

***Dermatophagoides farinae* house dust mite allergen challenges reduce stratum corneum ceramides in an experimental dog model of acute atopic dermatitis**

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Background – Ceramides are essential stratum corneum (SC) lipids and they play a pivotal role in maintaining effective cutaneous barrier function.

Objectives – The present study aimed at determining the effect of a *Dermatophagoides farinae* house dust mite (Df-HDM) allergen challenge on SC ceramides of atopic dogs experimentally sensitized to these allergens.

Animals – Six Df-HDM-sensitized atopic Maltese–beagle dogs were used.

Methods – Prechallenge SC was obtained by cyanoacrylate stripping. One week later, the dogs were challenged topically with Df-HDM allergens, which resulted in mild to moderate inflammation 24 h later. Two weeks after challenge, SC of lesional and nonlesional skin was obtained. Finally, SC was collected from challenge sites 2 months after lesion resolution. The different SC lipids were quantified blindly by thin-layer chromatography.

Results – Significantly lower amounts of ceramides [AH], [AP], [AS], [NP], [EOP], [NS] and [EOS] were observed in lesional SC compared with prechallenge samples, while no significant effect was found on the amount of other lipids, including cholesterol and free fatty acids. The ceramide profile of nonlesional skin generally showed the same postchallenge reduction pattern. Ceramide amounts returned to normal within 2 months after lesion remission.

Conclusion and clinical importance – These findings suggest that the allergic reactions caused by Df-HDM allergens lead to a selective reduction of SC ceramides, not only at sites of inflammation but also at sites away from those of allergen application. There is normalization of ceramide amounts after inflammation subsides. These observations suggest that the deficiency of ceramides observed in canine atopic skin occurs, at least in part, secondary to inflammation.

Introduction

Although mostly <10 µm thick, the stratum corneum (SC) represents the most important component of the skin barrier. It consists of corneocytes embedded in extracellular lamellar lipids mainly composed of ceramides, cholesterol and free fatty acids.¹ Recent studies clearly pointed out that a disturbed barrier function might be involved in the induction and maintenance of atopic dermatitis (AD) in humans and dogs.^{2,3} Electron microscopic visualization of the intercellular lipids of dogs suffering from AD clearly demonstrates a heterogeneous deposition of lipid lamellae compared with that of normal canine SC.⁴ This observation prompted further studies of ceramide content and profiling, because these lipids are thought to play a central role in the disturbed skin barrier of human AD. Indeed, perturbations of the ceramide

homeostasis in canine AD have been repeatedly reported,^{5–7} and are linked to increased transepidermal water loss and decreased barrier function.^{2,5}

The present study was performed to determine the effect of a mite allergen challenge on the SC ceramide profile of six atopic Maltese–beagle crossbred dogs (MBA) experimentally sensitized to the house dust mite *Dermatophagoides farinae* (Df-HDM). This experimental canine AD model reproduces acute atopic skin lesions⁸ and it has been used, for example, to study the effect of histamine H₁ and H₄ receptor antagonists on skin lesion development.⁹ The advantage of this model is the selective induction of skin lesions by topical administration of Df-HDM allergen onto the axillary region, thereby allowing for sampling not only of lesional but also of distant nonlesional skin.

To test the hypothesis that allergenic challenge would result in decreased SC ceramide content, not only at the site of mite application but also away from it, we aimed to study the variation of SC lipid profiles before and after Df-HDM allergen challenge in mite-allergic dogs. We also studied the recovery of SC ceramides 2 months after

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lesion resolution. Our results provide evidence suggesting that inflammation causes a temporary reduction in ceramides and that changes also occur at sites distant from allergen application sites. Observed changes return to normal once inflammation subsides.

Materials and methods

All aspects of this study were approved beforehand by the North Carolina State University Institutional Animal Care and Use Committee.

Experimental animals

Six MBA dogs were enrolled in this study. This inbred line of dogs was selected because it is known to exhibit spontaneous hypersensitivity to food allergens and to develop AD-like signs upon challenge with such allergens.¹⁰ Moreover, our previous studies confirmed the easy sensitization of these dogs to Df-HDM allergens and the development of AD-like skin lesions following epicutaneous Df-HDM allergen challenges.⁸ These dogs do not usually develop skin lesions, apart from occasional bacterial folliculitis, if not provoked with an allergen to which they are hypersensitive. In all, there were five intact males and one spayed female. The average age was 6.5 years (range 3.8–12.8 years).

Sensitization protocol.

The hair was clipped 1 day before the SC was removed from the right axilla and groin with 10 consecutive tape strips performed using a 2.5-cm-wide clear adhesive tape (Scotch packaging tape; 3M Deutschland GmbH, Neuss, Germany). Each successive strip was performed with a new tape area to ensure appropriate stickiness and SC removal.

Following tape stripping, 500 µg of lyophilized Df whole mite powder (Greer laboratories, Lenoir, NC, USA) was suspended in mineral oil. Twenty microlitres of this suspension was rubbed onto the previously tape-stripped right axilla once weekly for eight applications, this sensitization lasting 2 months. After monthly boosters (three boosters on average) by epicutaneous Df-HDM application, all dogs enrolled in this study had elevated Df-specific IgE, positive Df intradermal test reactivity and positive skin lesions at the site of Df-HDM application.

Dermatophagoides farinae house dust mite challenges.

Mite challenges were performed on the right axilla of each of the six dogs with 500 µg of lyophilized Df-HDM as described above. Twenty-four hours after challenge, skin lesions were assessed as follows: erythematous macules, oedema, papules/pustules and excoriations were scored as 0 (absent), 1 (faint, mild) or 2 (strong, severe). The grades for each lesion were added to yield a lesional score. The maximal lesional score achievable was eight points.

Separation of the stratum corneum

One week before challenge, 2 weeks after and 2 months following lesion remission, SC samples were obtained according to Reiter *et al.*⁶ One drop of cyanoacrylate (3M Vetbond Veterinary Tissue Adhesive; 3M Deutschland GmbH) was applied to the sampling area after cutting the hair and a glass slide was pressed onto the cyanoacrylate. Thirty seconds later, the glass slide was carefully removed and was stored at –20°C or in dry ice until analysis. This procedure was performed twice at the same site to ensure capture of most, if not all, of the corneocyte layers.

Lipid extraction

After defrosting at room temperature, hair shafts were removed from the sample and the SC surface was determined by measuring the removed area using an ordinary office scanner (Stylus DX5000; Epson, Meerbush, Germany). The cyanoacrylate resin was scraped from the glass slide using a razor blade and transferred into a glass tube with a Teflon cap. Lipid extraction was also performed according to Reiter *et al.*⁶ by adding 4 mL hexane:ethanol (95:5, v/v) under son-

ication for 20 min. The supernatant containing the SC lipids was filtered through a Minisart filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany), dried under nitrogen and stored at –20°C until analysis.

Thin-layer chromatography

Extracted lipids were dissolved in chloroform:methanol (2:1, v/v) and were analysed by planar thin-layer chromatography as previously described¹¹ with some modifications. The different lipid classes were analysed according to comigrated lipid standards. Phosphatidylcholine, oleic acid, glyceryl trioleate, cholesteryl oleate, ceramides [AS] and [NS], galactocerebrosides, cholesteryl sulfate and cholesterol were obtained from Sigma-Aldrich (Steinheim, Germany). Ceramides [EOS], [NP] and [AP] were obtained from Evonik (Essen, Germany) or were kindly provided by Herbert Fuhrmann (University of Leipzig). One microlitre of each lipid extract was applied on a thin-layer chromatography plate after precleaning with methanol and activating for 30 min at 80°C. Separation of the lipids was achieved in a horizontal development chamber (CAMAG, Berlin, Germany) at room temperature using three solvent systems, as follows: (i) chloroform:ethanol:acetic acid (80:18:2%); (ii) chloroform:methanol:acetic acid (91.4:4.3:4.3%); and (iii) hexane:diethylether:acetic acid (72.7:18.2:9.1%). All chemicals were of the highest purity available. Diethylether was purchased from Merck (Darmstadt, Germany) and acetic acid from AppliChem (Darmstadt, Germany). All other chemicals were obtained from Labscan (Dublin, Ireland). After development, chromatograms were air dried for 15 min, dipped into an aqueous solution of copper sulfate pentahydrate (125 g/L) and phosphoric acid (125 g/L) for 30 s (Merck) and heated to 180°C for 30 min. Afterwards, an ordinary office scanner (Stylus DX5000; Epson, Meerbush, Germany) was used to capture a digital image, which could be analysed using image analysis software (ImageJ, National Institutes of Health, USA, <http://rsb.info.nih.gov/ij/>). Comigrated lipid standards at four different concentrations (0.125, 0.625, 1.25 and 1.875 µg/µL) were used to calculate the amount of the lipids in the SC samples. The SC lipid quantities are reported in micrograms per square centimetre of SC area.

Statistical analyses

Assuming the hypothesis that ceramide levels decreased postchallenge and returned to normal after resolution of lesions, one-tailed Wilcoxon matched-pairs signed rank tests (Graphpad Prism 5.00; Graphpad Inc., San Diego, CA, USA) were used to compare the amount of the different lipid classes between prechallenge samples in the MBA dogs and those 2 weeks (at the Df-HDM application site and 5–10 cm away) and 2 months post-Df-HDM challenge. Furthermore, the lipid amounts of the 2 months postlesion resolution samples were compared with those of the 2 weeks postchallenge samples using one-tailed Wilcoxon matched-pairs signed rank tests. A significance level of $P < 0.05$ was adopted.

Results

The different lipid classes and cholesterol:ceramide ratio were compared before treatment and after 2 weeks and 2 months, respectively. Two weeks postchallenge, the total ceramide content, as well as that of all ceramide fractions examined, was significantly lower at the site of mite application compared with prechallenge values (Figure 1 and Table 1). Similar reductions in total ceramide and fraction contents were also seen at nonlesional sites, at least 10 cm away from allergen application. The SC cholesterol fraction was altered after Df-HDM challenge at the allergen application site, with an upregulation in three of six dogs and a downregulation in the other dogs, thereby resulting in a high standard deviation (Table 1). Two months after lesions were no longer present, five of six dogs showed cholesterol amounts compa-

table to those before Df-HDM challenge. The increased cholesterol:ceramide ratio seen after Df-HDM challenge in lesional and nonlesional skin was restored to the initial ratio 2 months after lesion resolution, as well. Glycosylceramides were almost undetectable in the SC samples, with a low amount found only in one of six dogs after Df-HDM challenge.

The amount of SC free fatty acids and all other nonceramide lipid classes did not show any significant differences after Df-HDM application, either at the Df-HDM application site or 10 cm away (Table 1).

In summary, the comparison of SC lipid fractions at the various sites and time points showed a fairly specific reduction in ceramide content after challenge compared with pre-allergen application. Not only was this ceramide

reduction seen at the application site (i.e. lesional skin) but it also occurred at sites distant from it. Ceramide amounts returned to normal within 2 months after lesion remission except for Cer[NS].

Discussion

The present study reveals that lower amounts of total ceramides and ceramide [AH], [AP], [AS], [NP], [EOP], [NS] and [EOS] fractions were observed in postchallenge lesional and nonlesional SC compared with prechallenge samples, while no significant effect was found in the amount of other lipid classes, including cholesterol, cholesterol esters, phospholipids, triglycerides and free fatty acids. Two months after lesion resolution, the amounts of

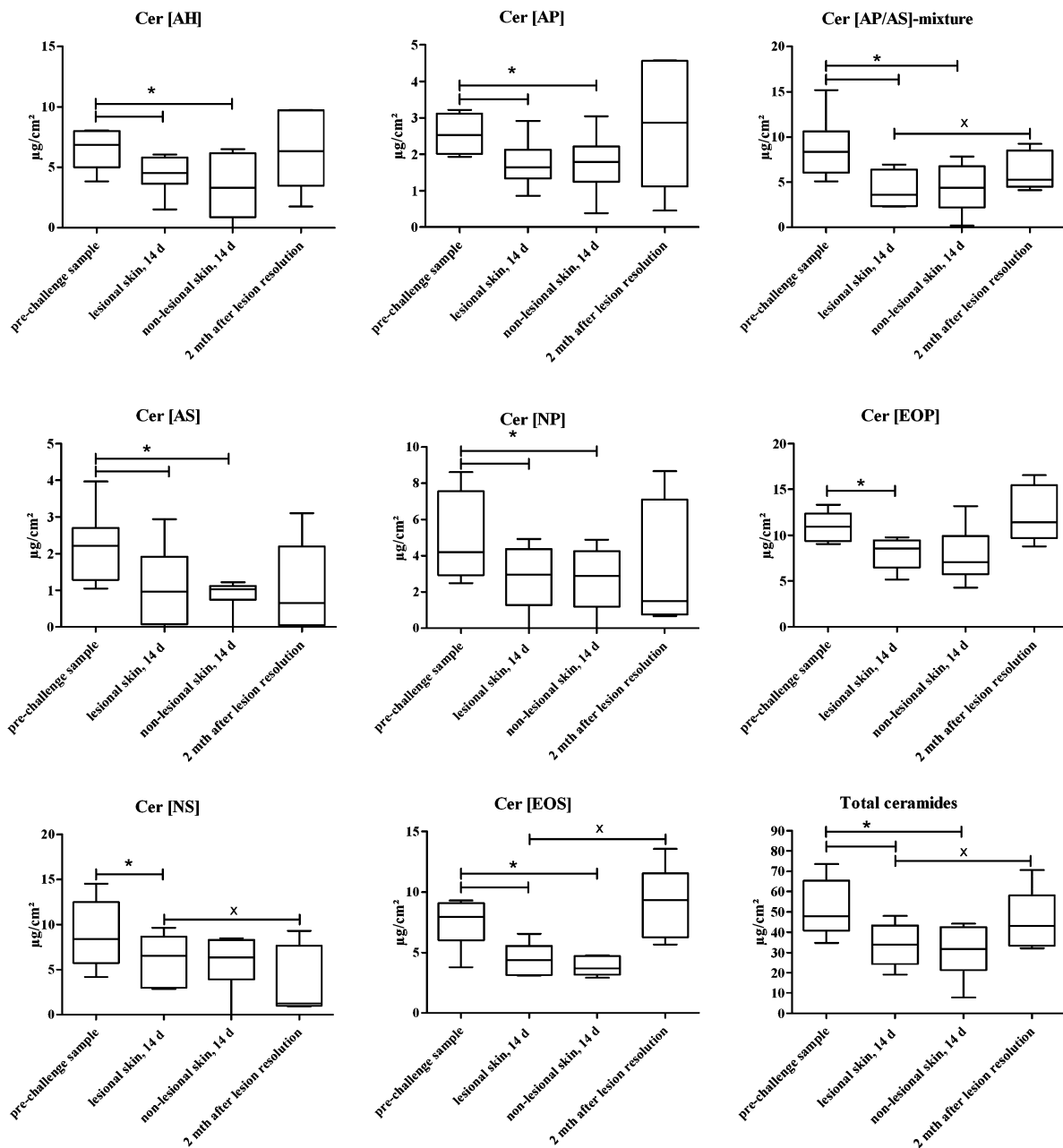


Figure 1. Amount of ceramide fractions of the stratum corneum of the right axilla of six Maltese-beagle dogs. Values are shown for the prechallenge sample, lesional skin on day 14 after *Dermatophagoides farinae* house dust mite (Df-HDM) application, nonlesional skin on day 14 after Df-HDM application and the Df-HDM application site 2 months after lesion resolution. * $P < 0.05$ versus control samples before HDM challenge. $\times P < 0.05$ versus 2 months after lesion resolution.

Table 1. Amount of nonceramide lipids and the cholesterol:ceramide ratio of stratum corneum of the right axilla of six Maltese–beagle dogs in the prechallenge sample and in lesional skin on day 14 after *Dermatophagoides farinae* house dust mite (Df-HDM) application, nonlesional skin on day 14 after Df-HDM application and the Df-HDM application site 2 months after lesion resolution

Lipid	Lipid ($\mu\text{g}/\text{cm}^2$)			
	Prechallenge sample	Lesional skin on day 14	Nonlesional skin on day 14	Two months after lesion resolution
Phospholipids	1.4 $\pm$ 1.7	1.0 $\pm$ 1.7	1.0 $\pm$ 2.0	0.01 $\pm$ 0.2
Cholesteryl sulfate	1.0 $\pm$ 1.7	0.6 $\pm$ 1.4	0.0 $\pm$ 0.0	0.0 $\pm$ 0.1
Glycosylceramides	0.0 $\pm$ 0.0	0.1 $\pm$ 0.2	0.0 $\pm$ 0.0	0.2 $\pm$ 0.5
Cholesterol	85.1 $\pm$ 24.9	106.4 $\pm$ 62.6	77.5 $\pm$ 35.0	73.3 $\pm$ 32.2
Free fatty acids	15.6 $\pm$ 9.4	17.7 $\pm$ 12.9	17.8 $\pm$ 3.5	18.6 $\pm$ 5.6
Triglycerides	10.5 $\pm$ 7.5	16.5 $\pm$ 10.2	11.6 $\pm$ 9.7	10.9 $\pm$ 5.1
Fatty acid methyl ester	8.8 $\pm$ 5.5	9.2 $\pm$ 3.0	8.5 $\pm$ 2.0	13.3 $\pm$ 6.0
Cholesteryl oleate	18.5 $\pm$ 7.0	18.6 $\pm$ 6.8	21.4 $\pm$ 4.5	15.6 $\pm$ 7.1
Total ceramides	51.5 $\pm$ 14.4	33.8 $\pm$ 10.5*†	30.5 $\pm$ 13.4*	45.2 $\pm$ 15.3
Cholesterol:ceramide ratio	1.7 $\pm$ 0.5	3.3 $\pm$ 2.1*†	3.2 $\pm$ 2.4*	1.6 $\pm$ 0.2

Values are means $\pm$ SD. * P < 0.05 versus control samples before HDM challenge. † P < 0.05 versus 2 months after lesion resolution

ceramides were comparable to prechallenge values. These findings suggest that the inflammatory reaction caused by Df-HDM allergens leads to the selective reduction of SC ceramides, not only at sites of allergic inflammation but also at sites away from those of allergen application.

There are few studies evaluating the SC lipid profile of normal and atopic dogs. At first, Reiter *et al.*⁶ described only a decrease in the proportion of SC ceramide 1 (i.e. CER[EOS]) and ceramide 9 (i.e. CER[EOP]) in the skin of dogs with AD. This reduction in the SC ceramide proportion was also reported by Shimada *et al.*⁵ Furthermore, a recent publication established that the overall profile of free ceramides in the SC was altered, with the quantity of total free ceramide being decreased in both nonlesional and lesional skin of dogs with AD.⁷ A second paper¹² confirmed this reduction, not only of free but also of protein-bound SC ceramides in dogs with AD. In the present study, we compared the variation of SC lipids after challenge compared with pretreatment values in experimentally sensitized atopic dogs. Our postchallenge results at lesional (i.e. allergen application sites) and nonlesional (i.e. distant) sites are comparable to those of two studies of nonlesional AD canine SC lipids,^{7,12} because we established that the ceramide decrease was not limited to only two of its subclasses, but rather that it was observed throughout the different ceramide classes.

Electron microscopic observations of SC postfixed with ruthenium tetroxide revealed that, in normal human and canine skin, the lipid lamellae are arranged in a repeated pattern, with electron translucent bands in a broad–narrow–broad sequence.¹³ For a correct organization of SC extracellular lamellae, a tightly balanced composition of ceramides, free fatty acids and cholesterol is essential. Specifically, ceramide 1 [EOS], which contains the ω -6 fatty acid linoleic acid linked to a long-chain ω -hydroxy fatty acid, plays a central role because it favours the formation of a long periodicity phase and an ordered lipid lamellar packing.¹⁴ Additionally, ceramide [EOP], also a linoleate-containing ω -esterified ceramide, is thought to be centrally involved in the formation of the extracellular lamellar lipids.¹³ Apart from the absolute amount of ceramides, the ratio between ceramides and cholesterol is crucial for an optimal organization of SC extracellular

lamellae. A change in the proportion of cholesterol to ceramides leads to a 'swelling' of the long periodicity phase, as shown in *in vitro* experiments.^{13,15} In the present study, there was a tendency for a shift in the cholesterol:ceramide ratio that is indicative for an altered barrier function. Furthermore, it has recently been shown that a decrease in ceramide fraction is correlated with an increased transepidermal water loss in dogs with AD.⁵ The reasons for a disturbed SC ceramide profile after challenge with Df-HDM allergens remain speculative; it could be due to either a reduction of ceramide synthase and/or an increase of ceramide-degrading enzymes. The amount of ceramide in the SC is regulated by ceramide-generating enzymes, such as serine-palmitoyl transferase,¹⁶ sphingomyelinase² and β -glucocerebrosidase,¹⁶ and the degrading enzyme, ceramidase.¹⁷ An influence on ceramide synthesis by cytokines has been described for human epidermal sheets.¹⁸ Tumour necrosis factor- α and interferon- γ both increase ceramide synthesis in epidermal sheets, probably by enhancing acid sphingomyelinase and glucocerebrosidase activity, whereas the addition of interleukin-4 reduces ceramide amounts.¹⁸ Interestingly, these reduced ceramide levels are also accompanied by an enhanced transepidermal water loss.¹⁸ These data are corroborated by an *in vivo* study in mice.¹⁹ The intradermal injection of picomolar concentrations of interleukin-4 inhibited the recovery of the epidermal barrier function up to 24 h after barrier disruption by tape stripping or acetone application.¹⁹ The SC of atopic dogs has been reported to exhibit higher amounts of glycosylceramides than that of normal dogs,²⁰ which is probably the result of a deficient activity of β -glucocerebrosidase. Interestingly, in our study, we only found traces of glycosylceramides in the SC of our atopic dogs, with alteration in only one of six dogs in lesional skin after HDM challenge.

The pathophysiology of disturbed ceramide pattern and lipid barrier is further complicated by the fact that ceramides themselves act as bioactive substances. For instance, a (partial) role of a short-chain ceramide as a second messenger of interferon- γ -induced upregulation of intercellular adhesion molecule-1 and of human leukocyte antigen-DR in keratinocytes,²¹ as well as in keratinocyte differentiation,²² has been described.

The repeated observations of a reduction of the proportion^{5,6} and quantity^{7,12} of ceramides in nonlesional canine AD SC, compared with those of normal dogs, have led to a paradigm shift regarding the pathogenesis of this canine disease. Indeed, these studies suggested the existence of an SC lipid defect in canine AD that is suspected to lead to barrier dysfunction, increased water loss and epidermal allergen penetration. As discussed in two recent reviews,^{23,24} it is still unknown whether the observed lipid defect is primary (i.e. a genetic defect predisposing to AD and pre-existing lesions) and/or secondary to allergic inflammation itself. The finding that the SC lipid defect is seen in nonlesional AD skin is often interpreted as being of primary origin.⁶ However, nonlesional atopic canine skin can hardly be construed as being normal skin. Indeed, it contains more inflammatory cells^{25,26} and proinflammatory cytokines^{27–29} than normal canine skin; it is therefore clinically normal in spite of being microscopically and immunologically inflamed. At the time of this writing, a primary SC lipid defect in canine atopic skin has not been convincingly demonstrated. For this to be established, one would have to demonstrate that the lipid defect existed before the first atopic skin lesions ever existed. Revealing a lower amount of lipids in visibly lesional and nonlesional atopic SC compared with that of normal dogs does not imply the existence of a primary epidermal lipid defect, because the changes seen could merely reflect those occurring secondary to allergic skin inflammation.

In conclusion, our results demonstrate that, in an experimental mite-associated canine acute AD model, Df-HDM-induced inflammation led to a reduction in the amount of all ceramide fractions examined. This reduction occurred not only at the site of previous lesions but also at sites distant from allergen application, sites that were away from any skin lesion. Furthermore, 2 months after the spontaneous resolution of skin lesions the ceramide levels returned to normal levels. These results argue for the ceramide deficiency seen in nonlesional atopic skin being due, at least in part, to allergic inflammation. Moreover, the recent report of the normalization of transepidermal water loss, which is correlated with ceramide proportion,⁵ after successful treatment of canine AD (i.e. with AD lesions in remission)³⁰ is in agreement with our observation (i.e. a regeneration of the ceramide levels 2 months after lesion resolutions) that the barrier defect might be due, at least in part, to atopic inflammation.

It remains to be determined whether an SC lipid defect secondary to allergic inflammation is of clinical and/or pathogenic relevance. As a result, whether or not topical lipid interventions that restore this defect would be of clinical benefit, as sole or adjuvant therapy, can only be proved by well-designed and controlled clinical trials.

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Résumé

Contexte – Les céramides sont des lipides essentiels du stratum corneum (SC) et jouent un rôle clé dans le maintien effectif de la fonction de barrière cutanée.

Objectifs – Cette étude vise à déterminer l'effet des tests d'allergènes d'acariens de la poussière de maison *Dermatophagoides farinae* (Df-HDM) sur les céramides du SC de chiens atopiques expérimentalement sensibilisés à ces allergènes.

Sujets – Six chiens atopiques beagle-maltais sensibilisés à Df-HDM ont été inclus.

Méthodes – Le SC a été préparé par application de cyano-acrylate. Une semaine plus tard, les chiens ont été testés topiquement avec les allergènes Df-HDM, résultant en une inflammation moyenne à modérée 24h plus tard. Deux semaines après les tests, le SC de peau lésionnelle et non-lésionnelle était obtenu. Enfin, le SC a été collecté de sites testés 2 mois après la résolution des lésions. Les différents lipides du SC ont été quantifiés en aveugle par chromatographie en couche mince.

Résultats – De significativement plus faibles quantités de céramides [AH], [AP], [AS], [NP], [EOP], [NS] et [EOS] ont été observés dans les SC lésionnels en comparaison avec les échantillons prélevés avant les tests. Aucun effet significatif n'a été trouvé sur la quantité des autres lipides, comme le cholestérol et les acides gras libres. Le profil des céramides de la peau non-lésionnelle a montré généralement le même patron de réduction après-tests. Les quantités de céramides sont revenues à la normale en 2 mois après la rémission des lésions.

Conclusions et importance Clinique – Ces données suggèrent que les réactions allergiques dues aux allergènes Df-HDM mènent à une diminution sélective des céramides du SC, non seulement aux sites d'inflammation mais aussi aux sites éloignés de ceux d'application des allergènes. Une normalisation des quantités de céramides s'opère après disparition de l'inflammation. Ces observations suggèrent que la carence de céramides observée dans la peau de chien atopique apparaît, au moins en partie, secondairement à l'inflammation.

Resumen

Introducción – las ceramidas son lípidos esenciales del estrato corneo (SC) y juegan un papel importante en el mantenimiento de la efectividad de la función de barrera de la piel.

Objetivos – el presente estudio pretende determinar el efecto de la re-exposición a alérgeno derivado del ácaro del polvo del hogar, *Dermatophagoides farinae*, (Df-DHM) sobre las ceramidas del SC en perros atópicos experimentalmente sensibilizados a estos alérgenos.

Animales – seis perros atópicos sensibilizados a Df-DHM cruces de Maltés y Beagle se utilizaron en el estudio.

Métodos – el SC previo a la re-exposición se obtuvo mediante cinta adhesiva de cianoacrilato. Una semana más tarde los perros fueron re-expuestos tópicamente a alérgenos Df-DHM, lo cual resultó en inflamación leve a moderada a las 24 horas. Dos semanas tras la re-exposición se obtuvo SC de áreas con y sin lesión. Finalmente SC se obtuvo de las zonas de re-exposición dos meses tras la resolución de las lesiones. Los diferentes lípidos del SC fueron cuantificados a ciegas mediante cromatografía de capa fina.

Resultados – se observaron cantidades significativamente menores de ceramidas [AH], [AP], [AS], [NP], [EOP], [NS] y [EOS] en las zonas de lesiones comparadas con las muestras anteriores a la re-exposición, mientras que no hubo efecto significativo en la cantidad de otros lípidos, incluidos colesterol y ácidos grasos libres. El perfil de ceramidas de la piel sin lesiones generalmente mostraba la misma reducción tras re-exposición. Las cantidades de ceramidas volvieron a los valores normales dos meses después de la remisión de las lesiones.

Conclusión e importancia clínica – estos hallazgos indican que las reacciones alérgicas causadas por alérgenos de Df-DHM provocan una reducción selectiva de ceramidas en el SC, no solo en áreas de inflamación sino también en zonas lejos de las áreas de re-exposición. Se produce vuelta a la normalidad en las cantidades de ceramidas tras la resolución de la inflamación. Estas observaciones sugieren que la deficiencia de ceramidas observada en la piel de perros atópicos ocurre, al menos en parte, de forma secundaria al proceso inflamatorio.

Zusammenfassung

Hintergrund – Ceramide sind essentielle Lipide im Stratum corneum (SC) und spielen eine entscheidende Rolle, um eine effektive Hautbarriere aufrechtzuerhalten.

Ziele – Die gegenwärtige Studie zielte darauf ab, die Wirkung einer Allergenprovokation mit *Dermatophagoides farinae* Hausstaubmilben (Df-HDM) auf SC Ceramide von atopischen Hunden, die experimentell auf Allergene sensibilisiert worden waren, zu bestimmen.

Tiere – Sechs Df-HDM sensibilisierte Malteser-Beagle Mischlinge wurden verwendet.

Methoden – SC wurde vor der Provokation mittels Cyanoacrylat Stripping entnommen. Eine Woche später wurden die Hunde topisch mit Df-HDM Allergenen provoziert, was 24h danach eine milde bis moderate Entzündung verursachte. Zwei Wochen nach der Provokation wurde SC von veränderter und unveränderter Haut entnommen. Letztendlich wurde SC 2 Monate nach der Abheilung der Veränderungen von Provokationsstellen entnommen. Die verschiedenen Lipide des SC wurden mittels Dünnschichtchromatographie blind quantifiziert.

Ergebnisse – Signifikant niedrigere Mengen an Ceramiden [AH],[AP],[AS],[NP],[EOP],[NS] und [EOS] wurden im veränderten SC im Vergleich zu Proben vor der Provokation gefunden, während keine signifikanten Veränderungen im Bezug auf die Menge der Lipide, inklusive Cholesterol und freie Fettsäuren, gefunden wurden. Das Ceramidprofil von nichtläsionaler Haut zeigte generell dasselbe Muster wie nach der Provokation. Die Mengen der Ceramide waren 2 Monate nach Ausheilung der Veränderungen wieder normal.

Schlussfolgerung und klinische Bedeutung – Diese Ergebnisse weisen darauf hin, dass allergische Reaktionen, die durch Df-HDM Allergene verursacht waren, zu einer selektiven Reduzierung der Ceramide im SC, nicht nur an den entzündlichen Stellen, sondern auch an Stellen, die weg von der Applikationsstelle des Allergens lagen, führten. Nachdem die Entzündung nachlässt, kommt es zu einer Normalisierung der Ceramidmengen. Diese Beobachtungen weisen darauf hin, dass ein Mangel an Ceramiden, wie er bei der atopischen Haut des Hundes beobachtet wurde, zumindest teilweise sekundär nach einer Entzündung vorkommt.

要約

背景 セラミドは角質層(SC)に必須の脂質で、それらは効果的な皮膚バリア機能の維持にきわめて重要な役割を担っている。

目的 この研究の目的は実験的に *Dermatophagoides farinae* ハウスダストマイトアレルギー (Df-HDM) に感作されたアトピー性皮膚炎の犬における、ハウスダストマイト暴露のSCセラミドに対する影響を決定することである。

供与動物 6頭のDf-HDMに感作されたアトピーのマルチーズビーグル犬を用いた。

方法 暴露前のSCはシアノアクリレートストリップによって採取した。1週間後、Df-HDMアレルギーを犬に塗布して暴露させ、それにより24時間後に軽度から中程度の炎症が起きた。感作から2週間後、病変部と非病変部のSCを採取した。最後に、SCは病変が消失した2か月後に暴露感作部位から回収した。SC脂質を盲検で薄層クロマトグラフィーにて定量した。

結果 セラミド [AH], [AP], [AS], [NP], [EOP], [NS] と [EOS] が病変部SCで感作前サンプルと比較して有意に減少していたが、コレステロールと遊離脂肪酸を含む他の脂質の量の変化に有意差はなかった。非病変部皮膚のセラミドプロファイルは全体的に暴露後減少パターンを示した。セラミド量は病変が寛解した2か月以内に正常に戻った。

結論と臨床的な重要性 これらの所見はDf-HDMアレルギーによるアレルギー反応が炎症部位だけでなくアレルギー塗布部位から離れた部位でもSCセラミドの選択的減少に導くことを示した。炎症消失後のセラミド量の正常化がみられた。それらの所見は犬アトピー皮膚が生じたときのセラミドの低下は、少なくともその一部は、炎症から2次的に生じたことを示唆している。

摘要

背景 - 神经酰胺是角质层 (SC) 必不可少的脂质成份, 它们对维持有效的皮肤屏障功能起到关键的作用。

目的 - 选择实验室方法致敏的异位性患犬, 用美洲户尘螨 (Df-HDM) 过敏原激发, 目的是研究其角质层神经酰胺的作用。

动物 - 使用6只Df-HDM过敏的患异位性疾病的马耳他-比格串种犬。

方法 - 激发前用氰基丙烯酸盐粘合剂剥离法获得角质层。一周后, 犬局部施以Df-HDM过敏原, 24小时后导致轻度至中度炎症。两周后, 对病变和非病变皮肤角质层采样。最后, 2个月时从激发部位收集病变恢复的角质层。采用薄层色谱法对不同角质层脂质盲量化。

结果 - 病变角质层与激发前样本相比, 神经酰胺 [AH], [AP], [AS], [NP], [EOP], [NS] 和 [EOS] 数量明显降低, 没有发现其他脂质在数量上有明显不同, 包括胆固醇和游离脂肪酸。无病变与激发后皮肤的神经酰胺谱系均普遍减少。神经酰胺数量在病变缓解后的两个月内恢复正常。

结论和临床价值 - 这些结果表明, 由于Df-HDM过敏原引起的过敏反应导致角质层神经酰胺选择性减少, 不仅在炎症灶也在远离施用过敏原的部位。炎症消退后神经酰胺数量正常。这些观察结果表明, 至少在某种程度上, 犬异位性皮肤的神经酰胺减少继发于炎症。