efficas care™
for ALLERGIES and ASTHMA

Scientific Summary Targeting Multiple Allergic Diseases

Executive Summary

Extrinsic atopic dermatitis, allergic asthma, and allergic rhinitis are prevalent allergic diseases. These diseases can be distinguished by the location of their most important symptoms; skin, lungs and sinuses, respectively. They also share many characteristics, including the same underlying immune system dysregulation as evidenced by inappropriate T-cell mediated responses to innocuous antigens, peripheral eosinophilia, elevated serum IgE levels, chronic immune system activation, and over-production of inflammatory mediators, including leukotrienes, cytokines and chemokines.

The role of inflammatory mediators in allergic diseases is well characterized. In particular, leukotrienes, derived from the fatty acid arachidonic acid, play a significant role in the pathophysiology of allergic diseases. There are two classes of leukotrienes with different roles. Cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) directly promote allergic inflammation in target tissues and increase the synthesis of other proinflammatory mediators, such as cytokines. The chemotactic leukotriene, LTB_4, is a chemoattractant for immune cells and activates neutrophils leading to endothelial leakage.

It has been demonstrated that two different strategies to reduce the impact of leukotrienes on symptoms of allergic disease are effective: 1) blocking leukotriene receptors and 2) inhibiting the action of the leukotriene synthesis enzyme, 5-lipoxygenase (5-LO). The receptor blocking drugs target only one of the two classes of leukotrienes, yet appear to have a modest but clinically significant impact on disease symptoms. The leukotriene synthesis blocker blunts production of both classes of leukotrienes and also has a clinically significant impact on disease symptoms of atopic dermatitis (eczema), asthma and allergic rhinitis.

EFFICAS CARE is a specially formulated medical food based upon bioactive fatty acids with modes of action relevant for the dietary management of allergic diseases via reduction of leukotrienes. EFFICAS CARE has been shown in clinical trials to alter the pools of fatty acid precursors of leukotrienes, to significantly reduce the synthesis of leukotrienes by immune cells *ex vivo* in healthy individuals as well as in asthmatics. Efficas Care has also been shown during consumer trials to significantly improve disease management and the quality of life in individuals with asthma, allergic rhinitis and eczema. Since EFFICAS CARE blocks leukotriene synthesis and improves disease management, it is scientifically valid to target multiple allergic diseases with this product.

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The Features of Allergic Disease.

Allergic disease is a dysregulation of the immune system, characterized by

1. Inappropriate T-cell responses
2. Tissue edema
3. Imbalances in the numbers, locations and activation status of specific immune cell types and subtypes
4. Allergen sensitivity and production of allergen specific IgE, and
5. Overproduction of and hypersensitivity to inflammatory mediators, including leukotrienes and cytokines

Extrinsic atopic dermatitis (AD), allergic asthma (AS), and allergic rhinitis (AR) are prevalent allergic diseases that can be distinguished by the location of their most important symptom manifestations; skin, lungs and sinuses, respectively. These diseases have the same underlying, systemic origin and two or more of them may occur simultaneously or sequentially in the same individual (Kagi, 2001; Leung et al. 2004; Meltzer, 2000; Oettgen and Geha, 1999; Spergel and Paller, 2003). Allergic disease normally begins in infancy, with either food allergy or AD. Approximately 50-80% of children with AD will go on to develop allergic rhinitis and/or asthma, a phenomenon known as the atopic march (Rackal and Vender, 2004; Spergel and Paller, 2003). The systemic origin is a dysregulation of the immune system response to environmental antigens, mediated by T-cells (Borish and Steinke, 2003). Allergic conditions are characterized by elevated serum IgE levels, peripheral eosinophilia, T-helper lymphocyte imbalances, markers of immune system activation such as mast cell activation and excess synthesis of leukotrienes and cytokines.

Imbalances in Immune cell types and subtypes

A brief discussion of immune cells involvement in allergic disease is given below. Consult the references for more detail.

T-Helper Cell Subtypes

In allergic disease, Th-2 cells predominate over Th-1 cells. This chronic state of T cell imbalance leads to the overproduction of inflammatory cytokines such as IL-4, IL-5 and IL-13. IL-4 in particular contributes to symptoms of itch, as well as stimulating IgE production by B cells. IL-5 stimulates production of eosinophils. T-cell derived cytokines also contribute to proliferation of mast cells.

Eosinophils

Excess numbers of activated eosinophils in target tissues and in circulation is a hallmark of all allergic diseases (Borish and Steinke, 2003; Prussin and Metcalfe, 2003). These cells are the major source of inflammatory cysteinyl leukotrienes and also produce IL-13. Leukotrienes cause bronchoconstriction in the lungs, mucous production in sinuses, and promote permeability to inflammatory cells in skin. IL-13 induces airway hyperresponsiveness (Vladich et al., 2005). Eosinophils also release major basic protein, which is toxic to epithelial cells in the respiratory tract.

Mast Cells
Allergic individuals produce greater numbers of mast cells than nonatopic individuals (Borish and Steinke, 2003; Prussin and Metcalfe, 2003). Mast cells bear allergen specific IgE. When exposed to an allergen, mast cells become activated and degranulate, releasing histamine, cytokines and prostaglandins and leukotrienes (Holgate, 2000). Mast cell degranulation quickly produces the typical symptoms of allergic disease: itch, redness and swelling of the skin; bronchoconstriction, mucous production, swelling and cough in the lungs; itch, sneezing and mucous production in sinuses.

Basophils
Basophils produce inflammatory mediators, including histamine, leukotriene LTC_4, IL-4, and IL-13 (Prussin and Metcalfe, 2003). Basophils, like mast cells, express high-affinity IgE receptor. When activated, basophils release histamine, cytokines and leukotrienes. Elevated levels of circulating basophils are characteristic of atopic asthma and atopic dermatitis. LTB_4 induces degranulation of IL-13 primed basophils.

Langerhan's cells.
Skin of individuals with active AD, AS and/or AR contains Langerhan's cells expressing surface FcεR1 with bound IgE (Semper et al., 2003). Thus, skin abnormalities exist in all allergic disease, regardless of whether AD is present.

The role of inflammatory mediators in allergic disease

Table 1 summarizes the involvement of key cytokines and leukotrienes in allergic disease

Table 1. Inflammatory mediators in allergic disease.

Cytokine	Source	Impacts in allergic disease
IL-4	Th-2 lymphocytes, eosinophils, basophils	Enhances antigen-presenting capacity of B cells. Induces isotype switching from IgM to IgE. Influences T-cell differentiation and survival. Induces mast cell expression of LTC_4 synthase. Stimulates mucous production in airways. Stimulates production of leukotriene receptors.
IL-5	Th-2 lymphocytes	Promotes eosinophil differentiation and survival. Upregulates 5-LO
IL-13	Th-2 lymphocytes	Promotes IgE synthesis Promotes mucous secretion in airways.
Leukotriene		
LTB_4	Neutrophils, macrophages	Chemoattractant for other immune cells in respiratory tract and skin, which prolongs the inflammatory response in these tissues. Activates neutrophils leading to endothelial leakage and promotion of inflammation. Promotes synthesis of IL-5 by T cells. Induces degranulation of basophils.
LTC_4 & derivatives (cysteinyl leukotrienes)	Eosinophils, mast cells, macrophages, basophils	Provoke bronchoconstriction. Promote mucous production. Promote sensitivity to histamine. Increase permeability of vascular tissue to immune cells. Promote secretion of IL-4 and IL-5 by lymphocytes.

Leukotrienes (LTs) and the pathophysiology of allergic diseases.

Leukotrienes are inflammatory mediators (Brain and Williams, 1990; Samuelsson, 1983) that play a significant role in the pathophysiology of allergic diseases, including asthma, atopic dermatitis, allergic rhinitis and food allergy. Leukotrienes are derived from the fatty acid arachidonic acid (AA), which is concentrated in the membrane phospholipids of blood cells. There are two types of leukotrienes. Cysteinyl leukotrienes (LTC_4, LTD_4 and LTE_4) directly promote allergic inflammation in target tissues and increase the synthesis of other proinflammatory mediators, such as cytokines. The chemotactic leukotriene, LTB_4, is a chemoattractant for immune cells and activates neutrophils leading to endothelial leakage.

Key criteria (Busse 1996) that implicate LTs in the pathophysiology of AD, AS and AR include the following:

1. LTs are found in fluids removed from the involved tissues;
2. Tissues with receptors for LTs are found in the affected tissues;
3. The administration of exogenous LTs mimics the effects of AD, AS and AR in the relevant tissues;
4. Reducing LT activity, whether by inhibiting their synthesis or by blocking their receptors, ameliorates disease symptoms.

LTB_4 is produced primarily by neutrophils and monocyte/macrophages (O'Byrne 1997; Wenzel, 2003). The role of LTB_4 in allergic disease is multifactorial. First, LTB_4 is a chemoattractant for immune cells such as neutrophils, eosinophils and monocytes in the respiratory tract (Busse, 1996; Busse, 1998) and in skin (Bisgaard et al, 1986). These leukocytes are recruited from the general circulation to the site of inflammation in lungs, nasal tissues and skin. Second, LTB_4 activates neutrophils leading to endothelial leakage which further promotes allergic inflammation (Busse, 1998; Wenzel 2003). Finally, LTB_4 indirectly mediates pain (Bisgaard and Kristensen, 1985; Brain and Williams, 1990)

The roles of the cysteinyl leukotrienes, LTC_4, LTD_4 and LTE_4, are multifaceted (Bisgaard, 2001). When produced in lung tissue, they are powerful bronchoconstrictors; LTC_4, LTD_4 for example, are ca. 1000x more powerful than histamine (Bisgaard, 2001). Cysteinyl leukotrienes are produced by resident mast cells and alveolar macrophages, as well as by peripheral blood eosinophils (Bisgaard, 2001; Eapen and Busse, 2002). When produced in sinuses, cysteinyl leukotrienes promote the production of mucous, promote cell-mediated inflammation and promote synthesis of inflammatory cytokines (Bisgaard, 2001; Busse, 1998; Peters-Golden and Henderson, 2005). Airborne allergen exposure provokes leukotriene synthesis in nasal tissues (Busse, 1996). Cysteinyl leukotrienes also promote sensitivity to histamine by immune cells (Pynaert et al., 1999.) In skin, these leukotrienes increase the permeability of vascular tissue to inflammatory cells, and epidermal cells are sites of leukotriene synthesis (Rackal and Vender, 2004; Talbot et al., 1985).

In all of these allergic diseases, there is an overproduction of leukotrienes: the specific disease(s) present in any one individual may be determined in part by the location(s) of leukotriene synthesis.

Production of and response to leukotrienes by allergic individuals

Leukotrienes are important for innate immunity and the physiological response to pathogens (Peters-Golden and Coffey, 1999). However, excess production of leukotrienes promotes the inappropriate response to harmless antigenic substances that characterizes allergy.

Blood leukocytes of allergic individuals produce higher levels of leukotrienes upon stimulation than do the same types of cells from nonallergic individuals (Bisgaard, 2001; Sampson et al., 1992). Further, the response of allergic individuals to leukotrienes is exaggerated compared to the response by nonallergic individuals.

Leukotrienes in Atopic Dermatitis (AD).
Both LTB_4 and cysteinyl leukotrienes are implicated in the pathophysiology of AD (reviewed in Ruzicka, 1988, 1989). Cysteinyl leukotriene levels (urinary LTE_4) were significantly higher in patients with atopic dermatitis than in healthy volunteers (Fauler et al., 1993; Hishinuma et al. 2001). Others have documented increased levels of LTE_4 in urine from children with severe AD (Øymar et al., 2005) and from adults (Adamek-Guzik et al. 2002) during exacerbations but not during remission. Hon et al. (2004) found that disease severity as measured by SCORAD was significantly correlated with measures of urinary LTE_4 in children. In contrast, Sansom et al. (1997) reported no elevation in urinary LTE_4 levels in seven patients during and after a flare episode compared to a normal range from control subjects. LTB_4 can be isolated from skin lesions of atopic dermatitis (Fogh et al., 1989; Ikai and Imamura, 1993; Ruzika et al., 1986). Increased production of LTB_4 *in vivo* has been reported in the skin of atopic patients after allergen specific challenge (Bisgaard et al., 1985; Koro et al., 1999).

Neuber et al. (1991) and Hilger et al. (1991) have reported that the spontaneous release of LTB_4 and LTC_4 from neutrophils is three times higher in patients with AD than in controls. Cysteinyl LT release from basophils (Shichijo et al., 1995) and eosinophils (Schauer et al., 1995) isolated from AD patients is increased compared to healthy controls. Enhanced spontaneous and stimulated releasability of LTC_4 from leukocytes of patients with AD compared with normal controls has been reported (Ruzicka 1988; Sansom et al. 1997). Elevated spontaneous release of LTC_4 from circulating basophils has been documented in severe compared to mild AD (James et al. 1993). Peripheral blood leukocytes from subjects with AD have a lower threshold of stimulus required for leukotriene release, and release higher levels of leukotrienes when stimulated than do leukocytes from normal subjects (Ruzicka and Ring, 1987). Shimizu et al., (1994) hypothesized that the increased production of LTC_4 *ex vivo* was due to eosinophilia rather than due to enhanced releasability of the leukotrienes in AD.

Since AD commonly occurs in patients with other atopic diseases such as asthma, it has been noted that patients treated with a leukotriene antagonist for their asthma symptoms also had some improvement in their skin condition. Case studies (Angelova-Fischer and Tsankov, 2005; Carucci et al., 1998) and randomized controlled studies (Capella et al., 2001; Eustachio et al., 2002; Rackal and Vender, 2004) in subjects with AD confirmed this observation.

The important role of the enzyme LTA_4 hydrolase, which synthesizes LTB_4, in the pathogenesis and development of atopic dermatitis was evaluated by Okano-Mitani et al. (1996). Their findings include the following; The LTA_4 hydrolase activities in the supernatant fraction of peripheral blood polymorphonuclear leukocytes (PMN) were significantly higher in preparations of cells from severe AD patients than in those from moderate and mild AD patients and normal controls. LTA_4 hydrolase activities were also significantly higher in peripheral blood mononuclear cells (PBMC) from severe AD patients than in those from moderate and mild AD patients and normal controls. LTA_4 hydrolase activities in PMN were reduced after improvement of the disease in eight patients with severe or moderate AD. The enhanced ability of neutrophils from AD

patients to produce LTB_4 from exogenous LTA_4 *in vitro* was recognized previously (Hilger et al., 1991). See also the review by Ikai and Imamura, 1993.

Leukotrienes in Asthma.
The cysteinyl leukotrienes are potent bronchoconstrictors produced by immune cells resident in lung tissue. These mediators are direct causal agents of many of the symptoms of asthma, including airway smooth muscle constriction, airway hyperresponsiveness, eosinophil migration, vascular permeability, and edema (Brain and Williams, 1990; Busse, 1998; Busse and Kraft, 2005; Donnelly et al. 1995; O'Byrne 1997; Wenzel, 2003). Leukocytes from asthmatics produce 3 – 5x higher levels of leukotrienes than do leukocytes from healthy controls (Sampson et al., 1992). The 5-LO enzyme may be upregulated in asthmatic subjects (Mita et al., 1985). Further, the response to inhaled leukotrienes is exaggerated in asthmatics compared to healthy subjects (O'Byrne 1997). In asthmatic children, concentrations of cysteinyl leukotrienes in exhaled breath condensates were significantly higher than in control children (Baraldi et al., 2003; Zanconato et al, 2004; Zsuzsanna et al., 2002). Asthmatic children with exercise-induced bronchoconstriciton (EIB) had higher concentrations of cysteinyl leukotrienes in exhaled breath condensates than control children or asthmatic children without EIB (Carraro et al., 2005). Exaggerated production of cysteinyl leukotrienes is also evident in aspirin-induced asthma (Antczak et al., 2002).

Leukotrienes in Allergic Rhinitis (AR)
Leukotrienes mediate many of the typical symptoms of allergic rhinitis, including nasal congestion, mucous secretion and edema (Busse and Kraft, 2005; Peters-Golden and Henderson, 2005). CysLTs are overproduced by patients with allergic rhinitis within minutes of nasal allergen exposure (Peters-Golden and Henderson, 2005). Increased production of LTC_4 *in vivo* has been reported in the tear fluid (Bisgaard et al., 1985) and intact skin (Talbot et al., 1985) of atopic patients after allergen specific challenge. These lipid mediators interact with receptors, particularly the cysLT1 receptor, on eosinophils, mast cells, macrophages, and neutrophils (Steinke and Borish, 2004). They also stimulate production of other proinflammatory mediators, such as cytokines IL-4 and IL-5 (Steinke and Borish, 2004).

Peters-Golden and Henderson (2005) recently reviewed the significant role that leukotrienes play in allergic rhinitis. "Many of the cells involved in the pathophysiology of allergic rhinitis produce and release CysLTs. This production and release begin early in the allergic response, since unlike cytokines, which require transcription and translation before synthesis, all the enzymes necessary to produce CysLTs are already present in inflammatory cells. During the early phase response, mast cells and basophils are the primary source of CysLTs, which act both locally and systemically to stimulate the production, recruitment, and activation of additional inflammatory cells. The newly recruited inflammatory cells, predominantly eosinophils but also monocytes and macrophages, are the primary source of CysLTs during the late-phase reaction".

Strategies to reduce the impact of leukotrienes in allergic diseases.

Two different types of LT-modulating agents are 5-lipoxygenase inhibitors and LT receptor antagonists (LTRAs). Since the 5-lipoxygenase inhibitor acts at the initial step in the LT synthetic pathway, it has the ability to alter the production of all the leukotrienes (Chari et al., 2001; Julemont et al., 2003), including LTB_4, while the receptor antagonists target only the cysteinyl LTs, LTC_4, LTD_4, and LTE_4. There are several ways to assess leukotriene synthesis *in vivo* that have been used in clinical trials: LTB_4 production by stimulated whole blood *ex vivo* (e.g. Rubin et al., 1991; Surette et al, 2003b), urinary LTE_4 (e.g. Israel et al, 1993), and LTs in exhaled breath condensate (e.g. Baraldi et al., 2003; Zsuzsanna et al., 2002).

LT modulating agents have been assessed as monotherapy as well as in combination with other therapies. In most cases, LT modifying agents have a clinically significant benefit in combination with other therapies.

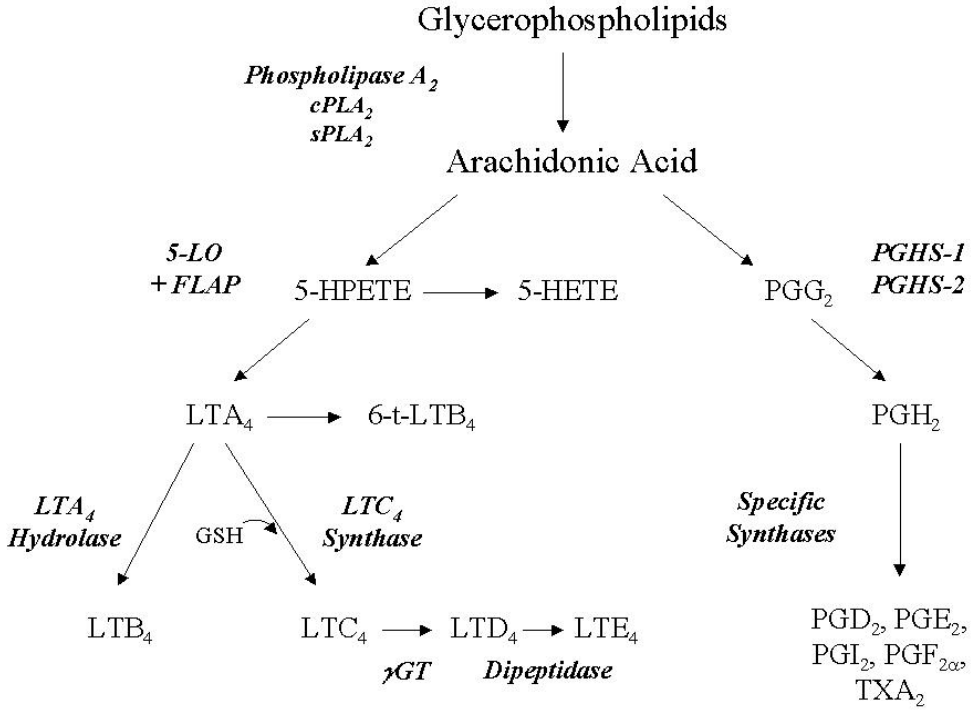


Fig. 1. Leukotriene (LT) biosynthetic pathway (left side of Figure). The enzyme 5-lipoxygenase (5-LO) acts upon free arachidonic acid as the first step in the pathway. The 5-lipoxygenase inhibitor, zileuton, as well as metabolites from the medical food EFFICAS CARE, act to inhibit 5-LO and thus blunt the entire leukotriene pathway. The leukotriene receptor antagonists, e.g. montelukast, do not impact synthesis of leukotrienes, but block the activity of the cysteinyl leukotrienes at the receptor.

Leukotriene Modulation in Atopic Dermatitis

Evidence in the literature provides a pathophysiological rationale for the use of cysLT receptor blockers in the treatment of AD (reviewed in Kagi, 2001). Case reports confirm that LTRs may reduce symptoms of AD even in severe cases (Zabawski et al., 1999) although an LTR had no significant impact on moderate to severe AD in one controlled trial (Veien et al., 2005). In addition, leukotriene synthesis inhibition would likely be beneficial, given the role of LTB_4 in AD. This topic was recently reviewed by Rackal and Vender (2004).

Impacts of 5-LO inhibition in atopic dermatitis include:

1. Significant reduction in disease dissatisfaction ($p=0.03$) (Woodmansee and Simon, 1999).
2. Significant reduction in objective skin scores ($p=0.03$) (Woodmansee and Simon, 1999).

Impacts of LTRAs in atopic dermatitis include:

1. Reduced body surface affected (Carucci et al., 1998)
2. Reduced severity of AD (Biswas et al., 2001; Capella et al., 2001; Friedman et al., 2000; Galindo et al., 2000; Pei et al., 2001;Yanase and David-Bajar, 2001; Zabawski et al., 1999).
3. Reduced itch (Capella et al., 2001; Friedman et al., 2000)
4. Steroid sparing (Carucci et al., 1998; Yanase and David-Bajar, 2001; Zabawski et al., 1999)

LTRAs have also been reported to reduce severity of urticaria (Erbagci 2002)

Leukotriene Modulation in Asthma

Impacts of 5-LO inhibition in asthma include:

1. Significant improvement in quality of life and asthma control in adult subjects (Israel et al., 1996).
2. Improved FEV_1 (Liu et al., 1996)
3. Reduced β-agonist use (Liu et al., 1996)
4. Reduced cold-air induced bronchoconstriction (reviewed in Bisgaard, 2001)
5. Reduced eosinophilia (reviewed in Bisgaard, 2001; Liu et al., 1996)
6. Reduced exercise-induced bronchoconstriction (reviewed in Horwitz et al., 1998)
7. 50% reduction in emergency room visits and hospitalizations (reviewed in Wenzel, 1988).

Impacts of LTRAs in asthma include:

1. Improved pulmonary function in adult patients with asthma (Barnes et al 1997; Barnes et al. 2001, Fish et al, 1997; Kemp et al. 1999; Reiss et al. 1998).
2. Significantly improved pulmonary function and reduced symptoms in children, irrespective of concomitant inhaled corticosteroid (ICS) use (Knorr et al. 1998; Knorr et al. 2001; Pearlman et al. 2000).

3. Significant tapering of ICS dose without loss of asthma control (Lofdahl et al. 1999).
4. Significantly reduced percentage of days with daytime asthma symptoms and reduced nighttime awakenings in children (Knorr et al. 2001; Pearlman et al. 2000).
5. Reduced airway hyperresponsiveness (Carratu et al. 2003; Rorke et al. 2002; Westbroek and Pasma, 2000; Yoshida et al. 2000).
6. Decreased daytime asthma symptoms and nocturnal awakenings in adult patients (Reiss et al. 1998).
7. Significantly improved the asthma quality of life in all four domains (activity, symptoms, emotional function, and exposure to environmental stimuli) (Reiss et al. 1998; Nathan et al. 1998).
8. Decreased eosinophil levels in blood, BAL fluid, and sputum of adults (Calhoun et al. 1998; Pizzichini et al. 1999; Reiss et al 1998) and decreased eosinophil levels in blood of children (Knorr et al 1998, 2001; Stelmach et al. 2002).
9. Significant reduction in the decreased pulmonary function after exercise in asthmatics with exercise-induced bronchoconstriction (Dahlen et al. 2002; Kemp et al. 1998; Pearlman et al. 1999).

Leukotriene Modulation in Allergic Rhinitis

Nasal congestion was reduced by inhibition of 5-LO (Knapp and Murray, 1994).

Impacts of LTRAs in allergic rhinitis include:

- Reduced rhinorrhea, nasal itching and nasal obstruction (Jiang, 2006).
- Reduced daytime nasal symptoms (congestion, rhinorrhea, pruritus, and sneezing) and nighttime symptoms (difficulty sleeping, nighttime awakenings, and congestion on awakening) (Patel et al., 2005)
- Reduced daytime nasal symptoms (congestion, rhinorrhea, pruritus, and sneezing), nighttime symptoms (difficulty sleeping, nighttime awakenings, and congestion on awakening) and daytime eye symptoms (Nayak et al., 2002; Philip et al. 2002; van Adelsberg et al. 2003)
- Significantly reduced daytime nasal symptoms, daytime eye symptoms, nighttime symptoms and composite symptom score when combined with loratidine compared to placebo (Meltzer et al., 2000; Nayak et al., 2002)
- Significantly improved rhinoconjunctivitis-specific quality-of-life score, which included significant improvement in domains such as activity, ocular symptoms, practical problems, and emotional symptoms (Meltzer et al., 2000; Nayak et al. 2002; Patel et al., 2005; Philip et al. 2002; van Adelsberg et al 2003).
- Decreased peripheral blood eosinophils (Philip et al., 2002)
- Decreased number of eosinophils in nasal lavage fluid of patients with both asthma and allergic rhinitis (Piatti et al. 2003).
- Reduced asthma and allergic rhinitis symptoms in patients with both asthma and allergic rhinitis (Piatti et al. 2003).

Leukotrienes and Quality of Life
The reduction of leukotriene levels in subjects with asthma (Biernacki et al., 2005; Busse and Kraft, 2005; Israel et al., 1996; Nathan et al., 1998), atopic dermatitis (Woodmansee and Simon, 1999), and allergic rhinitis (Patel et al., 2005) has been reported to improve quality of life.

EFFICAS CARE: Mechanisms of action relevant to allergic diseases.

EFFICAS CARE is a medical food that reduces production of leukotrienes by inhibiting the action of the 5-lipoxygenase enzyme. The role of this enzyme in leukotriene synthesis is illustrated in Figure 1, above.

Leukotriene Production and the Arachindonic Acid Pathway.

When immune cells are stimulated, fatty acids are cleaved from the membrane phospholipids by phospholipase[1]. Leukotrienes are formed from arachidonic acid (AA), in response to a stimulus such as allergen exposure. The free AA is a substrate for the enzyme 5-Lipoxygenase (5-LO)[2], the first enzyme in the pathway for synthesis of the bronchoconstricting cysteinyl leukotrienes, LTC_4, LTD_4, LTE_4, and the chemotactic leukotriene LTB_4 (Bisgaard, 2001). 5-LO produces a short-lived intermediate from AA, 5-HPETE. A synthase then produces LTA_4, another short lived intermediate which is a branch point in the leukotriene pathway. This intermediate is a substrate for LTA_4 hydrolase, producing LTB_4. The intermediate may also combine with a glutathione residue to produce LTC_4. Further processing of LTC_4 produces LTD_4 and LTE_4. Inhibition of the first enzyme in the pathway, 5-LO, reduces levels of all the leukotrienes (Julemont et al., 2003).

The same stimulus that releases AA from membranes will release other highly unsaturated fatty acids, such as DGLA and EPA. DGLA decreased LTB_4 production by human mononuclear leukocytes *in vitro* in a dose-dependent manner (Iverson et al., 1992). Unlike arachidonic acid and EPA, DGLA is not a substrate for 5-LO, but instead is converted by 15-LO to 15-OH-DGLA (aka 15-HETrE). 15-HETrE blocks conversion of AA to LTA_4 by direct inhibition of 5-LO (Guichardant et al., 1993) and is an even stronger inhibitor of LTB_4 production than is DGLA (50x). Inhibition of 5-LO activity in human neutrophils *ex vivo* has also been demonstrated in cells supplemented with GLA or DGLA (Chilton-Lopez et al., 1996). Neutrophils metabolize DGLA to 15-HETrE (Chilton-Lopez et al., 1996). Dietary GLA increases DGLA in phospholipids and decreases *ex vivo* elaboration of LTB_4 in healthy human volunteers (Surette et al., 2003a; Ziboh and Fletcher, 1992).

When EPA is liberated from membrane phospholipid, it competes with arachidonic acid for the 5-LO enzyme (Lands, 1992). Dietary EPA (provided in fish oil) has been demonstrated in multiple studies to actively reduce levels of AA in membrane

[1] Inhibition of phospholipase is the primary anti-inflammatory activity of corticosteroids.
[2] Arachidonic acid is a substrate for numerous enzymes that influence inflammation. For brevity, only the leukotriene pathway is introduced here.

phospholipids (Chapkin et al., 1991; Miller et al., 1991; Stulnig, 2003). Dietary EPA reduced AA levels in murine macrophages (Chapkin et al., 1991) but not in human neutrophils (Chilton et al., 1993). EPA inhibits the enzyme delta-5 desaturase that would otherwise produce AA from DGLA (Nassar et al., 1986), so that higher doses of GLA can be provided safely in a medical food as long as EPA is also provided in sufficient quantity. Finally, in contrast to DGLA, EPA is a substrate for 5-LO, and thus competes with AA for the enzyme's active sites. 5-series leukotrienes are produced from the action of 5-LO on EPA, but these have reduced bronchoconstricting activity compared to LTB_4, LTC_4, LTD_4 and LTE_4 (Schwartz, 2000). Peritoneal macrophages from mice fed fish oils produced fewer leukotrienes overall than those from control mice, and the ratio of series 4 to series 5 leukotrienes was dramatically altered in favor of series 5.

Clinical Studies Demonstrating Impact of the Medical Food on Leukotrienes
EFFICAS CARE, a medical food formulation containing specified amounts and ratios of GLA and EPA, has been demonstrated to significantly reduce leukotriene synthesis (LTB_4) by stimulated whole blood in healthy subjects and asthmatic subjects (Surette et al., 2003a,b).
Two preliminary clinical trials with the active ingredients were conducted to determine the minimum effective levels needed to reduce leukotriene biosynthesis and prevent increases in plasma arachidonic acid (Barham et al., 2000; Surette et al., 2003a). These were followed by three clinical studies conducted to evaluate the safety, tolerability and effectiveness of the formulated medical food product. These studies were: a single-center, randomized, double-blind, placebo-controlled, parallel-group, escalating-intake inpatient trial in healthy adult subjects (Surette et al., 2003a); a pharmacokinetic study in asthmatic children and adolescents (Efficas, unpublished); and a safety and efficacy study in adult asthmatics (Surette et al., 2003b). Results in these studies were consistent. The bioavailability of the bioactive fatty acids was significantly improved by formulating them into the emulsion product. It was also convincingly demonstrated that the product was well-tolerated and was effective in lowering the production of leukotrienes in healthy subjects and in those with asthma. The emulsified medical food providing 750 mg GLA + 500 mg EPA significantly reduced ($p< 0.05$) *ex vivo* whole blood leukotriene synthesis by 23% in asthmatic subjects (Surette et al., 2003b).

Proven Leukotriene Reduction in Healthy Subjects
In a placebo-controlled clinical study involving 47 healthy adults, Efficas Care significantly decreased leukotriene production within 14 days (Surette et al., 2003a). The clinical study also demonstrated that the medical food was well tolerated in this population.

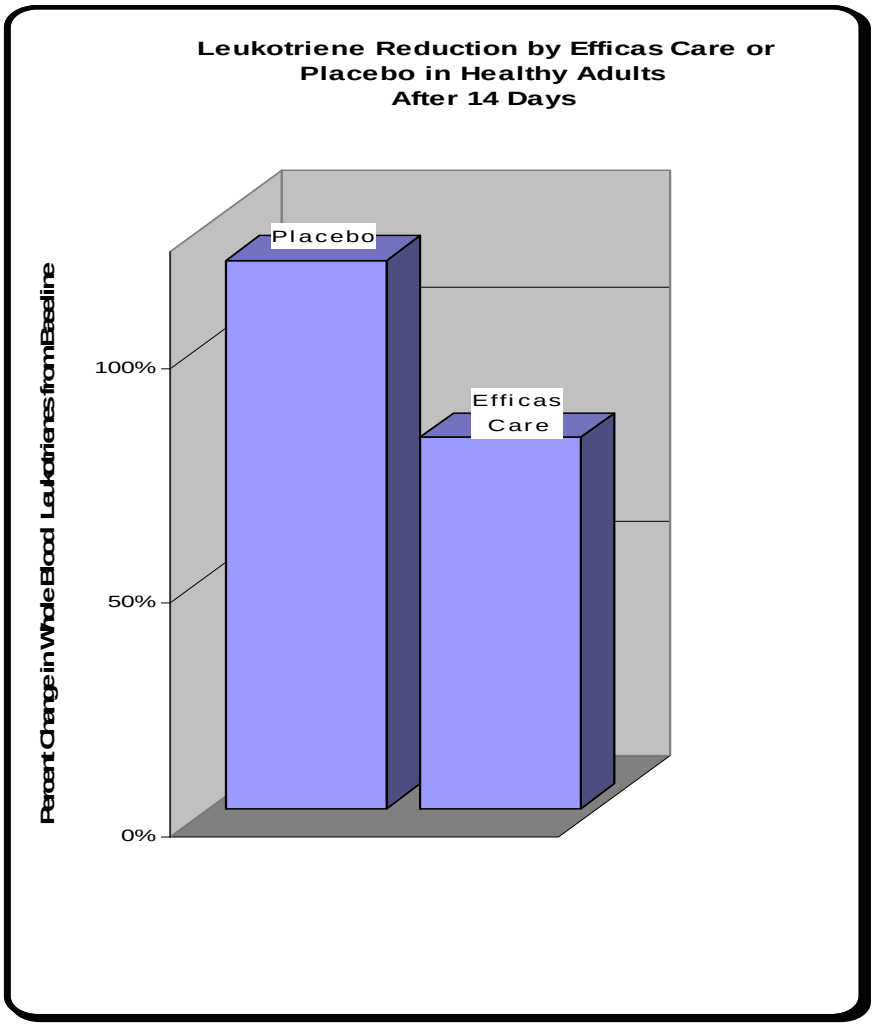
Leukotriene (LTB_4) Reduction ($p< 0.03$)
Efficas Care vs placebo – mean change from baseline -4.1 vs. 3.8 ng/ml per 10^6 neutrophils ($p< 0.03$)*

Whole blood leukotriene (LTB_4) concentrations (ng • ml $^{-1}$ • 10^6 polymorphonuclear neutrophils) in healthy adults consuming Efficas Care or placebo for 2 weeks. Values are mean (SD).

	Baseline (ng x ml^{-1} x10^6 PMN)		**Day 14** (ng x ml^{-1} x10^6 PMN)
Group	Mean (SD)		Mean (SD)
Placebo	22.2 (7.3)		26.0 (16.2)
10g Efficas Care™	19.8 (4.8)		15.7* (6.0)

*Significantly different compared to placebo determined by ANCOVA, $p<0.03$.

SD = standard deviation; PMN = polymorphonuclear neutrophils.



Proven Leukotriene Reduction in Asthmatic Patients

In a placebo-controlled clinical study involving 43 adult patients with mild to moderate atopic asthma, Efficas Care significantly decreased leukotriene production within 28 days (Surette et al., 2003b). The clinical study also demonstrated that the medical food was well tolerated in this population.

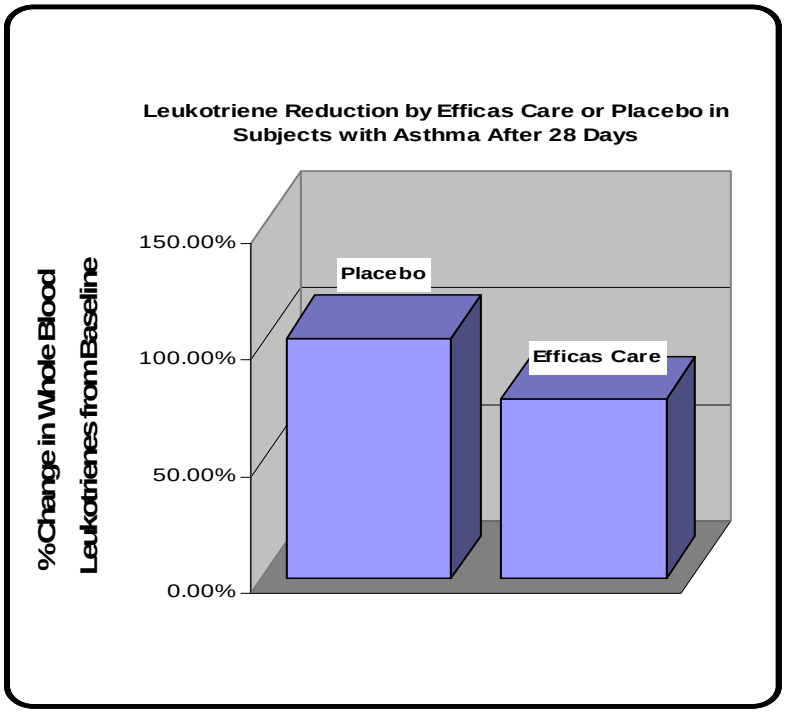
Leukotriene (LTB_4) Reduction ($p< 0.05$)

Efficas Care vs placebo – mean change from baseline -4.1 vs. 0.6 ng/ml per 10^6 neutrophils ($p< 0.05$)*

Whole blood leukotriene (LTB_4) concentrations (ng • ml $^{-1}$ • 10^6 Polymorphonuclear neutrophils) in patients with atopic asthma consuming Efficas Care or placebo for 4 weeks. Values are mean (SE).

	Baseline (ng x ml^{-1} x10^6 PMN)		**Day 28** (ng x ml^{-1} x10^6 PMN)
Group	Mean (SE)		Mean (SE)
Placebo	18.8±3.9		19.4±2.6
10g Efficas Care™	17.6±3.1		13.5±1.5 *

*Significantly different compared to placebo determined by ANCOVA ($p<0.05$).



Studies Demonstrating Impact of the Medical Food on Quality of Life and Disease Management

Patient assessments of their symptoms are an important measure of benefit (quality of life). The medical food improved subjective assessment of asthma in 8 of 11 patients (72%) versus 37.5% of subjects on placebo after 4 weeks of use (Surette et al., manuscript in preparation). An additional open label consumer study evaluated the impact on QOL of the medical food in subjects with asthma, allergic rhinitis or eczema. Mean scores for the validated QOL instruments MiniAQLQ and ACQ improved significantly ($p<0.001$) in subjects with asthma (Surette et al., manuscript in preparation). QOL scores also improved significantly ($p<0.001$) in subjects with allergic rhinitis and eczema (Efficas, unpublished).

Conclusion:

EFFICAS CARE has been shown in clinical trials to alter the pools of fatty acid precursors of leukotrienes, to significantly reduce leukotrienes in healthy individuals as well as in asthmatics and to improve the quality of life in subjects with asthma, allergic rhinitis and eczema. Leukotrienes contribute significantly to the pathophysiology of allergic diseases, including asthma, allergic rhinitis and eczema. The medical food, EFFICAS CARE, blunts leukotriene synthesis via a nutritional mechanism, and thus is suitable for use in multiple allergic diseases.

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