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REVIEW ARTICLE

Treating canine atopic dermatitis with unsaturated fatty acids: the role of mast cells and potential mechanisms of action

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Summary

Canine atopic dermatitis (CAD) is an inflammatory skin disorder that is characterized by pruritus and associated cutaneous changes. Treatment interventions include allergen avoidance, allergen-specific immunotherapy as well as a symptomatic therapy using glucocorticoids and antihistamines. In addition, a dietary intervention using polyunsaturated fatty acids (PUFA) has been shown to alleviate symptoms in some dogs. Although the beneficial effects of PUFA in the treatment of CAD have been known for several years, their mode of action remains unclear. This review discusses the evidential basis of the therapeutic use of dietary PUFA in the treatment of CAD. Particular emphasis will be placed on the role of cutaneous mast cells. In addition, recent evidence from *in vitro* studies on the regulation of mast cell exocytosis will be used to build a mechanistic model of the active principle of PUFA. It is proposed that dietary PUFA are integrated into mast cell membranes resulting in a reorganization of membrane microdomains. This may then be accompanied by functional changes of membrane-associated proteins such as the phospholipases D (PLD), enzymes having an important impact on mast cell exocytosis processes.

Keywords canine atopic dermatitis, polyunsaturated fatty acids, mast cells, lipid rafts, phospholipases D

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Introduction

Repeatedly, polyunsaturated fatty acids (PUFA) have been described to have beneficial effects in the treatment of canine atopic dermatitis (CAD) (Scott et al., 1997; Harvey, 1999; Saevik et al., 2004; Bensignor et al., 2008). It has been shown that the dietary supplementation of dogs suffering from CAD with PUFA reduces the clinical signs as pruritus in some cases and lowers the amount of immune-suppressive drugs needed to control the disease (Saevik et al., 2004). However, the mechanisms of PUFA action are not fully understood. This review will discuss the existing knowledge regarding the therapeutic use of dietary PUFA in the treatment of CAD with a focus on mast cell degranulation. Moreover, recent data will be integrated into a new mechanistic model illustrating the regulatory action of PUFA on mast cell exocytosis in CAD and other chronic inflammatory diseases.

Canine atopic dermatitis

Canine atopic dermatitis is an inflammatory skin disorder of dogs, which frequently occurs in industrialized countries (Hillier and Griffin, 2001). The predominant symptom of the disease is pruritus (Halliwell and Schwartzman, 1971). Self-trauma exacerbates the inflammatory process and leads to secondary skin lesions such as erythema, excoriations and lichenification (Griffin and DeBoer, 2001). These are commonly accompanied by bacterial or yeast infections (Griffin and DeBoer, 2001). Affected body areas include the ears, the paws, the ventral abdomen as well as inguinal and axillary regions (Griffin and DeBoer, 2001). The increase in the incidence of CAD may be due to the spread of indoor keeping, vaccinations and antiparasitic treatments (Hillier and Griffin, 2001). Predisposing factors for the occurrence of CAD are the breed (Labrador, Retriever, Boxer, German Shepherd, West Highland White Terrier, Cocker

Spaniel), the age (6 month to 3 years) and the season (pollen season) (Griffin and DeBoer, 2001). Disorders of the fatty acid metabolism, that is a deficiency of the enzymes Δ5-desaturase and Δ6-desaturase, are also considered as a predisposing or an aggravating factor of CAD (Fuhrmann et al., 2006). These enzymes are responsible for the synthesis of highly unsaturated n3 and n6 fatty acids from α-linolenic acid (LNA) and linoleic acid (LA) (Sprecher et al., 1995; Jakobsson et al., 2006), which are effective in the epidermal lipid barrier (Burr and Burr, 1930). Their dysfunction in the skin may result in a defective epidermal lipid barrier in atopic dermatitis, thus contributing to the pathogenesis of CAD (Schlotter et al., 2009).

It is thought that skin barrier defects facilitate penetration of the stratum corneum by allergens (Hill and Olivry, 2001; Olivry et al., 2010a). This may trigger antigen-presenting cells in the epidermis (Hill and Olivry, 2001; Olivry et al., 2010a). Immune cell-derived inflammatory mediators then activate keratinocytes, which in turn release additional cytokines and chemokines (Olivry et al., 2010a). At the same time, there is an IgE-mediated degranulation of cutaneous mast cells and an immigration of granulocytes, T lymphocytes and dendritic cells (Olivry et al., 2010a). The resulting dermal and epidermal damage in combination with self-trauma and secondary infections contribute to self-perpetuating inflammation and chronic skin lesions (Olivry et al., 2010a).

In meta-analyses, Olivry and co-workers have repeatedly set apart the effectiveness of various forms of therapy of CAD (Olivry et al., 2001, 2010a,b; Olivry and Bizikova, 2013). Allergen avoidance or an allergen-specific immunotherapy has been found to be effective in preventing the disease (Olivry and Sousa, 2001; Loewenstein and Mueller, 2009). Symptomatic therapy approaches include the use of glucocorticoids, antihistamines or the combination of both (Olivry and Sousa, 2001). Good results are reported for corticosteroids and cyclosporine A (Olivry and Sousa, 2001). These substances frequently lead to adverse side effects and need to be given long term. Therefore, the dose should be kept as low as possible. In this respect, PUFA supplements are considered as a valuable therapeutic addition due to their glucocorticoid-sparing effect (Olivry and Bizikova, 2013).

The role of mast cells in CAD

The development of CAD is thought to result from a complex reaction against allergens (Hillier and Griffin, 2001). Nevertheless, dogs suffering from CAD have been shown to be similar in their IgE levels compared to non-affected dogs (Lauber et al., 2012). Therefore, serological or intradermal IgE tests are not deemed sufficient for the initial diagnosis of CAD (Olivry et al., 2010a). Cells known to contribute to the pathogenesis of CAD include keratinocytes, dendritic cells, T lymphocytes and mast cells (Olivry et al., 2010a). The impact of mast cells in CAD is corroborated by three lines of evidence: (i) a correlation between the number of mast cells in the skin and the pruritus of dogs suffering from CAD (Auxilia and Hill, 2000), (ii) increased exocytosis by mast cells of atopic dogs (DeMora et al., 1996) and (iii) promotion of clinical signs of atopic diseases due to mediator release by dermal mast cells (Marsella and Olivry, 2001).

Mast cells are implicated in a variety of allergy and autoimmune disorders. The effector cells of the innate immune response are filled with vesicles, which contain numerous potent pro-inflammatory mediators as histamine, prostaglandins, leucotrienes as well as the enzymes hexosaminidase, tryptase and chymase (Hill and Olivry, 2001). Mast cell activation initiated by the binding of allergen-bound IgE antibodies to mast cell IgE receptors leads to an immediate release of these pre-formed mediators (Hill and Olivry, 2001). As a result, a local or systemic hypersensitivity reaction occurs (Hill et al., 2001).

Histamine has a multitude of effects on animals'organisms including the induction of vasodilation, an increase in capillary permeability as well as bronchoconstriction (Rossbach et al., 2009). Histamine thereby triggers inflammatory processes. Release of histamine by activated cutaneous mast cells is thought to induce pruritus in mice and men (Carr et al., 2009; Rossbach et al., 2009). However, in dogs, histamine injections into the skin have been reported not to provoke a scratching response (Carr et al., 2009; Rossbach et al., 2009). This dovetails with the fact that antihistamines given as monotherapy are hardly effective in the treatment of CAD (Baumer et al., 2011). Nevertheless, a chronic moderate increase in histamine might play a role in the pathogenesis of CAD.

Prostaglandins and leucotrienes are formed de novo from membrane phospholipids after mast cell activation (Boyce, 2007). The manifold biological effects of the lipid mediators include the modulation of smooth muscle contractility and vascular permeability, the sensation of pruritus and pain as well as neutrophil chemotaxis and platelet aggregation (Boyce, 2007).

Mast cell proteases affect the inflammatory process by modifying the extracellular matrix either directly or indirectly by regulating the activities of extracellular matrix-processing enzymes (Pejler et al., 2010).

The degradation of procollagen, collagen, vitronectin and fibronectin compromises the cellular adhesion to the extracellular matrix and facilitates the influx of inflammatory cells into the tissue (Pejler et al., 2010).

Treatment of CAD with PUFA

The link between dietary fatty acids and atopic diseases dates back to the year 1929 when Burr and Burr observed inflammatory processes in the dermis of rats, which were fed a fat-free diet (Burr and Burr, 1929). The skin inflammation was found to be alleviated by the supplementation of the essential fatty acids LNA (C18:3n3) and LA (C18:2n6) (Burr and Burr, 1930). *In vivo,* these fatty acids are elongated and desaturated resulting in eicosapentaenoic acid (EPA, C20:5n3), docosahexaenoic acid (DHA, C22:6n3) and arachidonic acid (AA, C20:4n6) (Sprecher et al., 1995; Jakobsson et al., 2006). These are important for skin health as well (Burr and Burr, 1930). For the synthesis of EPA, DHA and AA from LNA and LA, the catalytic activity of the enzymes Δ5-desaturase and Δ6-desaturase is needed (Sprecher et al., 1995; Jakobsson et al., 2006). In atopic dogs, a reduced conversion rate of LNA and LA to their metabolites as well as a diminished incorporation of the PUFA into skin lipids has been found (Olivry and Hill, 2001). This abnormal epidermal lipid metabolism has led to the proposal that an inadequate Δ5-desaturase activity and a Δ6-desaturase deficiency might be a possible cause for the development of CAD (Olivry and Hill, 2001; Fuhrmann et al., 2006). In fact, dogs suffering from CAD are reported to have a defective skin barrier in that they show higher transepidermal water loss (Olivry, 2011). Moreover, the administration of fatty acids was shown to result in an improvement of the skin barrier in a hairless mouse model (Fujii et al., 2013).

The impact of a PUFA supplementation on CAD pathogenesis has been investigated repeatedly. Several studies report that feeding of PUFA-enriched diets to atopic dogs results in a decrease in pruritus (Scott et al., 1997; Harvey, 1999; Saevik et al., 2004; Bensignor et al., 2008). Furthermore, in a delayed-type hypersensitivity skin test, a suppression of cell-mediated immune responses was observed (Wander et al., 1997). Beside, PUFA-enriched diets in general were demonstrated to improve the state of hair and skin even in healthy dogs (Marsh et al., 2000; Rees et al., 2001).

It is important to note that the beneficial effects of the unsaturated fatty acids were found to depend on the amount and the ratio of n6 to n3 fatty acids in the diet. A reduction in pruritus due to PUFA supplementation was seen when the PUFA were given in a 5 to 1 or 10 to 1 ratio for n6 and n3 respectively (Scott et al., 1997; Olivry et al., 2001; Saevik et al., 2004). Likewise, in healthy dogs, n6 to n3 ratios of 1.4 to 1, 5 to 1 and 10 to 1 but not higher were reported to result in a reduction in prostaglandin E2 or leucotriene B4 synthesis by stimulated monocytes, neutrophils and skin (Vaughn et al., 1994; Wander et al., 1997).

Clinical signs usually improve after 12 weeks of PUFA application (Olivry et al., 2001). Changes in membrane fatty acid pattern after dietary supplementation of PUFA have been reported after 8 to 12 weeks in dogs (Stoeckel et al., 2011, 2012). For monitoring a diet-induced increase in tissue n3 fatty acids, the analysis of plasma fatty acids is sufficient (Stoeckel et al., 2012). However, erythrocyte fatty acids should be analysed whether the effect of a dietary intervention on tissue n6 fatty acids is of interest (Stoeckel et al., 2012).

Taken together, there is compelling evidence that PUFA are involved in the pathogenesis of CAD. First, unsaturated fatty acids in general improve the condition of the skin. Second, PUFA modulate the immune system, thereby driving the immune response into an anti-inflammatory direction.

Impact of PUFA on mast cell mediator release

Even though PUFA supplementation is widely used in atopic patients, their mode of action remains unclear. Some interesting insights into the impact of PUFA on mast cell exocytosis were provided by *in vitro* studies using the canine mast cell line C2 (Gueck et al., 2003, 2004a,b; Seidel et al., 2005; Schmutzler et al., 2010; Basiouni et al., 2012, 2013). Due to the impaired function of the IgE receptor expressed by C2 cells (Hunter et al., 2009), the wasp venom peptide mastoparan, which triggers a G protein receptor, was used for stimulation. In a series of experiments, the consequences of a mast cell supplementation with PUFA in physiologically relevant concentrations were analysed. The data gained are of particular importance as the C2 used here exhibits diminished enzymatic activity of Δ5-desaturase (Seidel et al., 2005). This is similar to atopic patients. Mast cell enrichment with the n3 PUFA LNA was shown to decrease the stimulation-induced exocytosis of pro-inflammatory mediators compared to control cells (Gueck et al., 2003). LNA-supplemented mast cells were characterized by a decreased histamine release, a reduction in prostaglandin E2 production and a decrease in the activity of the pro-inflammatory enzyme tryptase (Gueck et al., 2003). Likewise, DHA enrichment of stimulated

mast cells resulted in a significant decrease in prostaglandin E2 release in comparison with stimulated control cells (Gueck et al., 2004a). This decrease in the liberation of pro-inflammatory mediators may account for the beneficial effects of n3 PUFA in treating CAD. In contrast, supplementation of C2 with the n6 PUFA AA was found to enhance both the spontaneous as well as the stimulation-induced increase in histamine and prostaglandin E2 levels as well as increased tryptase activity (Gueck et al., 2004b). Thus, a diet low in AA should be favoured for dogs suffering from CAD.

PUFA supplementation and mast cell membranes

Mast cell function crucially depends on the lipid composition of the plasma membrane separating the inner of a cell from the surrounding environment. Integrated or associated proteins such as ion channels, receptors and membrane-bound enzymes enable a directed exchange of substances as well as transmission of information from cell to cell. Typically, upon activation, several membrane proteins interact by forming protein complexes to fulfil their function (Davey et al., 2007, 2008). Hence, the lateral movement of membrane proteins within the lipid bilayer is of considerable importance to ensure proper reactions of a cell to exterior conditions.

The physical properties of a plasma membrane, for example the membrane fluidity, depend on the fatty acid pattern of the membrane phospholipids (Stillwell and Wassall, 2003; Ma et al., 2004; Wassall and Stillwell, 2008). An increase in unsaturated fatty acids leads to a higher membrane fluidity, thus affecting protein dynamics (Koenig et al., 1997; Smaby et al., 1997; Epand, 1998; Ma et al., 2004). In this regard, it is important to mention that dietary fatty acids in fact modulate the lipid composition of cellular membranes (Biondo et al., 2008; Schmitz and Grandl, 2008). A heightened supply of PUFA has been shown to result in an incorporation of the unsaturated fatty acids into the membranes of body cells (Biondo et al., 2008; Schmitz and Grandl, 2008).

Moreover, studies have shown that the membrane phospholipids are not distributed evenly within the cell membrane, leading to structurally and functionally distinct subdomains (Pike, 2003). These lipid rafts are characterized by an enrichment of cholesterol and saturated fatty acids as well as the predominance of particular membrane proteins including the high affinity IgE receptor (Pike, 2003). The lipid rafts are significant for the overall cellular performance and are implicated in a variety of cellular processes such as trafficking, signal transduction and molecular sorting (Simons and Toomre, 2000; Pike, 2003). With respect to the relevance of the microdomains as protein clustering centre and signalling platform, disturbances of lipid rafts, for example, by incorporation of unsaturated fatty acids, are believed to impact protein assembly and signal transmission (Simons and Toomre, 2000; Van and Leo, 2002; Siddiqui et al., 2007; Yaqoob, 2010). For example, the binding of antigen-bound IgE antibodies leads to an aggregation of IgE receptors, which, in turn, initiates mast cell activation (Davey et al., 2007, 2008). This assembly of IgE receptors usually takes place in the lipid rafts (Davey et al., 2007, 2008). A reorganization of lipid rafts therefore might affect mast cell activation, mast cell exocytosis and release of preformed mast cell mediators. *In vitro* investigations using canine C2 mast cells provide evidence that a PUFA supplementation leads to an incorporation of the unsaturated fatty acids in lipid rafts and non-raft membrane domains (Basiouni et al., 2012). The increase in PUFA content was further shown to go along with an increased unsaturation index of the membrane domains (Basiouni et al., 2012), which is a marker of membrane fluidity. Thus, there are numerous indications that the dietary supplementation of dogs suffering from CAD with PUFA alters mast cell function via a modulation of membrane microdomain lipid composition.

The role of the phospholipases D

An example of membrane-bound enzymes that might be affected by reorganization processes of the cell membrane due to PUFA supplementation are phospholipases D (PLD). Phospholipases in general are hydrolases that cleave phospholipids, the key component of cell membranes, into fatty acids and other lipophilic substances. Due to their enzymatic activity, phospholipases are of importance in the synthesis and degradation of biological membranes as well as the formation of cell organelles (Selvy et al., 2011; Peng and Frohman, 2012). In addition, phospholipases play a considerable role in intracellular signalling cascades as their reaction products are mediator substances and second messengers (Selvy et al., 2011; Peng and Frohman, 2012).

Phospholipases D cleave phosphatidylcholine by releasing phosphatidic acid and soluble choline (Selvy et al., 2011; Peng and Frohman, 2012). The reaction product phosphatidic acid is a short-lived bioactive signalling molecule that has been shown to be implicated in vesicular trafficking including endocytosis and exocytosis (Selvy et al., 2011; Peng and Frohman,

2012). In this context, PLD have been considered to be essential for cell degranulation (Selvy et al., 2011; Peng and Frohman, 2012).

To date, two isoforms of the PLD have been described in mammalian systems: PLD1 and PLD2 (Selvy et al., 2011; Peng and Frohman, 2012). Both differ in their basal activity as well as in their subcellular localization. PLD2 has a higher basal activity compared to PLD1 and is found in the plasma membrane regardless of its activation status (Colley et al., 1997; Selvy et al., 2011). PLD1 is co-localized with secretory vesicles and, upon stimulation, translocates to the plasma membrane (Brown et al., 1998; Choi et al., 2002). It is thought that PLD1 mediates the movement of secretory vesicles to and the fusion of these vesicles with the plasma membrane, whereas PLD2 is involved in membrane fusion processes only (Choi et al., 2002).

Impact of PUFA on PLD localization and activity

Phospholipases D have been proven to be relevant targets for the biological impacts of unsaturated fatty acids. PLD-dependent effects of PUFA have been described in intestinal cells (Awad et al., 1994), smooth muscle cells (Askari et al., 2002) as well as in immune cells of both the innate [neutrophils (Grenier et al., 2003), macrophages (Wang and Oram, 2005)] and the adaptive [lymphocytes (Bechoua et al., 1998; Kim et al., 1999; Diaz et al., 2002)] immune system. A PUFA-mediated modulation of the PLD also applies to mast cells. PUFA have been shown to affect the intracellular localization of PLD1 and to influence the enzymatic activity of total PLD (Gemeinhardt et al., 2009; Basiouni et al., 2013).

Mast cell activation is associated with a translocation of PLD1 from intracellular vesicular structures to the plasma membrane (Brown et al., 1998; Choi et al., 2002). The stimulation-induced movement of PLD1 is inhibited by the enrichment of mast cells with LA, LNA, EPA or DHA, but not with AA (Basiouni et al., 2013). As the translocation is considered to be essential for PLD1-mediated exocytosis, this observation provides an explanation for the decreased mediator release of mast cells supplemented with LA, LNA, EPA or DHA (Gueck et al., 2003, 2004a,b). Arachidonic acid has no inhibitory action on mast cell exocytosis which concords with the notion that AA is not suitable to prevent PLD1 translocation (Gueck et al., 2004b).

Furthermore, PUFA increase the stimulation-induced total PLD activity as shown for blood mononuclear cells (Bechoua et al., 1998), the fibroblast-like monkey cell line COS-1 (Gemeinhardt et al., 2009) as well as for the canine mast cell line C2 (Basiouni et al., 2013). All the PUFA tested including LNA, EPA, DHA, LA and AA emerged to promote the total enzymatic activity of mast cell PLD (Basiouni et al., 2013). Inhibition studies using isoform-specific inhibitors of either PLD1 or PLD2 provided further evidence that AA has an enhancing effect on both PLD isoforms (Basiouni et al., 2013). DHA, on the other hand, impacts on the PLD2 only (Basiouni et al., 2013). These divergent actions of DHA and AA provide valuable insights into the underlying mechanisms of mast cell regulation. DHA inhibits and AA promotes the mediator release by mast cells (Gueck et al., 2004a,b), thus PLD1 appears of special significance in the control of mast cell secretory events.

The question arises why certain PUFA do have divergent effects on mast cell PLD localization and activity. An answer may be found in the different interactions of unsaturated fatty acids with special signalling molecules. For instance, it has been reported that PLD1 is shifted together with protein kinase C alpha (PkCα) (Mochly-Rosen et al., 1990; Garbi et al., 2000; Hu and Exton, 2003). DHA as well as EPA is known to inhibit the translocation of PkCα (Nair et al., 2001; Denys et al., 2005) whereby they may also prevent the movement of PLD1. AA, in contrast, has been demonstrated to be a direct activator of PkCα (Khan et al., 1995; Lopez-Nicolas et al., 2006), which dovetails the inability of AA to arrest PLD1 translocation. Differential impacts of DHA and AA have also been described on the activators of PLD1. While AA is reported to have an activating effect on PLD1-stimulating mediators, namely the small GTPases ARF and Rho as well as PkCα (Fu et al., 1998; Araki et al., 2001), DHA has been demonstrated to promote ARF (Diaz et al., 2002), but to inhibit Rho and PkCα (Young et al., 2000; Yi et al., 2007).

Conclusion

Although beneficial effects of PUFA in the treatment of CAD have been known for several years, there is still a lack of knowledge regarding their mode of action. The positive effects of a PUFA supplementation on the skin barrier function are well described. Nonetheless, unsaturated fatty acids have an impact on numerous cells involved in the pathogenesis of CAD. These cells include keratinocytes, dendritic cells, T lymphocytes and mast cells. It appears that dietary PUFA are readily integrated into cell membranes, whereby modulating the properties of the lipid bilayers. As a result, there is a reorganization of membrane

microdomains, particularly lipid rafts. This leads to functional changes of membrane-associated such as the PLD. Phospholipases D, targets of unsaturated fatty acids, are of significance for the regulation of mast cell exocytosis processes and contribute to the pathogenesis of CAD. Both the localization and the activity are altered due to a PUFA enrichment of mast cells. These observations provide a molecular basis of the positive effects of dietary PUFA in down-regulating exaggerated mast cell degranulation.

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