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Artigo

Epidermal dysfunctions in canine atopic dermatitis: Clinical impacts and therapies

Disfunções epidérmicas na dermatite atópica canina: Impactos clínicos e terapias

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Abstract: Canine atopic dermatitis (CAD) is an immune-mediated disease characterized by inflammatory and pruritic processes. Recent theories propose that epidermal barrier failures can facilitate the entry of potential allergens, microorganisms and irritants, increasing the interaction with cells of the immune system, causing its excessive stimulation. Therefore, the present review aims to describe the main epidermal changes that contribute to the pathogenesis of CAD, as well as to present therapies used to recover the skin barrier. The most frequent changes in the epidermis of atopic animals involve transepidermal water loss, lipid and protein dysfunctions. Ceramides are lipids with structural functions, but they are also involved in cell signaling. These molecules, although present, have reduced concentrations in the skin of dogs with CAD, which is considered both primary and secondary defects of inflammatory processes. Other epidermal components, such as filaggrin, an essential protein in development and maintenance of skin barrier integrity, and antimicrobial peptides responsible for defense against pathogens and modulation of immune response, have mutations in atopic animals, contributing to the appearance of recurrent infections. These defects in cutaneous barrier may contribute to the pathogenesis of CAD, and such information helps in the treatment of this pathology, improving animal welfare with skin-healing therapies.

Key Words: Canine Atopy, Tegument disorders, Ceramides, Filaggrin, Antimicrobial Peptides

Resumo: A dermatite atópica canina (DAC) é uma doença imunomediada caracterizada por um processo inflamatório e pruriginoso. Teorias recentes propõem que falhas na barreira epidérmica podem facilitar a entrada de potenciais alérgenos, microorganismos e agentes irritantes, aumentando a interação com as células do sistema imunológico, estimulando-as excessivamente. Portanto, o objetivo da presente revisão é descrever as principais alterações epidérmicas que contribuem na patogenia da DAC, assim como apresentar terapias utilizadas para recuperar a barreira cutânea. As mais frequentes alterações na epiderme de animais atópicos envolvem a perda de água transepidérmica, disfunções proteicas e lipídicas. As ceramidas são lipídeos com funções estruturais, mas também estão envolvidas na sinalização celular. Tais moléculas, embora presentes, tem sua concentração reduzida na pele de cães com DAC, o que pode ser considerado tanto defeito primário quanto secundário ao processo inflamatório. Outros componentes epidérmicos, como a filagrina, uma proteína essencial no desenvolvimento e integridade cutânea, e os peptídeos antimicrobianos, responsáveis pela defesa contra patógenos e modulação de resposta imune, apresentam mutações em animais atópicos, culminando no aparecimento de infecções recorrentes. Esses defeitos na barreira cutânea podem contribuir na patogênese da DAC, sendo essas informações importantes no manejo terapêutico da doença, melhorando o bem-estar do paciente através de terapias repositoras de barreira cutânea.

Palavras Chaves: Atopia Canina, Desordens tegumentares, ceramidas, filagrina, peptídeos antimicrobianos.

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1. Introduction

Canine atopic dermatitis (CAD) is an inflammatory, pruritic and chronic disease present in veterinary clinical routine (HALLIWELL, 2006). The most commonly affected animals are young between six months and three years. Theories about immunopathogenesis involve a genetic predisposition and an epidermal dysfunction of affected animals, where a greater penetration of antigens would induce an exacerbated immune response, generating the clinical signs usually observed in canine atopy (WILHEM et al., 2011).

The main clinical manifestation of CAD is pruritus, which precedes other clinical signs and is responsive to corticosteroid therapy (BRUET et al., 2012). The commonly affected body sites are distal extremities and flexural areas of limbs, face, belly and ears (FAVROT et al., 2010). Associated with pruritus, atopic dogs may present with a variety of primary or secondary lesions, such as erythema, macules or papules, alopecia, excoriations, hyperpigmentation and lignification. Recurrent bacterial and fungal infections are common complications of this disease (GRIFFIN & DEBOER, 2001) since a failure of epidermal barrier favors the exacerbated proliferation of resident microorganisms in tegument (RODRIGUES-HOFFMAN et al., 2014).

Epidermal dysfunctions comprise lipid alterations, responsible for filling the intercellular space (HARA et al., 2000), and protein modification, responsible for the aggregation of keratinocytes in upper layers of epidermis (OSAWA et al., 2011) or defense against microorganisms (SCHAUBER & GALO, 2008). Such changes may influence the development and maintenance of allergy reaction and should be understood in order to better manage the clinical condition.

In this way, the present review aims to describe the main lipid and protein alterations present in epidermis of dogs with canine atopic dermatitis (CAD).

2. Development

2.1. Lipid changes

Ceramidss are molecules of lipid origin, composed of sphingosine and fatty acids. These molecules are mainly located in the cellular lipid bilayer, acting as structural and signaling elements (UCHIDA, 2014), limiting the penetration

of substances and pathogens (Figure 1), as well as restricting water movement outside the skin (JUNGERSTED et al., 2008). In experimental models of dogs with CAD, the reduction in lipid concentration was partially related to the development of an acute inflammatory response in individuals sensitized and exposed to allergens. The same study also revealed that the reduction of lipids present in stratum corneum (SC) of epidermis was detected both at sites close to and distant from the site of allergen application (HARA et al., 2000). These lipid changes are probably attributed to a decrease of ultra-long chain ceramides and free fatty acids, leading to a less dense and disorganized lipid lamellae (BREIDEN & SANDHOFF, 2014).

Lipid alterations are an important factor in the immunopathogenesis of this disease, since the reduction of its concentration in SC results in an increase in transepidermal water loss, culminating with a dry and fragile skin. These modifications impair the protective function of skin, increasing exposure to environmental allergens, which stimulate the immune response to hypersensitivity (Figure 1). These factors may, therefore, influence the development and maintenance of canine atopic dermatitis (SHIMADA et al., 2009), being the aim of new therapeutic approaches in order to reorganize the stratum corneum (CERRATO et al., 2016). In humans, it was demonstrated that type 2 cytokines, such as IL-4 and IL-13, globally change lipid metabolism in the skin, illustrating a potentially novel pathogenic function of IL-4/IL-13 activation in atopic dermatitis (BERDYSHEV et al., 2018).

Healthy dogs of different breeds can demonstrate quantitative variations in ceramide standards of stratum corneum (POPA et al., 2010), which may even be absent in such animals (POPA et al., 2011b). In the same study, the authors reported heterogeneity in lipid binding proteins in stratum corneum of atopic dogs, where such proteins and ceramides varied according to the epidermal layer, suggesting that their concentrations may be altered during the inflammatory crisis (POPA et al., 2011b).

Recently, a skin lipid profile, being docosahexaenoic acid (DHA) among these lipids, was identified as possible dermatitis biomarkers, which may be used as molecular indicators of treatment efficiency (FRANCO et al., 2018).

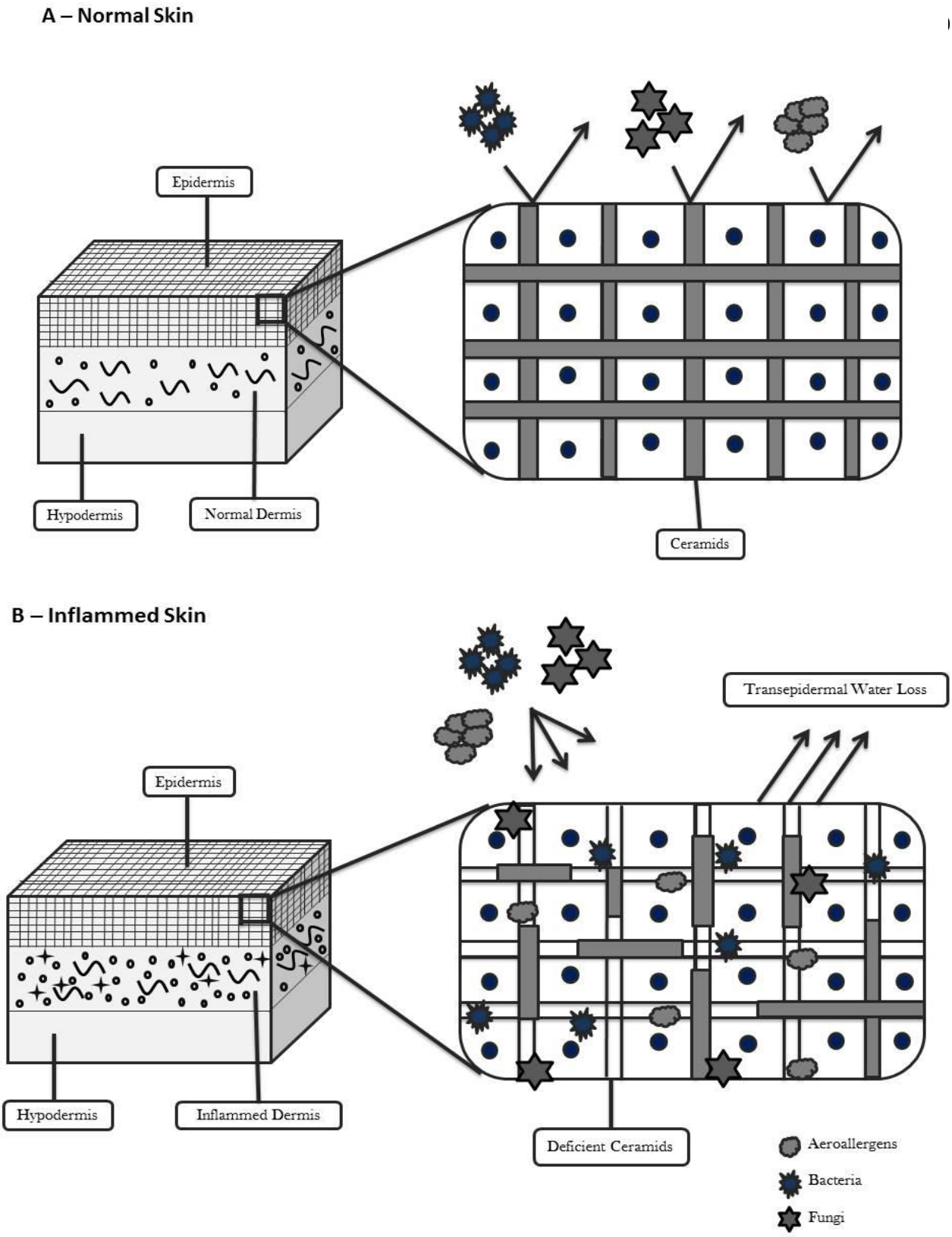

Figure 1. Structural aspects of skin with (B) and without (A) ceramids deficiency, allowing penetration of bacteria, fungi and aeroallergens, as well as transepidermal water loss. This will trigger an inflammatory response, ultimately leading to a pruritus crisis.

2.2. Protein changes

2.2.1. Filaggrin

Filaggrin is the main protein present in SC formation (KEZIC et al., 2008). This epidermal layer is formed by keratinized and anucleated cells, known as corneocytes, which are in constant desquamation (BANKS, 1992). The keratin intermediate filaments present in this layer are crosslinked with filaggrin, leading to keratinocyte flattening, a typical morphological characteristic of this epidermal stratum (KERSZENBAUM, 2006). It is important to note that such a protein is progressively degraded by enzymes, generating a pool of hydrophilic amino acids known as natural moisturizing factors (NMF). These factors include urocanic acid and carboxylic pyrrolidonic acid, which promote the uptake and entrapment of water and maintenance of acidic pH of the skin, therefore preventing adhesion and penetration of microorganisms (O'REGAN et al., 2008; IRVINE et al., 2011).

Studies of this protein in humans with atopy have demonstrated that mutations are among the major risk factors for disease development (OSAWA et al., 2011). It was also demonstrated that these mutations were associated with increased risk of contact allergic dermatitis development (TIMMERMAN et al., 2016). Relative to atopic dogs, reduced or undetectable expression of filaggrin portions, similar to what occurs in humans has been reported, suggesting a loss of function related to mutation (CHERVET et al., 2010).

Mutations in filaggrin gene contribute to dysfunction of the epidermal barrier due to the formation of disorganized SC, with abnormal structure and architecture. There are also alterations in lipid profile distribution, increased cutaneous pH and transepidermal water loss, as well as increased susceptibility to infections (IRVINE et al., 2011).

In humans, an important role of this protein has been reported in atopic patients without gene mutations (McGRATH, 2008). This fact is associated with the inflammatory process present in atopy, which increases the synthesis of histamine and cytokines, including IL-4, IL-17, IL-22, IL-25 and IL-31. These substances, together, are able to decrease the production of filaggrin or even reduce its genetic expression, suggesting an integrated role of different mechanisms in atopic dermatitis (CZARNOWICKI et al., 2014).

2.2.2. Antimicrobial peptides

Antimicrobial peptides (AMPs) are a group of small proteins, which are essential components of protection against

microorganisms in the skin, having microbicidal activity through the interaction and consequent rupture of membrane structures essential to pathogens (PALFFY et al., 2009). Clinically, the importance of AMPs can be evidenced by increased expression in infected skin, culminating in the resolution of pyoderma (MENENDEZ; BRETT-FINLEY, 2007; SCHITTEK et al., 2008). From the immunological point of view, AMPs are able to stimulate the process of phagocytosis, promote the chemotaxis and accumulation of cells of the immune system, such as monocytes and lymphocytes at the inflammatory site and coordinate the innate immune response (JENSSEN et al., 2006).

AMPs are primarily secreted by keratinocytes, sebocytes and mast cells (DINARDO et al., 2003; BRAFF et al., 2005; LEE et al., 2008). However, cells of the immune system, such as neutrophils and natural killers, may contribute to its production (AGBERBERTH et al., 2000). Th17 lymphocytes also play a role in AMPs synthesis, through secretion of IL-17A, which stimulates keratinocytes to produce these proteins (NOLI et al., 2014).

Recent works have demonstrated the decrease in mRNA expression of some AMPs and increased expression of others in atopic animals. These data may indicate their involvement in innate immunity of skin, in addition to suggesting that changes in the ratio between peptides may be the cause of increased infections in atopic animals (SANTORO et al., 2010). It is also known that atopic animals have a cutaneous microbiome different from healthy animals (RODRIGUES-HOFFMAN et al., 2014). However, further studies should be carried out to elucidate the actual participation of the AMPs in the alteration of microorganisms residing in the skin.

3. Clinical Impacts and Skin Barrier Repair

The most common clinical sign in CAD is pruritus, which may precede other lesions and responds to corticosteroid therapy. In addition to pruritus, affected dogs can present primary or secondary lesions. Some of the most common lesions seen in CAD are erythema, self-induced alopecia, excoriations, hyperpigmentation and lichenification. Additionally, bacterial and yeast infections have been reported as frequent complications (GRIFFIN & DEBOER, 2001). Those signs are partially associated with skin barrier dysfunction, which allows allergens and pathogens to penetrate the skin and overstimulate the immune system (ELIAS et al., 2008). Based on that, healing the skin and repairing the possible defects could delay

the pruritus crisis and help to improve the clinical signs. Skin deficiencies, associated clinical signs and proposed therapies are shown in Table 1.

Supplementation with oral essential fatty acids (EFAs) can influence superficial skin lipids and can improve the coat quality. Oral EFAs can also help to improve CAD clinical signs, although these benefits might not be seen in monotherapy and they can take several weeks to provide a good pharmacological response (OLIVRY et al., 2015). The supplementation of diets of dogs with atopic dermatitis for two months provided changes in stratum corneum lipids, making it closer to normal skin characteristics (POPA et al., 2011a).

Table 1. Skin barrier dysfunctions, clinical consequences and proposed therapies in dogs with canine atopic dermatitis.

Epidermal dysfunctions	Clinical Signs	Proposed Therapy*
Lipid Alterations	Itchy and Dry Skin Secondary Bacterial and Yeast infections	Supplementation with oral EFAs (POPA et al., 2011a). Topical application of EFAs (BLASKOVIC et al., 2014).
Filaggrin Mutations	Inflammation and pruritus	Use of emollient shampoos (OLIVRY et al., 2010). Use of post-bathing topical moisturizing (BORDEAU et al., 2007). Infection control (HILLIER et al., 2014). Pruritus control (OLIVRY et al., 2010).
AMPs Alterations	Secondary Bacterial and Yeast infections	Infection control and skin barrier healing (HILLIER et al., 2014).

Application of topical EFA formulations can also help to normalize the stratum corneum lipid barrier defects (OLIVRY et al., 2015). A

clinical trial using a spot-on therapy containing fatty acids and essential oils demonstrated its beneficial effect in alleviating the clinical signs of CAD, but changes in stratum corneum were not accessed post-treatment (BLASKOVIC et al., 2014). On the other hand, the use of a formulation containing ceramides, cholesterol and EFAs every three days for six applications to atopic dogs normalized stratum corneum abnormalities (POPA et al., 2012). Therefore, the use of EFAs via oral supplementation or topical application can be used as an adjuvant therapy in order to minimize lipid profile abnormalities.

The improvement of skin care and hygiene with emollient shampoo and topical moisturizers are associated with an antipruritic effect based on components, such as lipids, complex sugar and antiseptics, and mechanic removal of allergens. Phytosphingosine formulations have also provided a modest effect on skin lesions and pruritus in allergic dogs (OLIVRY et al., 2015). The frequency and intensity of bathing are important factors in relieving pruritus and should be associated with other therapies in order to heal the skin barrier.

The evaluation of the use of antimicrobial therapies should also be performed since secondary bacterial and yeast infections are common in dogs affected with atopic dermatitis. The treatment consists of topical and/or systemic drugs (OLIVRY et al., 2015). To improve the therapeutic efficacy, veterinarians should follow antimicrobial treatment guidelines (HILLIER et al., 2014) as well as watch out for irritating effects of topical antimicrobials, such as benzoyl peroxide, that might induce flares of CAD (OLIVRY et al., 2015).

4. Final Considerations

Epidermal alterations are important factors in pathogenesis of canine atopic dermatitis, and their understanding is necessary for a better clinical disease management. In addition, it is essential to restructure the skin barrier in order to increase the interval between crises of pruritus, conferring a better quality of life to the animal.

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