A moisturizer formulated with glycerol and propylene glycol accelerates the recovery of skin barrier function after experimental disruption in dogs

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Background – Moisturizers are foundational therapies for human atopic dermatitis. In veterinary medicine, the use of moisturizers has been recommended by an expert committee to alleviate skin dryness that would occur, for example, in canine atopic dermatitis (cAD). However, little is known regarding the effects of moisturizers on the skin barrier.

Hypothesis/Objectives – To investigate the effects of a moisturizer on skin barrier recovery in a canine model of chronic mechanical barrier disruption.

Animals – Six healthy beagle dogs maintained in a laboratory setting.

Methods and materials – A model of chronic skin barrier disruption was simulated by tape stripping on both sides of the thorax. The moisturizer then was applied twice daily for one week to one side of the thorax, while the other hemithorax was left untreated. The effects were evaluated by measurement of transepidermal water loss (TEWL) at various times during skin barrier recovery, and by histological assessment of the disrupted skin one week after moisturizer application.

Results – Overall, TEWL was reduced, epidermal thickness was lower, stratum corneum thickness was greater and the intensity of the dermal inflammatory infiltrate was reduced for treated sites.

Conclusions and clinical importance – These results suggest a potential benefit of the moisturizer for improving skin barrier function, which is frequently altered in chronic inflammatory dermatoses such as cAD.

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barrier; most published studies have evaluated the occlusive effects of lipid-containing topical formulations and not humectants. 13–19 Efficacy of moisturizers can be assessed either in clinical studies or in experimental models with artificially damaged skin. In humans, 20 laboratory animals 21 and dogs, 22 mechanical disruption of the skin barrier can be generated by removing the SC through repeated applications of adhesive tape, a procedure known as tape stripping (TS). The integrity and the restoration of the skin barrier function then can be assessed in different ways. One of the most common noninvasive methods used in human studies is the measurement of transepidermal water loss (TEWL), 23 which is well correlated with skin barrier impairment. 24 In dogs, TEWL also has been used widely for the evaluation of skin barrier integrity. 5, 22, 25, 26

Ermidrà spray (ICF; Palazzo Pignano, Italy) is one of many moisturizers available for veterinary use and is indicated for xerosis in dogs. The primary components of this product are humectants including glycerol, dexpanthenol and propylene glycol, which are either freely distributed in the product or micro-encapsulated by cyclodextrins and liposomes (which are intended to enhance penetration and provide progressive release of the moisturizing agent). The aim of this study was to investigate the in vivo effect of the moisturizer on the recovery of the skin barrier function and inflammation induced by TS, through evaluation of TEWL measurement and histopathological examination of skin biopsies.

Methods and materials

All procedures were approved by the institutional Animal Care and Use Committee of our institute. (Ethics committee reference number 1021).

Animals

Six healthy, male, 2-year-old beagle dogs weighing 7 to 12 kg were used for these studies. None of the dogs had a history or current evidence of skin lesions, and had not received any systemic or topical therapies within the three months preceding the study. The dogs belonged to a research colony housed indoors in a temperature- and humidity-controlled facility (25–28°C, relative humidity 40–60%) and were housed in individual cement runs which were cleaned twice daily. Twelve weeks before and throughout the course of the study the dogs were fed the same maintenance dry food regimen (Specific Adult medium breed, Dechra Veterinary Products; Montigny le Bretonneux, France) and tap water ad libitum. One week before the beginning of the study, the dogs were acclimatized to their environment.

Tape stripping

The TS of the SC for barrier disruption was performed using a commercial adhesive tape (19 mm Scotch Transparent Tape, 3M; Cergy, France). At each test site, a new piece of tape was applied, pressed with finger pressure for 10 s, then removed in one swift motion. 22

Experimental procedure

The lateral thorax was selected as the test site because it provided adequate surface area for TS, skin biopsy and a horizontal orientation for the evaporimeter probe. In addition, TS in this area triggered little response from the dog and was less likely to be licked. The hair was gently clipped using an electric hair clipper (Favorita II GT 104, Aesculap; Suhl, Germany) 24 h before the start of the study in order to minimize any effect of recent hair clipping. The same investigator performed all procedures and measurements in order to minimize variability. Six sites were evaluated on each hemithorax (12 sites in total). Sites were approximately 4 cm² and spaced 3 cm apart. Each site was stripped until TEWL reached 50 g/m²/h. This procedure was carried out once a day for seven consecutive days.

From Day 7 to Day 13, 1 mL of moisturizer was applied on the six sites located on the right hemithorax every 12 h. On the left hemithorax, sites were left untreated as a control.

TEWL measurements

The TEWL was measured four times at each of the 12 sites on Day 7: just before the first application of the moisturizer (T0) and 2, 4 and 8 h after application (T2, T4 and T8). TEWL then was measured once daily until Day 15 (T24 to T192). Measurements were made using a portable, battery-operated, closed, unventilated chamber evaporimeter (VapoMeter SWL4001TJ, Delfin Technolosy Ltd; Kuopio, Finland). The evaporimeter was activated by pressing a single button, after which its probe placed directly onto the skin, perpendicular to the surface. The 1 cm diameter of probe was positioned in the centre of the 2 cm diameter (4 cm²) test area for the measurement. The pressure applied to the probe was sufficient to prevent air leakage between the probe and the skin. The device automatically determined the measurement duration and signaled completion with an audible tone. 22 Measurements were repeated consecutively three times and the mean of the three measurements was used as a representative value.14, 16, 18, 22 When there was a variation >15% between the three measurements, they were repeated. All measurements were performed in an air-conditioned room where the temperature and humidity were identical to those in the dog’s living area. Before each measurement, the dogs were acclimatedized to experimental conditions for 15–20 min and they were not allowed to exercise during the hour before the measurements.

Histological examination

For five of the six dogs, one skin biopsy was collected from each hemithorax after the final TS and repeated on days 7 and 15 (end of study). Skin biopsy specimens were collected using a 6 mm skin punch under local anaesthesia induced by subcutaneous injection of lidocaine (Xylovet, Ceva, La Ballastière, France). Specimens were fixed in 10% neutral buffered formalin for 24 h, embedded in paraffin, routinely processed in the laboratory, sectioned at 4 µm thickness and stained with Haematoxylin & Eosin. All skin biopsy specimens were examined in a blinded fashion by two of the authors. One section of tissue was examined per slide. The following histopathological changes were evaluated and scored: thickness and nature of the SC, thickness of the epidermis and intensity of the dermal inflammatory infiltrate (Table 1). Epidermal and SC thicknesses were determined using ImageJ software https://imagej.nih.gov/ij/. Three measurements were performed randomly through the epidermis present on the section and the mean of the three values was taken as representative.

Statistical analysis

All analyses were performed using R Development Core Team (2008) R: A language and environment for statistical computing (R Foundation for Statistical Computing; Vienna, Austria). To test the effect of the moisturizer on the three quantitative variables TEWL, epidermal thickness and SC thickness, we used three generalized mixed models (linear, GLMM; additive, GAMM) using the lme4 function of the R/lme4 package and the gamm() function of the R/MGCV package, respectively. We chose GLMM and GAMM for two main reasons. First, mixed models were implemented because two grouping variables ("Dog" and "Site" on each dog) needed to be specified with a random effect to take into account the nonindependence between measures made at the same site and on the same dog. Second, we selected a GAMM over a GLMM for the first model because one explanatory variable ("Time") was expected to have a nonlinear effect on the response variable (log-transformation of the
TEWL. Furthermore, GLMM and GAMM also allowed use of a continuous autoregressive correlation structure for the “Time” covariate.

In the TEWL GAMM, the log-transformation of the TEWL was used as the response variable with a normal distribution because it was normally distributed for nine of 12 groups of measures (T0 to T192, Shapiro–Wilk normality test), whereas the TEWL was normally distributed for only three of 12 groups of measurements. The treatment (moisturizer or control) was used as an explanatory variable with a fixed effect in order to test the impact of the moisturizer on the TEWL. “Time” was used as an explanatory variable with a non-linear fixed effect because TEWL was expected to decrease faster in the first days than in the later days when the skin barrier was closer to full recovery. “Site” and “Dog” were used as random effects to take into account nonindependence between measures made at the same time and on the same dog. We used a continuous autoregressive correlation structure for the “Time” covariate in order to take into account the correlation between the successive measures of TEWL made at the same site of the same dog.

Epidermal thickness was used as the response variable with a normal distribution in the GLMM model (Shapiro–Wilk normality test, \( P = 0.4953 \)). The treatment (moisturizer or control) was used as an explanatory variable with a fixed effect in order to test the impact of the moisturizer on epidermal thickness. “Dog” was used as a random effect to take into account the nonindependence between measures made on the same dog.

The SC thickness was used as response variable in the GLMM model, with a normal distribution (Shapiro–Wilk normality test, \( P = 0.6911 \)). The treatment (moisturizer or control) was used as an explanatory variable with a fixed effect in order to test the impact of Ermidr on the SC thickness. “Dog” was used as a random effect to take into account the nonindependence between measures made on the same dog. All equations are provided in the Supporting Information.

Finally, to test the impact of the moisturizer on the categorical variable “Intensity” of the dermal inflammatory infiltrate (including categories 0 to 3) we used a Kruskal–Wallis rank sum test.

### Results

#### TEWL measurement

Before disruption of the skin barrier, the mean TEWL of the six dogs was 11 g/m²/h. Overall, TEWL was lower for moisturizer-treated sites than control sites (Figure 1a). Results from the TEWL GAMM showed this difference was significant for the quantitative variable tested (TEWL; Table 2). Moreover, a faster decrease in TEWL was observed during the first 24 h for moisturizer-treated sites (Figure 2a, b). Interestingly, a short rise of the TEWL was noticed, based on visual inspection, at T8 on the control sites, yet this rise was not observed on moisturizer treated sites. On Day 15, TEWL values had returned to normal on both moisturizer treated and control sites. For the TEWL GAMM, “Time” also had a significant effect, where TEWL decreased with time (Figure S1).

#### Histopathological results

The application of the moisturizer decreased the intensity of epidermal hyperplasia. Overall, the epidermal thickness was thinner for moisturizer-treated sites (Figure 1c). Results from the epidermal thickness GLMM showed this difference was significant for the quantitative variable tested (Table 2). In the control sites, the epidermis retained a more proliferative appearance with the presence of small rete ridges (Figure 3b).

The application of the moisturizer also increased the restoration of the SC. Overall, SC thickness was greater for moisturizer-treated sites (Figure 1b). Results from the SC thickness GLMM showed that these differences were significant for the quantitative variable tested (Table 2). In the moisturizer-treated sites, the SC had more layers and had a basket weave appearance closer to normal (Figure 3a). However, in the control sites, the SC was thinner and more compact (Figure 3b). Finally, the application of the moisturizer was associated with a significant decrease in the intensity of the dermal inflammation (Kruskal–Wallis \( \chi^2 = 10.5281 \), d.f. 1, \( P = 0.001176 \)) (Figure 1d). The inflammatory infiltrate induced by chronic barrier disruption was almost absent in Ermidr–treated sites, whereas it persisted, with moderate intensity, in control sites (Figure 3a, b).

#### Discussion

This canine model of chronic mechanical skin barrier disruption allowed us to investigate the effect of the moisturizer Ermidr on both the restoration of the skin barrier, assessed by TEWL measurements, and the inflammatory modifications induced by chronic disruption, assessed by histopathological examination.

The study showed that the moisturizer tested could be indicated for promoting skin barrier restoration and for alleviating cutaneous inflammation. All parameters evaluated, including TEWL, epidermal thickness, SC thickness and dermal inflammatory intensity scores, improved in treated sites compared to control sites. Interestingly, a short duration peak in TEWL was noticed, based on visual inspection at 8 h on the control sites; this may have represented a measurement error.

In murine models, three phases of barrier recovery with distinct metabolic activities occur after acute barrier disruption. The first phase is initiated by secretion of a preformed pool of lamellar bodies which leads to a rapid decrease of TEWL values. In the second phase, increased lipid synthesis and accelerated lamellar body formation and secretion lead to a slower decrease of TEWL values. During the final phase, increased...
keratinocyte proliferation and differentiation complete the skin barrier recovery and the TEWL value returns to baseline levels. However, in chronic barrier disruption the second phase could be stronger than in the acute situation, leading to a small increase in TEWL values rather than a slower decrease. The use of moisturizers might avoid this TEWL increase during the restoration of the skin barrier.

Few clinical and experimental studies have been conducted using moisturizers on dogs and even fewer have used TEWL measurements to evaluate the skin barrier integrity. Results have been inconsistent. Randomized, double-blinded, placebo-controlled studies have evaluated the effects of topical blackcurrant emulsion (enriched in essential fatty acids, ceramides and 18-beta glycyrrhetinic acid), a topical lipid complex therapy, and topical demethicone on barrier function of dogs with cAD. All failed to demonstrate significant improvement in skin barrier function as measured by TEWL. By contrast, TEWL values were significantly decreased in

Figure 1. (a–c) Boxplots of treated and control sites: (a) for transepidermal water loss (TEWL), (b) for stratum corneum thickness and (c) for epidermis thickness.

For each boxplot the median (line within the box), first and third quartiles (box), nonoutlier range (whiskers) and outliers (dot) are shown. (d) Classification of inflammatory cells at treated and control sites as assessed by histopathological investigation. The intensity of the dermal inflammatory infiltrate was categorized as: 0 no infiltrate; 1 mild infiltrate; 2 moderate infiltrate; and 3 severe infiltrate.

| Model type | Response variable | Explanatory variable | Model coefficient | 95% confidence interval | P-value |
|------------|-------------------|----------------------|-------------------|------------------------|---------|
| GAMM¹     | log(TEWL)         | Treatment = moisturizer | -0.081            | [-0.0978; -0.0643]     | < 2x10⁻¹⁶ |
|           |                   | Time (s) = n.a.       |                   | n.a.                   |         |
| GLMM²     | Epidermal thickness | Treatment = moisturizer | -8.767            | [-10.53; 7.008]        | 7.31x10⁻¹⁴ |
| GLMM³     | SC thickness      | Treatment = moisturizer | 7.95              | [5.176; 10.72]         | 5.77x10⁻⁷ |

n.a., not assessed; TEWL, transepidermal water loss.
¹Generalized additive mixed model (GAMM): log(TEWL) ~ s(Time) + Treatment.
²Generalized linear mixed model (GLMM): Epidermal thickness ~ Treatment.
³Generalized linear mixed model: SC thickness ~ Treatment.
*Approximate significance of smooth terms given by the gaml() function.

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two open clinical studies evaluating a ceramide-based moisturizer in dogs with cAD, and a spray containing unsaturated fatty acids and essential oils. One study demonstrated a positive impact on skin inflammation after application of an emulsion in a canine model of chronic barrier disruption, and failed to show any impact on TEWL measurements.

Humectants, by absorbing water, can stimulate a water flux which creates a stimulus for barrier repair. One of the main hygroscopic components of the moisturizer is glycerol. Glycerol interacts with the SC lipid structures or with proteins, altering their own water-bonding and hydrophilic properties. It also promotes lamellar body secretion, modifies the plasticity of SC lipids, and has keratolytic effects. The moisturizer also contains propylene glycol which is known to have a partial occlusive effect. This combination allows for the creation of an artificial film on the skin surface until the defective barrier is repaired in order to maintain adequate stratum corneum hydration. Moreover, it is possible that some components of the moisturizer also could directly stimulate lipid metabolism. Dexpanthenol, for example, is a precursor of vitamin B5, a component of coenzyme A that catalyzes early steps in the synthesis of fatty acids and sphingolipids which are of critical importance for SC lipid bilayers. Lastly, based on the results of the present study, the moisturizer could act on the changes induced by the chronic skin barrier disruption, namely the epidermal hyperplasia and dermal inflammation, which also promote SC restoration. Numerous epidermal cytokines probably are involved in the inflammatory changes observed in chronic barrier disruption. Some components of the moisturizer, such as dexpanthenol and zinc gluconate, have anti-inflammatory properties that could contribute to the decrease of dermal inflammation.

In conclusion, the application of the moisturizer Ermidr both improved the restoration of the skin permeability barrier and decreased the inflammatory changes noted in a model of chronic SC barrier disruption. Because similar lesions and functional changes are observed in inflammatory dermatoses, the results of this study are useful in understanding the effects of moisturizers on the skin barrier and their significant role in the management of inflammatory dermatoses, particularly cAD.
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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Supplementary Material

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Résumé

Contexte – Les hydratants sont des traitements fondamentaux de la dermatite atopique humaine. En médecine vétérinaire, l’utilisation d’hydratants a été recommandée par un comité d’expert pour soulager la sécheresse cutanée qui pourrait se développer, par exemple, dans la dermatite atopique canine (CAD). Cependant, les effets des hydratants sur la barrière cutanée sont peu connus.

Hypothèses/objectifs – Déterminer les effets d’un hydratant sur la restauration de la barrière cutanée dans un modèle canin de dysfonction de barrière mécanique chronique.

Sujets – Six chiens beagles sains de laboratoire.
Matériaux et méthodes – Un modèle de dysfonction de barrière cutanée a été simulé par test à la cellulose adhésive sur les deux faces du thorax. L’hydratant a ensuite été appliqué deux fois par jour pendant une semaine à un coté du thorax, tandis que l’autre coté n’était pas traité. Les effets ont été évalués par la mesure de la perte d’eau trans-épidermique (TEWL) à des temps différents au cours de la réparation de la barrière cutanée et par examen histopathologique de la peau lésée une semaine après application de l’hydratant.

Résultats – La TEWL a été réduite, l’épaisseur épidermique était plus faible, l’épaisseur de la couche cornée était plus importante et l’intensité de l’infiltrat inflammatoire dermique était réduite pour les sites traités.

Conclusions et importance clinique – Ces résultats suggèrent un bénéfice potentiel de l’hydratant pour améliorer la fonction barrière cutanée, fréquemment altérée dans les dermatoses inflammatoires chroniques telles que la CAD.

RESUMEN
Introducción – las cremas hidratantes son terapias fundamentales para la dermatitis atópica humana. En medicina veterinaria, el uso de humectantes ha sido recomendado por un comité de expertos para aliviar la sequedad de la piel que ocurre, por ejemplo, en la dermatitis atópica canina (cAD). Sin embargo, se sabe poco sobre los efectos de las cremas hidratantes en la barrera cutánea.

Hipothesis/Objetivos – investigar los efectos de un humectante sobre la recuperación de la barrera cutánea en un modelo canino de alteración crónica de la barrera mecánica.

Animales – Seis perros beagle sanos mantenidos en un laboratorio.

Métodos y materiales – se simuló un modelo de alteración crónica de la barrera cutánea mediante cinta adhesiva en ambos lados del tórax. La crema hidratante se aplicó dos veces al día durante una semana a un lado del tórax, mientras que el otro hemitórax no se trató. Los efectos se evaluaron mediante la medición de la pérdida de agua transepidermática (TEWL) en varios momentos durante la recuperación de la barrera cutánea, y mediante la evaluación histológica de la piel alterada una semana después de la aplicación de la crema hidratante.

Resultados – en general, la TEWL se redujo, el grosor epidérmico fue menor, el grosor del estrato córneo fue mayor y la intensidad del infiltrado inflamatorio dérmico se redujo para los sitios tratados.

Conclusiones e importancia clínica – estos resultados sugieren un beneficio potencial de la crema hidratante para mejorar la función de barrera cutánea, que con frecuencia se altera en dermatosis inflamatorias crónicas como la cAD.

Zusammenfassung
Hintergrund – Feuchtigkeitscremes stellen in der Humanmedizin für die atopische Dermatitis eine fundamentale Therapie dar. In der Veterinärmedizin wurde die Verwendung von Feuchtigkeitscremes von einem ExpertenKomité empfohlen, um die Trockenheit der Haut zu mildern, zum Beispiel bei der atopischen Dermatitis (cAD). Es ist jedoch wenig bekannt über die Auswirkungen der Feuchtigkeitscremes auf die Hautbarriere.

Hypothese/Ziele – Eine Untersuchung der Wirksamkeit von Feuchtigkeitsspendern auf die Wiederherstellung der Hautbarriere nach chronischer mechanischer Zerstörung der Hautbarriere in einem Hundemodell.

Tiere – Sechs gesunde Beagles, die in einem Untersuchungslabor gehalten wurden.

Methoden und Materialien – Das Modell einer chronischen Zerstörung der Hautbarriere wurde mittels Klebestreifenabtragung an beiden Seiten des Thorax simuliert. Der Feuchtigkeitsspender wurde danach zweimal täglich eine Woche lang auf einer Seite des Thorax aufgetragen, während die andere Thoraxseite unbehandelt blieb. Die Auswirkungen wurden mittels Messung des transepidemischen Wasserverlustes (TEWL) zu unterschiedlichen Zeiten während der Erholung der Hautbarriere evaluiert und mittels histologischer Beurteilung der zerstörten Haut eine Woche nach Anwendung des Feuchtigkeitsspenders beurteilt.

Ergebnisse – Insgesamt war die TEWL reduziert, die epidermale Dicke niedriger, die Dicke des Stratum corneum erhöht und die Intensität des dermalen Entzündungsinfiltrates an mit behandelten Stellen reduziert.

Schlussfolgerungen und klinische Bedeutung – Diese Ergebnisse weisen auf eine mögliche positive Auswirkung des Feuchtigkeitsspenders zur Verbesserung der Funktion der Hautbarriere hin, die bei chronischen entzündlichen Dermatosen wie bei der cAD häufig verändert ist.
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Resumo

Contexto – Os hidratantes são terapias fundamentais para a dermatite atópica em humanos. Na medicina veterinária, o uso de hidratantes foi recomendado por um comitê de especialistas para aliviar o ressecamento da pele que pode ocorrer, por exemplo, na dermatite atópica canina (DAC). No entanto, pouco se sabe sobre os efeitos dos hidratantes na barreira da pele.

Hipótese / Objetivos – Investigar os efeitos de um hidratante na recuperação da barreira cutânea em um modelo canino de ruptura mecânica crônica da barreira.

Animais – Seis cães beagle saudáveis mantidos em laboratório.

Métodos e materiais – Um modelo de ruptura cutânea foi simulado utilizando fita adesiva em ambos os lados do tórax. O hidratante foi aplicado duas vezes ao dia por uma semana em um lado do tórax, enquanto o outro hemitórax foi deixado sem tratamento. Os efeitos foram avaliados pela medição da perda de água transepidêmica (TEWL) em vários momentos durante a recuperação da barreira cutânea e pela avaliação histológica da pele alterada uma semana após a aplicação do hidratante.

Resultados – De maneira geral, a TEWL foi reduzida, a espessura epidérmica foi menor, a espessura do estroma corneano foi maior e a intensidade do infiltrado inflamatório dérmico foi reduzida nos locais tratados.

Conclusões e importância clínica – Esses resultados sugerem um benefício potencial do hidratante para melhorar a função da barreira cutânea, que é frequentemente alterada nas dermatoses inflamatórias crônicas, como a DAC.