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Transepidermal Water Loss, Stratum Corneum Hydration and Transmission Electron Microscope Observation on Acetone Damaged Canine Skin Barrier Model

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Abstract : The purpose of this study is to establish experimental canine skin barrier disruption model in dog. The study was designed to investigate the predictive properties of acetone damage using as effect parameters transepidermal water loss (TEWL) and stratum corneum (SC) hydration. To compare the structures of SC intercellular lipids in normal and acetone damaged skin, TEM observations were performed. Six clinically normal, male Beagles without dermatological problems were chosen for this study. Acetone damage was performed at 48hrs after clipping. Efficacy measurements for TEWL and skin hydration were performed before (t_1) and after (t_2) damage in a temperature- and humidity-controlled room (20-22°C, 50-60%). TEWL and SC hydration values were decreased in the acetone damaged model compared with non damaged skin. In TEM observation of acetone damaged sample, the intercellular lipid lamellae exhibit abnormal and incomplete structure compared with those of normal skin. It seems that acetone damage would be one of canine skin epidermal barrier disruption model for the study of canine atopic dermatitis (AD) as well as dry skin in veterinary dermatology research.

Key words **:** Canine, Skin barrier, Acetone, TEM, TEWL, stratum corneum hydration.

Introduction

Various skin disorders such as atopic dermatitis (AD), psoriasis, ichthyosis and severe xerosis are characterized by impaired stratum corneum (SC) barrier and increased Trans epidermal Water Loss (TEWL) (2,6,15,16). To study of the xerosis associated with itch following barrier perturbation, such as senile xerosis, seasonal xerosis, AD, uremic xerosis, human immunodeficiency virus xerosis, familial amyloidosis and congenital ichthyosis, many researchers made experimental dry skin animal model with acetone, sodium lauryl sulphate (SLS) and tape stripping (7,14). Pinnagoda *et al.* (16) found a correlation coefficient between the outcome of a 24 hr patch test and the outcome of a 4 day repeated SLS patch test in their study on a dish washing liquid. Barrier disruption has been accomplished with an acute acetone treatment model for human dry skin research (5,17). Inman *et al.* (8) performed some pioneering work with TEM observations and showed structures of SC intercellular lipids in normal and atopic dogs, and provided the evidence for continuity, as well as thickness variations of the intercellular lipid lamellae.

The purpose of this study is to establish experimental canine disrupted skin model for veterinary skin research. The study was designed to investigate the predictive properties of acetone damage using as effect parameters TEWL, the recovery rate, and as effect parameters skin hydration, the capacitance of hydration, to evaluate the inner ultra structurally changed epidermis of canine skin by TEM.

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Materials and Methods

Experimental animal

Six clinically normal, 2-4-year-old, male Beagle without dermatological problems and with a normal blood health profile were chosen for this study. The dogs were fed Natural Choice® Adult chicken and rice (Nutro Co., USA) for 12 weeks prior to testing and were kept in wire-floor cages in a room held at 27-32°C and 40-57% relative humidity. Clipping of the hair coat was performed on the shoulder back of each dog with a standard pair of shaving clippers (Oyster pro76®, No 40, Oyster Co., USA). Loose hair was brushed away before the probe was applied. The clipped shoulder back of each dog was divided into two parts for this experiment. Prior to this study, we obtained the approval of the Animal Ethics Committee of Kyungpook National University, in accordance with the guidelines of the National Research Council of Korea.

Acetone damage

Acetone damage was performed at 48h after clipping. One spot out of 2 spots on shoulder back was brought into contact twice with 5 mL acetone (Merck, Darmstadt, Germany) for 2.5 mins. No rubbing occurred. Skin contact was made by fixing Pyrex tubes (Extrelut® 15 mL, Merck, USA) filled with acetone on the skin while the dogs were gently moving. The acetone was then discarded. Evaluations were done before (t_1) and after (t_2) acetone treatment.

TEWL and SC hydration measurement

Dogs were acclimatized at least 20 min before efficacy measurements, and measured the efficacy of the TEWL and skin hydration before and after damage in a temperature- and humidity-controlled room (20-22°C, 50-60%). Physical and psychological stress was avoided before and during the experiments. On both treated and non-treated skin, TEWL and SC hydration measurements were carried out. TEWL was measured with an evaporimeter (VapoMeter® SWL2g; Delfin Technologies Ltd, Finland). The VapoMeter uses a closed chamber system, i.e. it computes a TEWL value (g/m^2/h) from the progressive increase in relative humidity inside the chamber within 10-12s. A Corneometer CM 825® (Courage+Khazaka Electronic GmbH, Germany) was used to determine SC hydration value (a.u) by capacitance measurements.

Table 1. TEWL and SC hydration values in the acetone damaged and no damaged skin

Time (hour)	TEWL (g/m^2/h)		SC hydration (a.u)	
Time (hour)	No damage	Acetone	No damage	Acetone
Before (t_1)	6.32 ± 1.35	6.57 ± 1.55	24.52 ± 2.72	26.94 ± 3.19
After (t_2)	6.33 ± 1.44	**4.65 ± 1.27	25.33 ± 2.36	**25.06 ± 2.87

(**: $P < 0.01$)

Transmission electron microscope (TEM)

For TEM observations, skin biopsy specimens were frozen in refrigerator and stored at -70°C until processing. Frozen skin sections (20 μm) were cut on a cryostat, mounted on positively charged slides, air dried, and fixed in Trump's fixative (4% formaldehyde, 1% glutaraldehyde in phosphate buffer). Sections were postfixed in 0.25% phosphate-buffered ruthenium tetroxide (Polysciences, USA) for 45 minutes at 4°C to preserve and stain epidermal lipid. The sections were dehydrated through graded ethanol solutions, cleared in acetone, and infiltrated with and embedded in Spurr's resin. Thin sections (800-1,000Å) were mounted on copper grids and examined on a Hitachi. H-7000 transmission electron microscope (Hitachi, Japan) operating at an accelerating voltage of 80 kV.

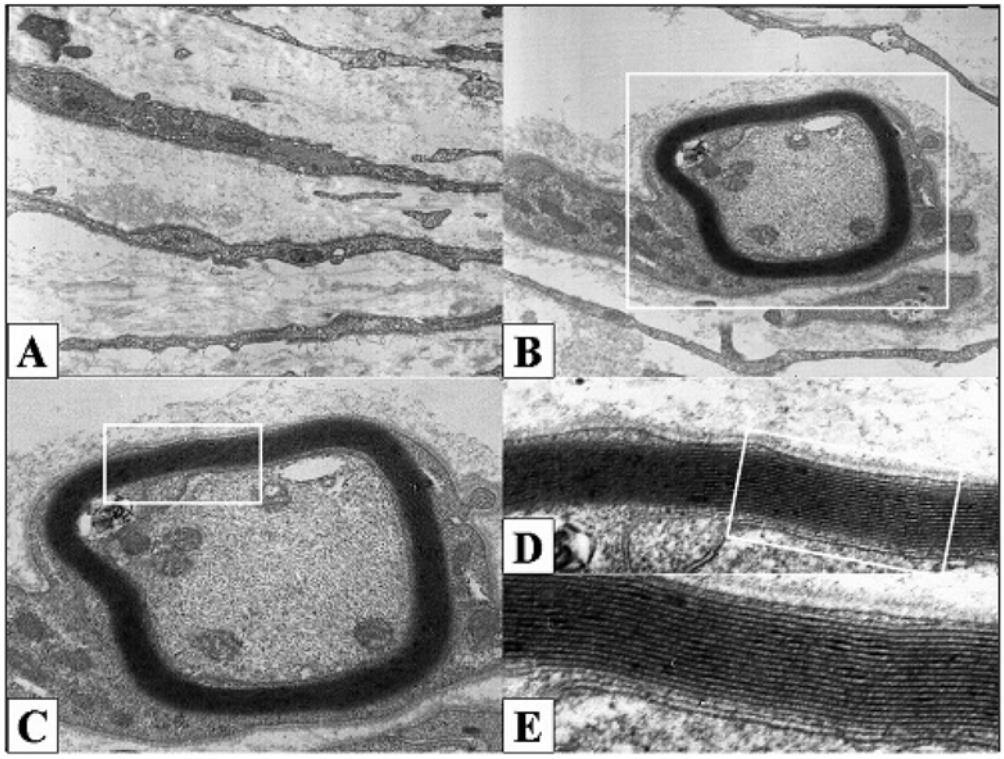

Fig 1. TEM of SC in no damaged skin. (Ruthenium tetroxide postfixation). *A.* Normal SC (× 9k). *B.* Lipid lamellae can be seen in the space between keratinocytes (white box) (× 10k). *C.* Detail of B. Lipid lamellae can be seen in the space between keratinocytes (white box) (× 20k). *D.* Detail of C. Well-formed lipid lamellae fill the intercellular spaces (white box) (× 30k). *E.* Detail of D. Ultrastructure of lipid bilayer in SC. Intercellular lipid lamellae are composed of alternating layers of polar lipid heads (electron dense, black) and nonpolar regions (electron lucent, white) (× 60k).

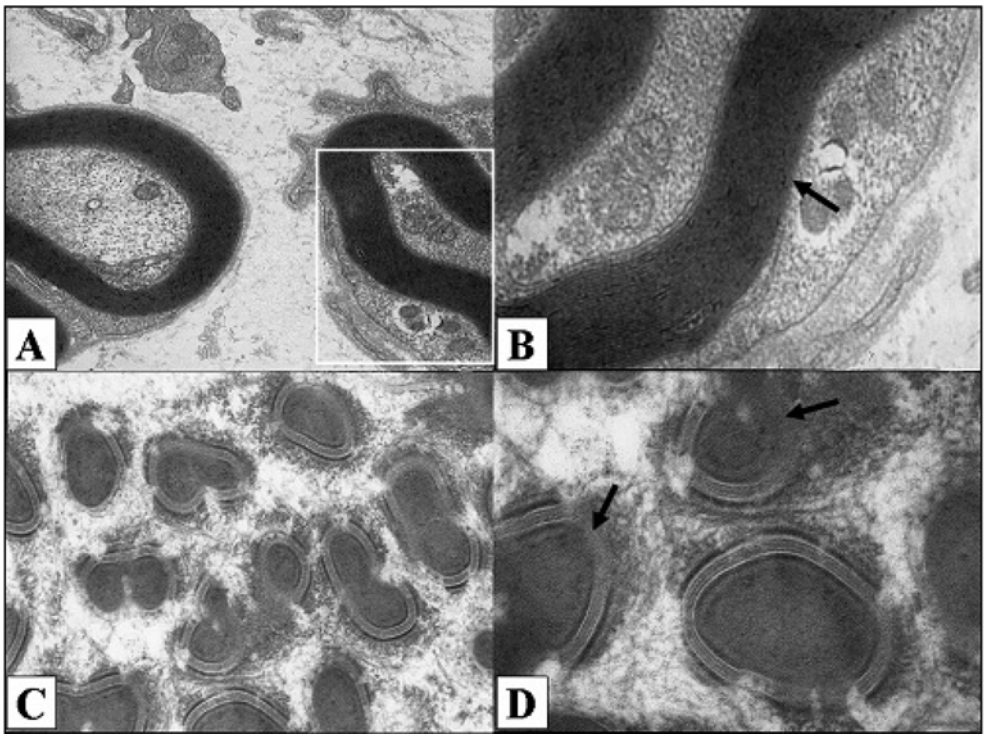

Fig 2. TEM of SC in acetone-damaged skin. (Ruthenium tetroxide postfixation). *A*. The intercellular lipid lamellae usually exhibit an abnormal and incomplete structure (white box) compared with those of no damaged skin (× 20k). *B*. Detail of A. The intercellular lipid lamellae usually exhibit an abnormal and incomplete structure (black arrow) compared with those of no damaged skin (× 40k). *C*. The intercellular lipid lamellae usually exhibit an abnormal and incomplete structure (black arrow) compared with those of no damaged skin (× 30k). *D*. Detail of C. The intercellular lipid lamellae usually exhibit an abnormal and incomplete structure (black arrows) compared with those of no damaged skin (× 60k).

Statistical analysis

To compare the effect on TEWL and SC hydration, a paired t-test (SPSS 10.0) was used to compare the effects between acetone damaged and no damaged skin at the same time. Statistical significance was set at $P < 0.05$.

Results

The effects of skin damage

In a paired t-test, TEWL and SC hydration values were statistically significant decreased ($P < 0.01$) in TEWL and SC hydration values were observed between before and after acetone damaged compared with non damaged skin (Table 1).

TEM

Ruthenium tetroxide postfixation TEM permitted the visualization of skin barrier ultra-structures, especially, SC intercellular lipids as multilayered lamellae in normal canine skin. In no damaged skin sample, lipid lamellae can be seen in the space between keratinocytes and well-formed lipid lamellae fill the intercellular spaces in SC (Fig 1). In addition, ultrastructure of lipid bilayer also can be seen in SC. Intercellular lipid lamellae are composed of alternating layers of polar lipid heads (electron dense, black) and nonpolar regions (electron lucent, white). In acetone damaged sample (Fig 2), the intercellular lipid lamellae exhibit and abnormal and incomplete structure compared with those of normal skin (Fig 1).

Discussion

In human, there are various skin barrier disruption models to study various skin diseases. Acute barrier disruption by organic solvent treatment, detergents, or tape stripping leads to the loss of the calcium gradient because of the passive loss of calcium from the upper epidermis (11,13). Abnormalities in the basic composition of the SC have been detected by noninvasive methods and showed an impairment in skin barrier function, TEWL (1,12) and reduction in SC hydration (1,3). Acetone damage results in the skin barrier disruption by superficial lipid extraction. Barrier disruption has been accomplished with an acute acetone treatment model to mimic dry skin conditions (5,17). Acetone can remove only the skin surface lipids, whereas the mode of action of SLS included the skin barrier disruption of the SC structure by deep lipid extraction (5,17). The present study was performed to evaluate the impact of distinct TEWL for barrier recovery as well as SC hydration before and after acetone damage. TEWL and SC hydration values were decreased in the acetone damaged model

compared with non damaged skin.

To compare the structures of SC intercellular lipids in normal and acetone damaged dog skin, TEM observations were performed. In acetone damaged sample, the intercellular lipid lamellae exhibit abnormal and incomplete structure compared with those of normal skin in this study. TEM of ruthenium tetroxide- fixed skin revealed extensive lipid lamellae deposition in the intercellular spaces of the SC in normal human, mouse and pig (10). The continuity and thickness of the intercellular lipid lamellae were significantly less in nonlesional atopic than in normal canine skin (8). Ruthenium tetroxide postfixation TEM seems to a suitable technique for investigating skin barrier ultra-structures, especially, SC intercellular lipids as multilayered lamellae in canine skin.

Epidermis is composed of several morphologically distinct layers such as basal, spinous, and granular cell layers (4). Disruption of this barrier function by compounds (e.g., detergents, solvents) or disease states (e.g., ichthyoses, AD, atopic xerosis) can alter the relative concentration of these lipids and result in an increase in TEWL (2,9). Although in the last 20 years, enormous progress has been made in elucidating the lipid organization in the SC, it is not fully understood in human dermatology. In this study, these preliminary observations suggest that the epidermal lipid barrier is disrupted in acetone damaged canine skin, and especially, the damage of lipid lamellae seems to be associated with the change of TEWL and SC hydration before and after acetone damage in canine skin. It seems that acetone damage would be one of canine skin epidermal barrier disruption model for the study of canine AD as well as dry skin. In addition, this acetone damaged skin model could be also used for veterinary dermatology research in skin barrier recovery shampoo, rinse and lotion for dry skin problems. Further study should be required to study the more detailed structures and compositions in canine normal and acetone damaged skin barrier.

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아세톤에 의해 손상된 개 피부장벽 모델에서의 경표피수분소실도 및 각질층보습도 평가와 전자현미경적 관찰

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요 약 : 본 연구는 실험적으로 개의 피부장벽 손상 모델을 평가하기 위해 아세톤을 이용하여 개 피부장벽의 손상을 유도하고 손상의 정도를 경표피수분소실과 각질층보습도 수치를 측정하였다. 손상 후의 각질층세포간 지질구조는 전자현미경을 이용하여 평가하였다. 피부 및 임상병리학적 검사에서 건강한 2~4세령의 수컷 비글 개 6마리를 사용하였으며 아세톤에 의해 피부는 삭모 후 48시간후 에 손상되었다. 경표피수분소실도와 각질층보습도는 아세톤 손상전보다 손상후에 유의성 있게 저하되었다. 아세톤 손상표피의 전자현미경학적 평가에서는 손상된 각질세포간 비정상적이고 불완전한 지질층판이 관찰되었다. 본 연구를 통하여 아세톤을 이용한 개의 피부장벽손상은 개 아토피나 건성피부 연구를 위한 손상모델 가능성이 있는 것으로 판단된다.

주요어 : 개, 피부장벽, 아세톤, 전자현미경, 경표피수분소실, 각질층보습도.