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Effects of Petrolatum and Glycerin on Acetone Damaged Canine Skin Barrier

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Abstract : The purpose of this study is to investigate whether the effects of topically applied petrolatum and glycerin on the barrier repair of acetone-induced skin damage in 6 beagle dogs. To confirm the effects of petrolatum and glycerin on acetone disrupted skin models, we performed to evaluate the characteristics of transepidermal water loss and SC hydration and scanning electron microscopic observations. TEWL and SC hydration measurements were carried out 3, 6, 12, 24, 48h after applying petrolatum and glycerin during recovery from acute disruption. Our results showed that there were some different effects between petrolatum and glycerin on the acetone damaged skin such as barrier function repair process and SC hydration status. The results indicate that the significant improvement could be observed in glycerin apply more than petrolatum after acetone damages, and further study will be required.

***Key words* :** Dog, acetone, skin barrier, petrolatum, glycerin

Introduction

Skin barrier function is clearly linked with transepidermal water loss (TEWL) and SC hydration as a linear relationship could be shown between the total amount of lipids removed from the stratum corneum and the degree of barrier disruption, expressed as a change in values of TEWL and SC hydration (1,16). In human skin research, there have been studied various skin barrier disruption models (by acetone-, sodium lauryl sulphate-, tape stripping damage-) to study various skin diseases, such as senile xerosis, seasonal xerosis, atopic dermatitis, uremic xerosis, human immunodeficiency virus xerosis, familial amyloidosis, congenital ichthyosis. In this respect, various skin disorders such as atopic dermatitis, psoriasis, ichthyosis and severe xerosis are characterized by impaired SC hydration and increased TEWL (2,3,10,15).

Glycerin may present crystal formation of SC lipids and preserve their normal structure even when the skin is underhydrated. Petrolatum is common occlusive substances like beeswax, lanolin, and various oils (4,9). Recovery with humectants or occlusive substance can be studied after irritation induced by chemicals, including SLS, or by tape stripping. In veterinary skin research, however, despite the high incidence of skin diseases associated with skin barrier disruption in the dog, to our knowledge, only few reports are available on the relationship between barrier disruption test and recovery test with humectants or occlusive substances.

The study was designed to study the efficacy of petrolatum as occlusive substance and glycerin as humectant on canine skin barrier function disturbed by the acetone damage.

Materials and Methods

Experimental animals and environment

Five clinically normal 2 - 4 year old, male Beagle dogs with no skin-associated problems and a normal blood health profile were chosen for this study. The dogs were fed Choice® Adult chicken and rice (Nutro Co., USA) for 12 weeks prior to testing and were kept in wired-floored cages in a room at 27-32 C° and 40-57 % relative humidity. The cages were inspected every 2 h during the day and any feces was removed. On the day of testing, the dogs were carried by hand to the test room and the measurement sites were visually inspected. They were not permitted to exercise and allowed to acclimatize at an ambient temperature of 20-21 C° and at a relative humidity of 40-48 % for 1 h before measurement.

Clipping and acetone irritation

Clipping of the hair coat was performed on the upper back (dorsal part of T2-T10 between the top of the scapular ridges) of each dog with a standard pair of shaving clippers (Oyster pro76®, No 40, Oyster Co., USA) (Fig 1). Loose hair was brushed away before measurement. The clipped are was divided into four parts. Barrier disruption was performed on the one of the 3 spots on the upper back of each dog by using acetone (Merck, Darmstadt, Germany). The spot was brought into contact twice with 40 × 40 mm² gauze soaked with 5 mL acetone for 2.5 min without any rubbing, and then the gauze was discarded.

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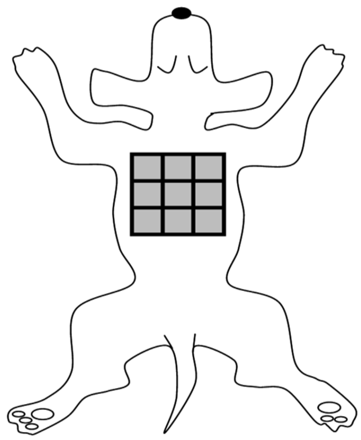

Fig 1. Schematic representation of the test sites on the upper back of dog. Clipping of the hair coat was performed on the upper back (dorsal part of T2-T12 between the top of the scapular ridges) of each dog. An area of $40 \times 40\ mm^2$ were marked, tape stripping and acetone damage were undertaken on these area for skin disruption. All test were randomized, as well as the sequence of application sites.

Topical application of petrolatum and glycerol

A single application of the glycerin ($1\ ml/cm^2$, Glycerin, Junsei, Japan) and petrolatum ($1\ g/cm^2$, Petroleum Jell, Puretest, USA) were performed on randomaly selected damaged skin site, respectivly. After application, measurements were carried out 3, 6, 24 and 48h. Damages(clipping only, acetone, or tape stripping) and single applications (DW, petrolatum, or glycerin) were performed 48h after clipping. Subsequent TEWL measurements were performed before (t_1=0), after (t_2=0) damage and treatment, following 3, 6, 24 and 48h after the damage and treatments. Physical and psychological stress was avoided before and during the experiments. All test skin spots were randomized, as well as the sequence of application spots.

Measurement of TEWL and SC hydration

Efficacy measurements for transepidermal water loss (TEWL) and skin hydration were carried out before (t_1) and after (t_2) tape stripping, and 3, 6, 12, 24, 48h after ion application in a temperature and humidity controlled room, 20-22 C^o and a 50-60 % and dogs were acclimatized for at least 20 min before efficacy measurements were carried out. Physical and psychological stress was avoided before and during the experiments. TEWL was measured using an evaporimeter (VapoMeter, Delfin Technologies Ltd, Finland) based on the guidelines given by Nuutinen et al. (12,13) The results were expressed as $g/m^2/h$. Electrical capacitance of the stratum corneum (SC) was measured with a capacitance meter (CM 825, Courage & Khazaka, Cologne, Germany). The capacitance was expressed in arbitrary units. Three readings were performed on each body site per set of measurements of each biophysical parameter.

Scanning electron microscopic observation

For SEM observations, the skin biopsy specimens were fixed in 2.5 % glutaraldehyde in buffer for 1 hr at room temperature (RT). The samples were washed in several changes of buffer for 4hours and rinsed for two times of 15minutes, postfixed in osmium tetroxide also for 2 hr at RT, and washed in distilled water. The samples were dehydrated in ethanol (60-90 %) for 10-20minutes, replaced on isoamyl acetate and then critical point-dried or air-dried. All samples were mounted on aluminum stubs, coated with ions (silver. Au) particles in a sputter coater, and examined with a Hitachi S-2500 scanning electron microscope (Hitachi, Japan).

Statistical analysis

Statistical analyses were conducted using paired samples t-test for the epidermal recovery effect of different ions on TEWL and capacitance in Beagle dog. All values were expressed as mean ± SD (SPSS 12.0 for Window, significance $P < 0.05$.).

Results

TEWL

The effects of petrolatum and glycerine on transepidermal water loss of canine epidermal barrier were showed in Fig 2. The application of petrolatum and glycerine significantly decreased TEWL value at 3, 6, 12, 24 and 48 h after barrier disruption compared with control. In the comparison the effects between petrolatum and glycerin on canine epidermal barrier recovery, the application of glycerine significantly

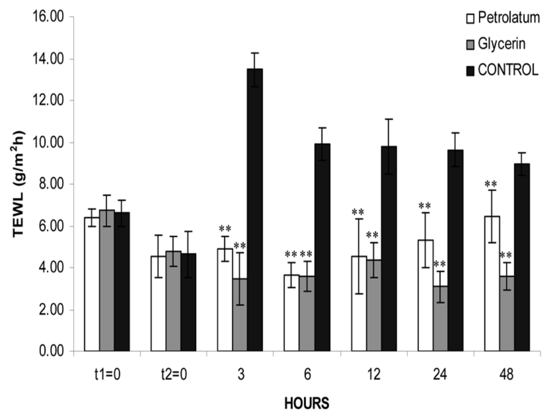

Fig 2. The effects of petrolatum and glycerin on transepidermal water loss of canine epidermal barrier recovery. Petrolatum and glycerine were spread on the upper back skin of Beagle dogs and measurements were carried out 3, 6, 24, 48 h after application. The application of petrolatum and glycerine significantly decreased TEWL value at 3, 6, 12, 24 and 48 h after barrier disruption compared with control. In the comparison the effects between petrolatum and glycerin on canine epidermal barrier recovery, the application of glycerine significantly decreased TEWL value than petrolatum at 3, 24, 48 h after barrier disruption ($n = 6$, $**P < 0.01$).

decreased TEWL value than petrolatum at 3 ($P < 0.05$), 24 and 48 h ($P < 0.01$) after barrier disruption ($n = 6$, $*P < 0.05$, $**P < 0.01$).

SC hydration

The effects of petrolatum and glycerine on canine epidermal hydration were showed in Fig 3. Petrolatum significantly decreased SC hydration value at 6 and 12 h and increased SC hydration value at 3 and 48 h compared with control. Whereas, glycerin significantly improved the SC hydration value at t_2, 3, 6, 12, 24, 48 h compared with control. The application of glycerine significantly ($P < 0.01$) increased SC hydration value than petrolatum at t_2, 3, 6, 12, 24 and 48 h after barrier disruption ($n = 6$, $*P < 0.05$, $**P < 0.01$).

SEM observation

Fig 4. shows scanning electron microscopic observation on canine skin surface 48 h after acetone damage and applications of petrolatum (G, H, I) and glycerin (D, E, F) compared with control (A, B, C). The irregular skin surfaces, hairs and some particle of petrolatum and glycerin were shown on each petrolatum and glycerin applied skin surface, respectively. Whereas, the disrupted skin surface and some scales were showed on the surface of control skin.

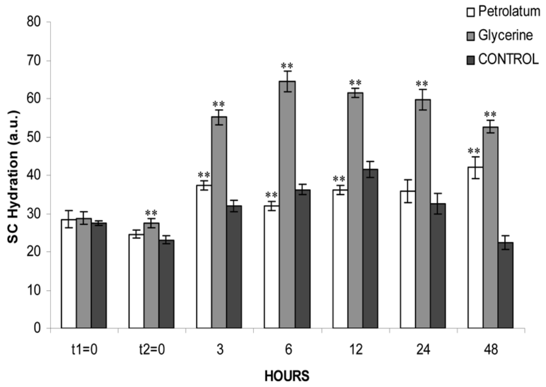

Fig 3. The mean values for capacitance of the stratum corneum (SC) taken from 6 beagle dog at various times ; before ($t_1 = 0$) and after ($t_2 = 0$) and 3, 6, 12, 24 and 48 h after topical applications of petrolatum, glycerin and distilled water (control) on acetone damaged canine skin. Results are expressed in arbitrary units. Petrolatum significantly decreased SC hydration value at 6 and 12 h and increased SC hydration value at 3 and 48 h compared with control. Whereas, glycerin significantly improved the SC hydration value at t_2, 3, 6, 12, 24, 48 h compared with control. The application of glycerin significantly ($P < 0.01$) increased SC hydration value than petrolatum at t_2, 3, 6, 12, 24 and 48 h after barrier disruption ($n = 6$, $*P < 0.05$, $**P < 0.01$).

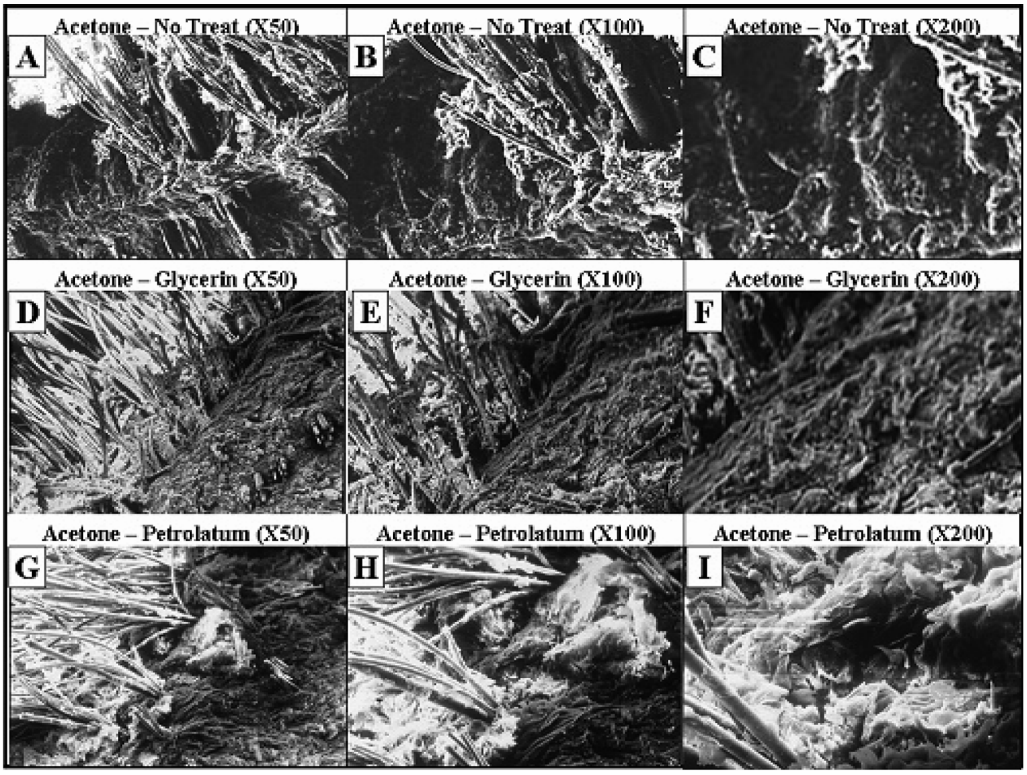

Fig 4. Scanning electron microscopic observation on canine skin surface 48 h after acetone damage and applications of petrolatum (G, H, I), glycerin (D, E, F) and distilled water (A, B, C). Petrolatum and glycerin particles can be seen on petrolatum and glycerin applied disrupted skin surfaces, whereas, superficial disruptions and some scales can be seen on distilled water applied the surface of skin (Osmium tetroxide postfixation. × 50, × 100, × 200).

Discussion

Acetone stress model has been used for barrier function and its recovery after and acute barrier insults, based primarily on the removal of surface lipids (both polar and non-polar SC lipids) by acetone treatment in human skin barrier research (5,18). In response to barrier disruption, a homeostatic repair response is initiated within the nucleated epidermis, which results in the rapid return of lipids to the SC and the return of the barrier function to normal (7,8,11,14,17). Zhai et al. (18,19) reported the characteristic of human barrier recovery after acetone perturbation, and suggested that this model provides simple methods of examining chemicals accelerating or inhibiting repair of these forms of acute skin damages in man.

As measured by TEWL and SC hydration, the differences could be found between petrolatum and glycerin the effect of the occlusive and moisturizing efficiency. In our study, the application of glycerine significantly decreased TEWL value and increased SC hydration value than petrolatum after barrier disruption by acetone over the time period. The conclusion of TEWL study showed that glycerin was more capable of restoring the damaged barrier than petrolatum. In the result of SC hydration, glycerin also more improved the hydration than those of petrolatum, therefore glycerin was more capable of skin hydration than petrolatum. These differences suggest that glycerine is more effective than petrolatum to epidermal barrier recovery and SC hydration on acetone damage.

Mason et al. (6) demonstrated scanning electron microscopic studies of the living canine epidermis and SC in dogs. In the comparison of skin surface - structures of acetone damage in our study, the characteristics of morphology of skin surface and scale were different between petrolatum and glycerin applications compared with control. Therefore, SEM could be a suitable technique for investigating skin barrier surface-structures, especially, morphology of skin surface on canine disrupted skin model study.

Different irritation patterns (sodium lauryl sulphate, tretionin, ultraviolet and dithranol etc.) and different measurement modalities (Laser-Doppler perfusion imaging, Laser-Doppler flowmetry, visual scoring, clorimetric measurements, Mexameter HB scale etc.) will be more needed to study canine skin disrupted models.

Conclusion

We utilized TEWL and SC hydration measurements to document the recovery of the skin barrier function and morphological observations in order to ascertain the different effects between petrolatum and glycerin on acetone damaged canine skin. Our results indicated that there were some differences between petrolatum and glycerin on acetone damage during the barrier function repair process. The results indicate that petrolatum and glycerin would be useful for canine skin barrier research such as various skin disruption studies. Further study will be required to confirm the histopathological and pharmacological changes of canine skin barrier recovery with petrolatum and glycerin.

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