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Altered lipid properties of the stratum corneum in Canine Atopic Dermatitis

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ABSTRACT

Skin barrier disruption plays a role in the pathogenesis of atopic dermatitis (AD) in humans. However, little is known about skin barrier (dys-) function in Canine Atopic Dermatitis. The properties of lipids located in the outermost layer of the skin, the stratum corneum (SC) are considered to be important for the barrier. In the present study the lipid composition and lipid organization of the SC of AD dogs and control dogs were examined. The lipid composition of lesional AD skin as compared to control skin, showed a reduced free fatty acid level and a decreased ratio of ceramide[NS] C44/C34, in which C44 and C34 are the total numbers of carbon atoms of the sphingosine (S) and non-hydroxy (N) acyl chains. As a consequence of the observed changes in lipid composition in AD lesional skin the lamellar organization of lipids altered and a shift from orthorhombic to hexagonal lipid packing was monitored. Simultaneously an increased conformational disordering occurred. These changes are expected to compromise the integrity of the skin barrier. The C44/C34 chain length ratio of ceramide[NS] also showed a decreasing nonlinear relationship with the AD severity score (CADESI). Taken together, canine atopic skin showed alterations in SC lipid properties, similar to the changes observed in atopic dermatitis in humans, that correlated with a disruption of the skin barrier. Hence lipids play an important role in the pathogenesis of Canine Atopic Dermatitis.

1. Introduction

Atopic dermatitis (AD) in dogs, like in humans is a genetically predisposed chronic inflammatory and pruritic skin disease [1,2]. The pathogenesis of canine AD is not well understood and one of the paradigms is that skin barrier dysfunction may facilitate allergen penetration into the epidermal layers and subsequently induction of both innate and adaptive immune responsiveness causing clinical symptoms in sensitive individuals [1,3]. This may further deteriorate the barrier function, influence the microbiome of the skin and may lead to exacerbation of clinical symptoms as observed in AD in humans [4–6].

The stratum corneum (SC), the outermost layer of the epidermis, acts as the primary physical barrier of the skin. The “brick and mortar” structure of the SC consists of corneocytes (the bricks) embedded in a lipid matrix (the mortar) [7]. Integrity of the SC, particularly the lipid matrix, is important in maintaining the skin barrier function [8–11]. Previous studies have shown changes in lipid properties in non-lesional and lesional skin of human AD [9,12–16]. The main lipid classes are ceramides (CERs), free fatty acids (FFAs) and cholesterol (CHOL) [17–19]. Studies of human SC revealed that CERs, FFAs and CHOL assemble in two crystalline lamellar phases with repeat distances of approximately 6 and 13 nm, referred to as the short (SPP) and long periodicity phases (LPP), respectively [20,21]. The lipids within the lamellae may be organized in an orthorhombic lateral packing (very dense), a hexagonal lateral packing (less dense) or a liquid packing (high conformational disordering). Whereas the orthorhombic pattern is most abundantly present in SC of healthy human skin, it was shown that the fraction of lipids forming a hexagonal lateral packing is increased in SC of AD skin compared to that in control skin [9,14,22]. The altered lipid organization in AD skin can be correlated with the changes in lipid composition in the SC [23]. In more detail, a reduction in the skin barrier function in AD patients correlated with i) a decrease in total lipid content in SC [13,24], ii) a reduced chain length of the FFAs and the CERs [9,14], iii) an increase in the fraction of lipids forming a

Abbreviations: AD, atopic dermatitis; CADESI, Canine Atopic Dermatitis Extent and Severity Index; CT, control skin; NLS, non-lesional atopic skin; LS, lesional atopic skin

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hexagonal packing [9].

Currently only limited information is available concerning the lipid composition [6,25] and lipid organization [26–28] in SC of dog skin. Since in humans the impaired skin barrier plays an important role in the pathogenesis of AD, in the present study we examined the lipid composition, the lamellar and lateral organization in SC of lesional and non-lesional skin of AD dogs as well as control animals. Lesional atopic skin showed changes in the lipid composition and organization similar to those observed in atopic dermatitis in humans.

2. Material and methods

2.1. Animals

Three control dogs and five AD dogs were included in this study. The control Beagle dogs, owned by the Utrecht University Animal facility unit, aged between 1 and 3 years. The AD dogs (Bedlington beagle crossbreeds), owned by the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, aged between 2 and 9 years. The atopic dogs met the diagnostic criteria for AD and other causes of pruritus were ruled out [29]. The severity of AD lesions was evaluated by the third version of the CADESI (Canine Atopic Dermatitis Extent and Severity Index) at each site (local score) and 62 body sites (total score) as described previously [30,31].

2.2. Skin biopsies and SC isolation

Prior to taking biopsies of the skin, hair was shaved at two sites that are commonly lesional in AD dogs (axilla and inguinal) and one site commonly non-lesional (trunk). Skin biopsies (10×10 cm^2) of both control and AD dogs were taken, by surgical blade excision immediately after euthanasia for purposes unrelated to this study. The SC was isolated from skin biopsies with small modifications of the method described by Tanojo et al. [32]. Briefly, subcutaneous fat was removed and the remaining part of the skin was stretched on a polystyrene foam block. A dermatome was used to cut the skin at the proper thickness of 0.3–0.6 mm. The dermatomized skin was collected on filter paper soaked with 0.1% trypsin solution (in PBS) in a petri dish at 4 °C. After 24 h, dishes were placed at 37 °C for 3 h and the SC was peeled off from the epidermis at room temperature. Subsequently the SC was washed with 0.1% trypsin inhibitor solution (in PBS) and stored at room temperature in a plastic bag containing silica gel and argon gas until further analyses.

2.3. Small angle X-ray diffraction

To examine the lamellar lipid organization in SC, small angle X-ray diffraction (SAXD) was used. Measurements were performed at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) at station BM26B. Prior to the measurements, the SC was hydrated for 24 h over a solution of 27% NaBr. The SAXD patterns were detected with a Pilatus 1 M detector (1043×981 pixels) and a sample to detector distance of 2 m, for a period of 2×150 s. The scattering profile of the X-rays of the SC samples was recorded as a function of its scattering vector (q) defined by $q = 2\pi \sin\theta/\lambda$ (λ is the wavelength of the X-rays, either 0.1033 or 0.124 nm, and θ is the angle of the scattered X-rays) [33]. The diffraction pattern of a lamellar phase is characterized by a series of equidistant peaks. The position of each peak can be denoted by its q-value or by its corresponding spacing, which is equal to $2\pi/q$. When dealing with a lamellar phase, the diffraction peaks attributed to such a phase are located at an equidistant position in the diffraction curve. This means that the nth order peak is located at a q-value being $n.q_1$ (the position of the 1st order diffraction peak of that lamellar phase). For calibration silver behenate/Cholesterol was used.

2.4. Fourier transform infrared spectroscopy (FTIR)

To analyze the lateral lipid organization, a Varian 670-IR spectrometer equipped with a broad band mercury-cadmium-telluride detector was used and the spectral resolution was 1 cm^{-1}. Absorption of infrared light of wavelengths ranging between 400 cm^{-1} and 4000 cm^{-1} was recorded [34]. Each spectrum was an average of 2560 scans and was collected during a temperature increase of 1 °C between 0 °C and 90 °C. Using the software Varian Resolution Pro 4.1.0.101 [35–37], all spectra were baseline-corrected and deconvoluted before analysis. After correction we focused on two regions of the spectra. To obtain information about conformational ordering of the chains, the positions of the CH_2 symmetric stretching within the wavelength range 2840–2860 cm^{-1} were determined. The changes in the position of the CH_2 symmetric stretching vibrations as function of temperature were determined as described previously [36]. The positions of the stretching vibration provide information on the conformational ordering. When the lipids are highly ordered, the CH_2 symmetric stretching frequencies are < 2850 cm^{-1}. When the lipids exhibit a high conformational disordering, the liquid phase, the peak positions of the CH_2 symmetric stretching vibrations are higher than 2853 cm^{-1}. The temperatures of the transitions from the orthorhombic to the hexagonal phase and the hexagonal to the liquid phase were determined from the plots. The midpoint temperature was taken as the transitional temperature by curve fitting with five linear functions to use a six-pair-parameter of the temperature and frequency [35].

To obtain information about the lateral packing, also the scissoring vibration in the spectrum was monitored. For CH_2 scissoring vibration, appearance of vibrations at both the frequencies 1463 and 1473 cm^{-1} (Table 1) indicate the presence of an orthorhombic phase in the sample, whereas the presence of vibration at approximately 1467 cm^{-1} only, represents a hexagonal or liquid phase.

2.5. Lipid extraction

To determine the lipid composition, SC lipids were extracted by the method described by Thakoersing et al. [40] using a modified Bligh and Dyer extraction procedure. The organic phases collected were dried under a stream of nitrogen gas at 40 °C dissolved in chloroform: methanol (2:1) and stored at − 20 °C.

2.6. High performance thin layer chromatography (HPTLC) and mass spectrometry (MS)

The lipid composition focusing on CERs, FFAs and CHOL present in the SC were analyzed by HPTLC. Using this approach 8 different CER subclasses or combinations of subclasses can be separated. CERs consist of a sphingoid base and an acyl chain, nomenclature according to Motta et al. [41]. The base can be either sphingosine (denoted by S), phytosphingosine (P), 6-hydroxyspingosine (H) or dihydroxysphingosine (dS), the acyl chain is either non-hydroxy (N), α-hydroxy (A) or ω-hydroxy acyl chain ester linked to a linoleate (EO). Hence CER subclasses to be distinguished are CER[EOS], CER[NS/NdS], CER[EOP], CER[NP], CER[EOH], CER[AS/NH], CER[AP], CER[AH] [42,43], in which CER[NS] and CER[NdS] together with CER[AS] and CER[NH]

Table 1
The most prominent infrared absorption frequency regions in FTIR analyses [10,38,39].

Frequency/cm^{-1}	Assignment	Remarks
2846–2855	CH_2 symmetric stretching	Frequency increases when chain becomes disordered
1463 and 1473	CH_2 scissoring	Orthorhombic phase
1467	CH_2 scissoring	Hexagonal phase
1466	CH_2 scissoring	Disordered phase