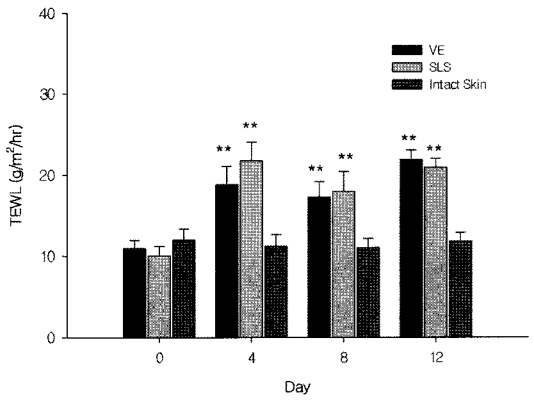

Fig 1. The changes of TEWL after topical application of VE and SLS in canine skin. *: p<0.05, **: p<0.01. VE: 1.25% SLS + vehicle, SLS: 1.25% Sodium lauryl sulphate

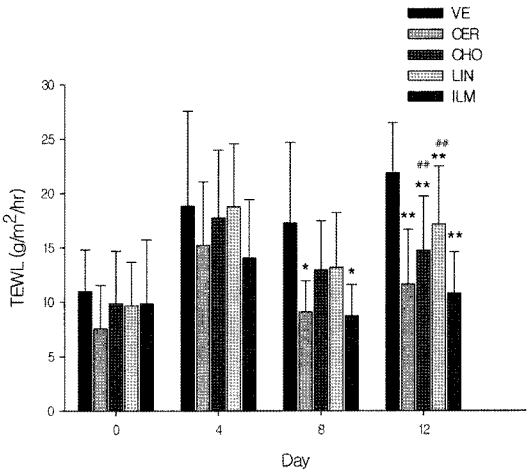

Fig 2. Canine TEWL measurements after topical application of CER, CHO, LIN and ILM on SLS-damaged skin. *(p<0.05) and **(p<0.01) represent a relation between vehicle and each lipid agent. #(p<0.05) and ##(p<0.01) show represent a relation between each lipid agent. VE: vehicle, CER: 2% ceramide, CHO: 2% cholesterol, LIN: 2% linoleic acid, ILM: 2% intercellular lipid mixture

Skin hydration

Topical application of vehicle and SLS resulted in a significantly decreased skin hydration on day 12 ($p < 0.01$). No significant difference between vehicle and SLS was observed. It was considered that vehicle didn't affect on SLS induced damages. Each lipid agent protected or increased skin hydration. CER and ILM significantly increased skin hydration on day 4, 8 and 12 ($p < 0.01$).

On day 12, CHO significantly increased skin hydration ($p < 0.05$) however, CER and ILM significantly increased more than CHO ($p < 0.01$). LIN didn't show effects on a decreased skin hydration by SLS during experimental period ($p < 0.05$, $p < 0.01$). (Fig 3 & 4)

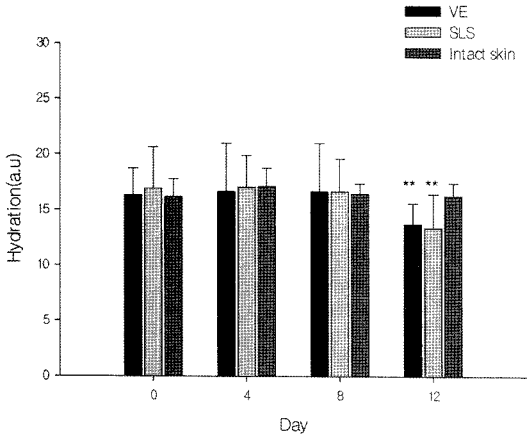

Fig 3. Canine skin hydration measurements after topical application of VE and SLS. **: p<0.01). VE: vehicle, SLS: 1.25% sodium lauryl sulphate.

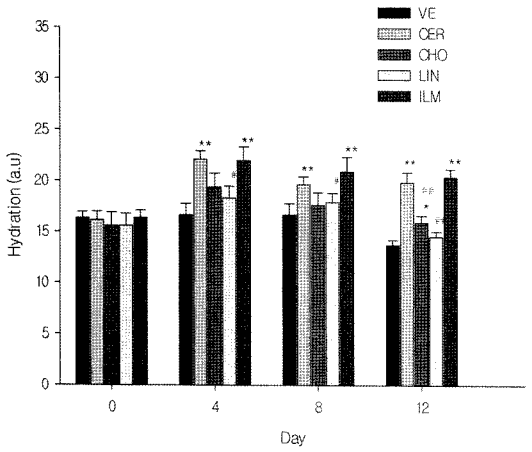

Fig 4. Canine skin hydration measurements after topical application of CER, CHO, LIN and ILM. *(p<0.05) and **(p<0.01) show represent a relation between vehicle and each lipid agent. #(p<0.05) and ##(p<0.01) represent a relation between each lipid agent. VE: vehicle, CER: 2% ceramide, CHO: 2% cholesterol, LIN: 2% linoleic acid, ILM: 2% intercellular lipid mixture.

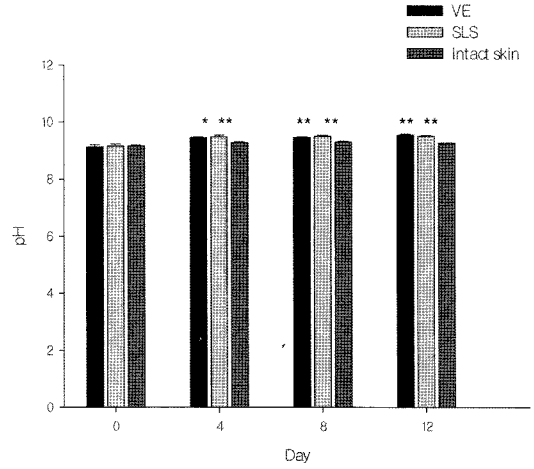

Fig 5. The changes of skin pH after topical application of VE and SLS. *: P<0.05, **: P<0.01. VE: vehicle, SLS: 1.25% sodium lauryl sulphate.

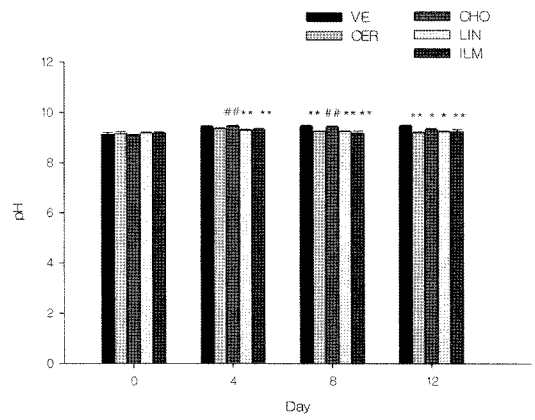

Fig 6. The change of skin pH after topical application of CER, CHO, LIN and ILM. *(p<0.05) and **(p<0.01) show a relation between vehicle and each lipid agent. #(p<0.05) and ##(p<0.01) represent a relation between each lipid agent. VE: vehicle, CER: 2% ceramide, CHO: 2% cholesterol, LIN: 2% linoleic acid, ILM: 2% intercellular lipid mixture.

Skin surface pH

Topical application of SLS on intact skin resulted in a significantly increased skin pH. Topically applied agents progressively reduced the increased skin pH by SLS. On day 4, 8 and 12, vehicle and SLS significantly increased skin pH, respectively ($p < 0.05$, $p < 0.01$). During experimental period, it was observed a significantly decreased skin pH in LIN and ILM ($p < 0.01$). CER significantly reduced the increased skin pH by SLS on day 8 and 12 ($p < 0.01$). On day 4 and 8, LIN and ILM significantly decreased more than CHO ($p < 0.05$). Especially, all applied agents significantly decreased skin pH on day 12($p < 0.05$, $p < 0.01$). (Fig 5 & 6).

Double fold skin thickness

Skin thickness was measured using double fold skin thickness method. Vehicle and SLS showed a significantly decreased skin thickness as compared with intact skin ($p < 0.01$). Skin thickness significantly decreased by SLS and vehicle. CER and ILM significantly increased the skin thickness as compared with vehicle ($p < 0.01$). The protective effects of CER and ILM were more excellent than CHO and LIN during experimental period ($p < 0.05$, $p < 0.01$). (Fig 7 & 8)

Histopathology

Significant decreases of the number of keratin layers, the thickness of stratum corneum and epidermis were detected in SLS and vehicle as compared with intact skin respectively ($p < 0.01$). However, the number of keratin layers, the thickness of stratum corneum and epidermis were significantly increased ($p < 0.01$ or $p < 0.05$) in all 4 types of treated agents in this study, as compared with vehicle except for the numbers of

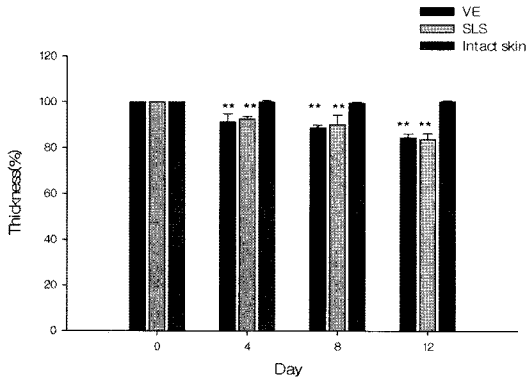

Fig 7. The changes of skin thickness after topical application of VE and SLS. It was measured using double fold skin thickness method. *: P<0.05, **: P<0.01. VE: vehicle, SLS: 1.25% sodium lauryl sulphate.

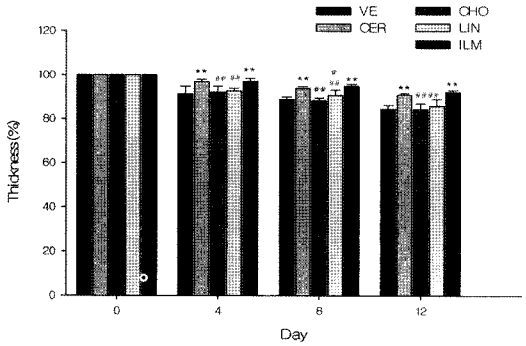

Fig 8. The changes of skin thickness after topical application of CER, CHO, LIN and ILM. *(p<0.05) and **(p<0.01) show represent a relation between vehicle and each lipid agent. #(p<0.05) and ##(p<0.01) represent a relation between each lipid agent. VE: vehicle, CER: 2% ceramide, CHO: 2% cholesterol, LIN: 2% linoleic acid, ILM: 2% intercellular lipid mixture.

keratin layers in CHO and thickness of stratum corneum in LIN, in which they were also non-significantly increased as compared with vehicle respectively.

The numbers of keratin layers in stratum corneum in SLS and vehicle were decreased as -75.36% and -73.91% as compared with intact skin respectively. However, they were increased as 144.44%, 38.89%, 44.44% and 272.22% in CER, CHO, LIN and ILM as compared with vehicle respectively.

The thicknesses of stratum corneum in SLS and vehicle were decreased as -64.38% and-62.55% as compared with intact skin respectively. However, they were increased as 126.75%, 27.43%, 7.83% and 172.85% in CER, CHO, LIN and ILM as compared with vehicle respectively.

The thicknesses of epidermis in SLS and vehicle were decreased as -63.18% and -58.64% as compared with intact skin respectively. However, they were increased as 52.10%, 7.41%, 17.73% and 95.45% in CER, CHO, LIN and ILM as compared with vehicle respectively. (Fig 9, Table 1)

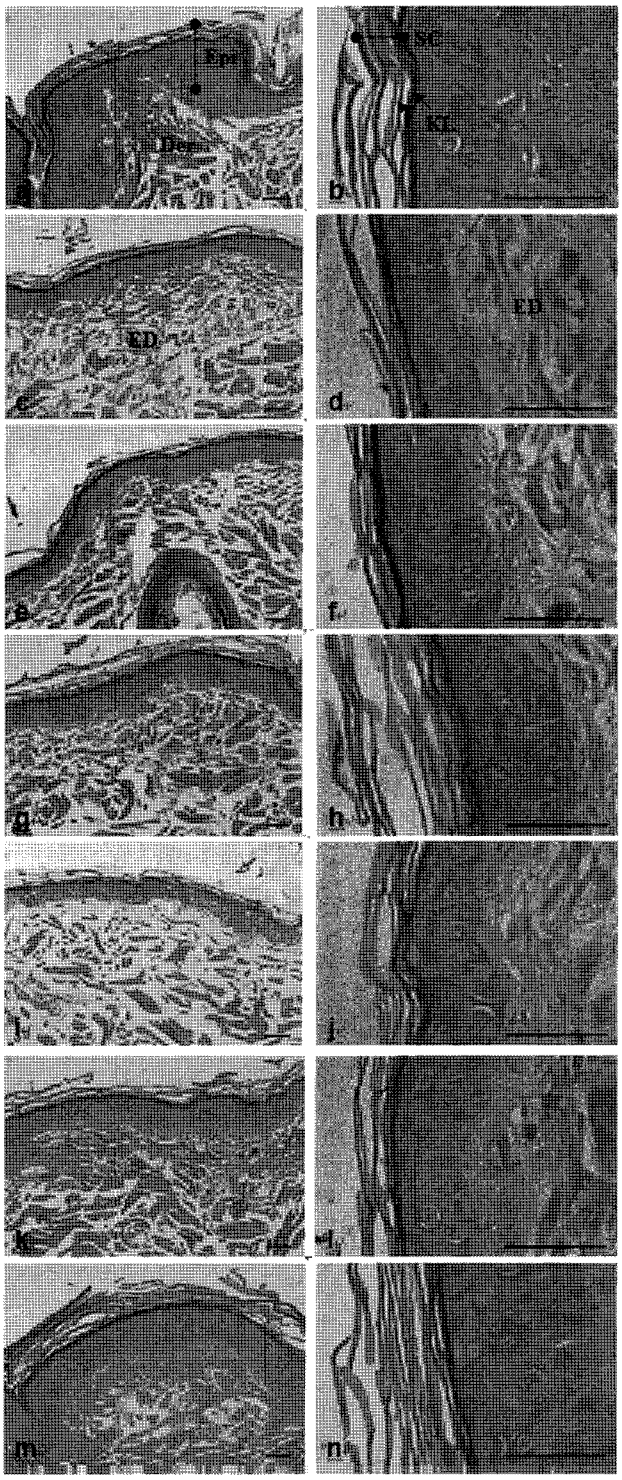

Fig 9. Histopathological profiles of intact skin (a,b), 1.25% SLS (c,d), vehicle (e,f), 2% ceramide (g,h), 2% cholesterol (i,j), 2% linoleic acid (k,l) and intercellular lipid mixture (m,n). The losses of keratin layers in SC and edematous changes (ED) in upper parts of dermis (Der)-contact dermatitis were detected, and consequently, the thickness of epidermis (Epi) and SC were decreased in 1.25% SLS and vehicle. However, these changes of microenvironment by SLS were markedly protected by topical application of lipids. All H&E stain; Scale bars = 80 μm.

Table 1. The changes of number of keratin layers in the SC and thickness of the SC and the epidermis

Groups	Number of keratin layers in stratum corneum	Thickness (μm) of	
		Stratum corneum	Epidermis
Group			
Intact skin	13.80 ± 2.17	28.04 ± 2.42	84.80 ± 6.32
SLS	3.40 ± 0.55** (−75.36%)	9.99 ± 1.12** (−64.38%)	31.23 ± 3.54** (−63.18%)
VE	3.60 ± 0.89** (−73.91%)	10.50 ± 0.89** (−62.55%)	35.07 ± 1.20** (-58.64%)
Treated groups			
CER	8.80 ± 1.30**,## (144.44%)	23.80 ± 2.43**,## (126.75%)	53.35 ± 3.13**,## (52.10%)
CHO	5.00 ± 0.71**,# (38.89%)	13.38 ± 1.39**,## (27.43%)	37.67 ± 1.75**,## (7.41%)
LIN	5.20 ± 0.84**,## (44.44%)	11.32 ± 0.95**,## (7.83%)	41.29 ± 1.52**,## (17.73%)
ILM (3:1:1)	13.40 ± 2.07## (272.22%)	28.64 ± 2.65*,## (172.85%)	68.55 ± 9.55**,## (95.45%)

Mean ± S.D. of five × 400 microscopic fields
* $p < 0.05$ and ** $p < 0.01$: A comparison with intact skin
# $p < 0.05$ and ## $p < 0.01$: A comparison with vehicle.
VE: vehicle+1.25% SLS, SLS: 1.25% sodium lauryl sulphate, CER: 2% ceramide+1.25% SLS, CHO: 2% cholesterol+1.25% SLS, LIN: 2% linoleic acid+1.25% SLS, ILM: 2% intercellular lipid mixture+1.25% SLS
(%): VE and SLS were compared with intact skin, respectively.
CER, CHO, LIN and ILM were compared with VE, respectively.

Nsmission transmission electronic microscopy; TEM

Specimens from skin biopsy were achieved on day 12. In intact skin, the well-maintained SC layer and corneodesmosome density were observed. SLS induced exfoliations of corneodesmosomes from the SC. Quite similar structure of SC was observed in vehicle and SLS, and the intercellular spaces of the SC were loose, and corneodesmosome density was declined. All the agents reduced degradations of corneodesmosome density. Especially, CER and ILM reduced degradations of corneodesmosome density more than CHO and LIN. (Fig 10)

DISCUSSION

The SC provides the major skin barrier to water loss and permeation of environmental substances, as well as contribution of mechanical protection (1,2). The skin barrier function was damaged by SLS in this study. During experimental period, no significant difference was observed between SLS and vehicle in all the measurement, it means, vehicle didn°Øt affect on SLS-damaged skin barrier function.

It was demonstrated that any one of the three components was increased about threefold to achieve a 3 : 1 : 1 molar ratio, barrier recovery was accelerated and, optimum lipid mixtures for the acceleration of barrier recovery include, in addition to ceramide and cholesterol, both nonessential and essential fatty acid (11,20,21). The 2% lipid concentration is the highest level that remains soluble in this vehicle and represents the same formulation used in previous studies (20,21). Previous studies have shown that application of any one or two of intercellular lipid on damaged skin impedes rather than facilitates the rates of barrier recovery in human (11,20,21).

However, different results were observed as compared with human study. CER and ILM significantly decreased TEWL more than CHO and LIN ($p < 0.05$, $p < 0.01$). Especially, CER showed effects as much as ILM. These results showed that CER and ILM were very effective on SLS-damaged canine skin barrier function. SLS influenced the skin barrier function by extraction of lipid from SC, which may be restored to be filled by topical application of lipids.

The TEWL increase after SLS irritation is not only due to extraction of lipids but is also a consequence of inflammation and spongiosis (13,16). SLS impairs the SC and exerts a direct toxic effect on the keratinocytes. In response to these changes, preformed cytokine interleukin-1α (IL-1α) is released from the SC, keratinocytes, langerhans cells, melanocytes and fibroblasts as the first step in the inflammatory cascade. IL-1α stimulates other keratinocytes and fibroblasts to produce and release more IL-1α and other pro-inflammatory cytoskine (TNFα, IL-6). Hyperkeratotic reaction of the SC and epidermis to repeated SLS exposure, often described as the skin hardening phenomenon (23, 24). The ceramides were known to modulate a secretion of PGE_2 in response to the action of IL-1. In previous studies, it was demonstrated that sphingosine enhances IL-1-mediated PGE_2 production in human dermal fibroblasts (8, 23,24,25). The ceramides also have a role of mediating TNFα action. TNFα appears to play a key role in the initiation of a protective as well as cognate immune response against bacterial and viral intracellular pathogens (26). These suggest that ceramides may be involved in modulating local immune functions (8). In previous studies, it was also demonstrated that daily treatment with SLS significantly increased the expression of intercellular adhesion molecule 1 (ICAM-1) by keratinocytes, and topical applied ceramide suppressed ICAM-1 expression as compared with untreated skin (17,26).

The changes of TEWL and SC hydration weren't paralleled in general. In this study, we could observe that SLS induced the increased TEWL and decreased SC hydration on

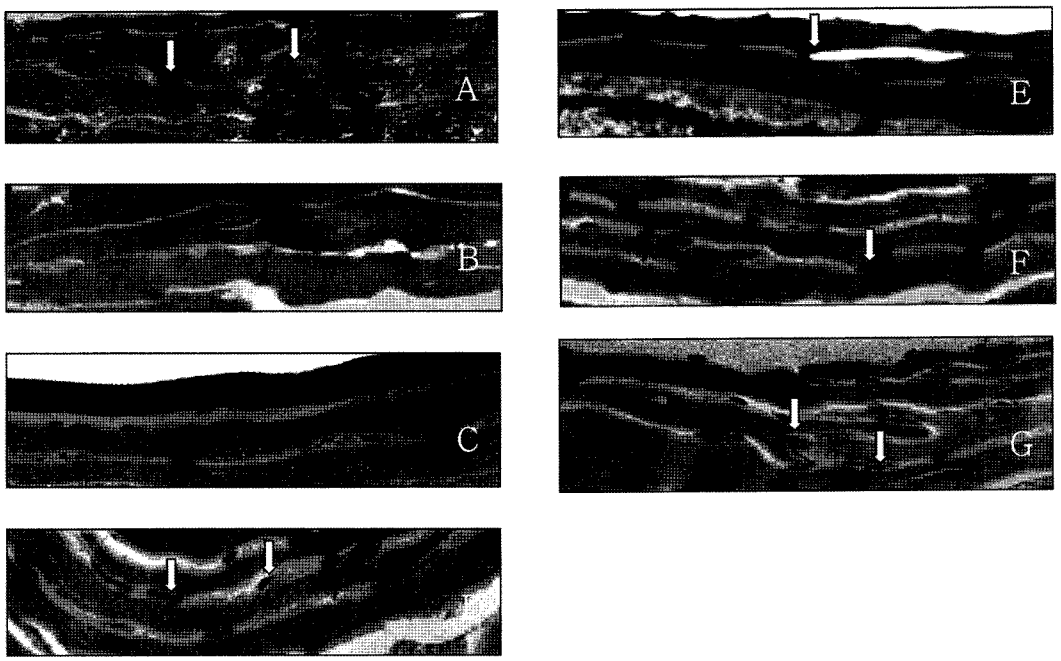

Fig 10. TEM of the stratum corneum (osmium tetroxide postfixation). SLS induced exfoliations of corneodesmosomes in the SC. A) Intact skin site. The well-maintained corneodesmosomes density of the SC is seen. B) Sodium lauryl sulphate (SLS)-applied site. The corneodesmosome density of the SC was reduced by SLS. C) Vehicle (VE)-applied site. The corneodesmosomes density of the SC was degraded by VE. Most of corneodesmosomes were exfoliated as compared with intact skin. D) Ceramide (CER)-applied site. Coneodesmosome density was declined as compared with intact skin. However, CER reduced the degradations of corneodesmosome density more than CHO and LIN. E) Cholesterol (CHO)-applied site. Corneodesmosome density was reduced as compared with intact skin. However, CHO didn't have effects as much as CER and ILM. F) Linoleic acid (LIN)-applied site. LIN reduced the degradations of corneodesmosome density of SC. G) Intercellular lipid mixture (ILM)-applied site. ILM protected exfoliations of corneodesmosomes on SLS-damaged skin. ILM seemed to have excellent effects on SLS-damaged skin. White arrow is corneodesmosome. Magnification: 10,000 ×.

canine skin. On day 12, it was observed the significantly decreased SC hydration in vehicle and SLS as compared with intact skin ($p < 0.01$). During experimental period, CER and ILM had an excellently protective effects on the decreased skin hydration by SLS ($p < 0.01$). LIN didn't show effects on SLS-damaged canine skin as compared with CER and ILM in skin hydration ($p < 0.05$, $p < 0.01$). It was considered that topically applied CER and ILM markedly reduced the extractions of intercellular lipid in SC. CER and ILM seems to be a very useful moisturizer.

The skin has long been known to display an acid surface-acid mantle-, which plays a role of permeability barrier function and cutaneous anti-microbial action and anti-inflammatory action. The skin surface pH is maintained and determined by amino acid, bicarbonate, filaggrin breakdown products and free fatty acid (12). However, Canine skin tends to have the highest pH of any mammalian species. In addition, beagles have a quite alkaline skin surface (pH 7.5-8.5) as compared with other breeds (mean pH 7.4), and the pH of the skin surface of dogs could increase by more than 1.0 pH unit within 1 min after they became very anxious (9). The skin surface pH of more than mean average pH 9.0 was explained by these. The skin surface pH was easily affected by irritant or skin disorder such as organic solvent, detergent, atopic dermatitis and xerosis. The increased skin surface pH resulted in a permeability barrier abnormality and altered SC cohesion (9,12). Since skin surface pH of beagle is close to alkali, beagle skin barrier function may be easily affected against exogenous stimuli as compared with other breeds. The skin pH was increased by SLS and vehicle ($p < 0.05$, $p < 0.01$). During experimental period, LIN significantly decreased the skin surface pH by its acidic pH ($p < 0.01$). On day 8 and 12, CER significantly prevented to increase skin pH by SLS ($p < 0.01$), and ILM significantly prevented to increase skin pH by SLS during experimental period ($p < 0.01$). CER and ILM seem to be good for repair of damaged skin barrier function as compared with CHO. Skin pH directly regulates epidermal permeability barrier homeostasis, and SC integrity and cohesion (18). Epidermal homeostasis is maintained not only by rate of cell production, but also through the rate of cell loss. Cell may be lost through two different mechanisms: necrosis or alternatively by a programmed cell death, termed apoptosis. Ceramides are considered in apoptosis and differentiation of keratinocyte, and ceramides may be considered in skin homeostasis. CER and ILM seemed to precipitate a repair of SLS-damaged skin, which may decrease the skin pH.

It was demonstrated that the thicknesses of the epidermis

and the SC were decreased by SLS in previous studies (8,15, 26). In this study, quite similar results were detected in skin thickness. We measured skin thickness using double skin fold method on day 0, 4, 8 and 12. Vehicle and SLS induced a decrease of skin thickness during experimental period ($p < 0.01$). CER and ILM prevented the decreased skin thickness as compared with vehicle and SLS during experimental period ($p < 0.01$). CHO and LIN didn't have a significant effect on the decreased skin thickness by SLS ($p < 0.05$, $p < 0.01$). CER and ILM were considered to recover the SLS-damaged skin.

Histological profiles demonstrated a significant diminution of the number of SC layer in SLS-damaged skin. It was shown that losses of keratin layers in SC and edematous changes in upper layer of dermis as results of SLS treatment, which is often observed in irritant contact dermatitis in human, and consequently, thickness of the epidermis and the SC were decreased in histological profiles (8,15,26,29,31). Quite similar histological profiles were detected between SLS and vehicle, it means, vehicle didn't or slightly affected on SLS-damaged skin. All lipids reduced diminutions of keratin layer in SC and thickness of SC as compared with SLS and vehicle. Quite similar results were detected between CHO and LIN. CER and ILM showed an excellent effect on SLS-damaged skin as compared with CHO and LIN. Especially ILM showed quite similar figure as compared with intact skin. It was considered that these lipids directly protect the losses of keratin layers.

Corneodesmosomes, the modified desmosomes of the SC, are largely responsible for the strong corneocyte cohesion, and are crucial for a proper barrier function of the epidermis. Their degradation at the epidermal surface is major importance for a normal desquamation process (25). It is composed of desmogleins and desmocollins (Dsc) as transmembrane components, with desmoplakins, plakoglobins and band 6 protein as cytoplasmic components. Desmogleins, a member of the cadherin family adhesion molecules, are classified into three isoforms called desmoglein-1 (Dsg1), Dsg2 and Dsg3 (17). Dsg 1 and Dsc 1 have been known to distribute in the uppermost layers of epidermis. The expression of Dsg-1 in canine skin was intense as compared with Dsg-3 (1). The desquamation process is a precisely controlled-cascade of events, is regulated by serine proteases in the intercellular regions of SC such as stratum corneum chymotryptic enzyme (SCCE) and stratum corneum tryptic enzyme (SCTE) (13). The increased skin pH by SLS seems to activate these enzymes. These activated enzymes seemed to induce an abnormal desquamation of the SC, and TEM profiles showed the degradations of corneodesmosome density. Ceramides are not only play an important role in skin barrier function but also involve in cell adhesion and epidermal differentiation, and play an important role of skin barrier homeostasis in which they have effects in repair of abnormal skin barrier function. CER and ILM significantly decreased the increased skin pH by SLS, and seemed to precipitate skin barrier homeostasis, which were considered to inactivate SCCE and SCTE and prevented the degradations of corneodesmosome density.

These results provided direct functional and morphological evidence that the canine skin barrier dysfunction by SLS were primarily due to decrease in the amount of lipid lamellae in the SC. And topically applied lipids have an effect on SLS-damaged canine skin barrier function, especially CER and ILM. As a whole, CER showed effects as much as optimized lipid mixture in canine skin. Skin structure, thickness, composition and epidermal turnover rate of dog were different with human. Therefore, the different results seemed to be observed in canine skin as compared with human. We recommend considering the difference between human and dog sufficiently. We performed to confirm the effect of ceramide using pseudoceramide 3 in this study. Whether other ceramide type and natural ceramide have similar effects on barrier repair or not, remains to be investigated in further experiments.

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개에서 sodium lauryl sulphate에 의한 손상 피부에 대한 각질세포간 지질의 국소적용 효과

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요 약: 세라마이드, 콜레스테롤, 자유지방산은 각질세포간 주요 지질로써 피부장벽의 생성과 유지에 있어 중요한 역할을 담당한다. 그러나, 개에서 각질세포간 지질에 대한 역할은 거의 알려져 있지 않다. 본 연구는 개에서 1.25% sodium lauryl sulphate (SLS)에 의한 피부 장벽손상 유발후 2% 세라마이드, 2% 콜레스테롤, 2% 리놀레익산과 이들 세가지 지질의 혼합제 (혼합제)의 장벽손상 복구를 평가하고자 실시하였다. 피부장벽기능은 표피경유수분소실(TEWL), 피부 수화도, 피부 산도, 피부 두께 측정을 통하여 평가하였고 최종적으로 조직학적 분석과 투과 전자 현미경 (TEM)을 통하여 피부 장벽구조를 평가하였다. SLS는 개의 피부에 효과적으로 피부장벽 손상을 유발하였다. 표피경유수분소실은 세라마이드와 혼합제 적용부위에서 계면활성제 및 대조군과 비교하여 유의적인 감소를 나타내었다 ($p < 0.05$, $p < 0.01$). 표피경유수분소실은 12일째에 모든 지질 적용군에서 유의적으로 감소하였으나 특히 세라마이드와 혼합제에서 콜레스테롤과 리놀레익산보다 유의적으로 감소하였다 ($p < 0.01$). 실험 기간 동안, 피부 수화도는 세라마이드와 혼합제에서 유의적 증가를 보였다 ($p < 0.01$). 조직학적 분석에서도 각각의 지질들은 피부손상을 회복시켰다. 특히 세라마이드와 혼합제의 효과가 탁월하였고 각질층의 두께도 대조군에 비해 유의적으로 증가되었다. 피부 산도는 세라마이드, 리놀레익산, 혼합제에서 유의적으로 감소하였다 ($p < 0.05$). 따라서 세라마이드와 혼합제의 국소적용은 계면활성제에 의한 피부 장벽 손상에 효과가 탁월함을 확인하였으며 아토피와 같은 염증성 피부염과 외부 자극에 의한 피부 장벽 손상시에도 세라마이드 또는 혼합제의 병용이 효과적일 것으로 사료된다.

주요어: 세라마이드, 지질, 피부장벽, SLS, 개