Effects on leukotriene Biosynthesis

Efficas Care™ is a proprietary medical food for the dietary management of asthma and allergic rhinitis (upper airway allergy). This product blocks the conversion of arachidonic acid to leukotrienes, molecules produced by cells of the immune system that are involved in the pathogenesis of inflammatory and allergic disorders like asthma and allergic rhinitis. Efficas Care™ is specially formulated to provide specific amounts and ratios of highly bioavailable fatty acids that are consumed once daily in a naturally flavored emulsion. This proprietary blend of fatty acids is clinically proven to safely and effectively block the production of leukotrienes, substances known to cause asthma attacks and allergy symptoms, in humans.

Background and Mechanism of Action

a. Regulation of Leukotriene Biosynthesis
Arachidonic acid (AA) is a polyunsaturated fatty acid that is found in the membranes of all cells, including those of the immune system. AA can be transformed by cellular enzymes into the prostaglandins and leukotrienes which possess important biological activity in many tissues (Figure 1). Phospholipase A2 (PLA2) releases arachidonic acid from the sn-2 position of cell membrane phospholipids. Free arachidonic acid may be metabolized by 5-lipoxygenase (5-LO) to leukotriene (LTA)₄, a substrate for the terminal enzymes of the leukotriene pathway, or by one of the isoforms of prostaglandin endoperoxide synthase (PGHS; cyclooxygenase) to PGH₂, a substrate for the terminal enzymes of prostanoid biosynthesis (Figure 2).
Figure 1. The arachidonic acid pathway: precedent for safe and effective intervention. Leukotrienes and prostaglandins have been implicated in diverse physiological processes, including asthma, allergic rhinitis, eczema, inflammation, carcinogenesis, hemostasis, parturition, maintenance of renal function, pain and fever. Other products of arachidonic acid include hydroxyeicosatetraenoic acids (HETEs), lipoxins, epoxyeicosatrienic acids, and isoprostanes, which also may contribute to and modulate inflammatory responses.

b. 5-LO pathway

5-LO generates the unstable intermediate 5S-hydroperoxyeicosatetraenoic acid (5-HPETE) that is reduced to 5-HETE or is converted by the sequential action of 5-LO to an epoxide, LTA₄. 5-LO is present in a soluble fraction of cells. After cell activation and in response to a Ca²⁺ flux, 5-LO translocates to the nuclear envelope, where arachidonic acid, released by PLA₂, is presented to 5-LO by 5-LO activating protein (FLAP). LTA₄ is processed to LTB₄ by cytosolic LTA₄ hydrolase, or to LTC₄ by LTC₄ synthase, an integral perinuclear membrane protein that conjugates glutathione (GSH) to LTA₄. LTA₄ also undergoes non-enzymatic hydrolysis to 5S,12R- and 5S,12S-dihydroxy-6-trans-LTB₄ diastereoisomers (6-t-LTB₄).
Figure 2. Metabolism of arachidonic acid by 5-LO and PGHS. The abbreviations are explained and the pathways are described in the text.

LTB₄ and LTC₄ are exported from the cell by specific carrier systems. Extracellularly, the glutamic acid residue of LTC₄ is released from the GSH moiety by γ-glutamyl-transpeptidase (γ-GT) to generate LTD₄, from which the glycyl residue is cleaved by a dipeptidase to form LTE₄. The cysteinyl leukotrienes, LTC₄, LTD₄, and LTE₄ act at specific G protein-coupled receptors (GPCR), CysLT1 and CysLT2, to elicit their effects, which include contraction of bronchial smooth muscle, vasodilatation, and mucus secretion within the airways. LTB₄ is a potent chemotaxin acting at a specific GPCR, BLT1. A second receptor for LTB₄, BLT2, has also been described. In addition, LTB₄ may act as a ligand for a nuclear receptor, peroxisome proliferator-activated receptor (PPAR) α. LTB₄ may also act as a ligand for a nuclear receptor, peroxisome proliferator-activated receptor (PPAR) α. 

Arachidonic acid is classified as an essential fatty acid of the n-6 family. These fatty acids are essential since they cannot be produced by humans and must be consumed in the diet. The left side of Figure 3 shows the pathway by which dietary linoleic acid (LA), the initial member of n-6 family of essential fatty acids, can be transformed to AA. Since AA is derived from dietary lipids, there has been a large research effort over the last 20 years to understand how AA metabolism can be controlled by dietary manipulation.

Dietary LA is the primary source of n-6 fatty acids in human diets and its conversion to AA is tightly controlled by limiting the Δ-5 and Δ-6 desaturation enzyme steps.
Researchers have attempted to exploit these control mechanisms by supplementing people’s diets with oils containing the metabolic intermediate, gamma-linolenic acid (GLA), which is a minor constituent of human diets \(^{17, 20}\). Since GLA is a product of the \(\Delta-6\) desaturase, providing dietary GLA bypasses the \(\Delta-6\) desaturase regulatory step. This GLA is elongated to form dihomo-gamma-linolenic acid (DGLA) which is then converted to AA by \(\Delta-5\) desaturase. However, key inflammatory cells lack \(\Delta-5\) desaturase activity resulting in an accumulation of DGLA relative to AA in these cells \(^{15}\) (Figure 4).
DGLA can also be converted by lipoxygenases and cyclooxygenases to products that act as modulators of the conversion of AA to leukotrienes \(^{14,15}\). Therefore, supplementation of the diet with GLA leads to the accumulation of natural inhibitors of leukotrienes within inflammatory cells. Dietary GLA is a key ingredient of Efficas Care\(^{\text{TM}}\).

In addition to reducing leukotriene production by inflammatory cells, supplementation of human diets with GLA also results in an increase in circulating AA concentrations since \(\Delta-5\) desaturase activity in other tissues such as the liver converts dietary GLA to AA. Thus with time, the consumption of dietary GLA leads to an elevation of circulating AA levels that can potentially reverse the ability of DGLA to interfere with the synthesis of leukotrienes. However, the n-3 fatty acid, eicosapentaenoic acid (EPA) (Figure 3), is a natural inhibitor of the \(\Delta-5\) desaturase reaction. EPA limits the conversion of DGLA to AA by competing for and inhibiting the \(\Delta-5\) desaturation step (Figure 5). Therefore, when consumed in the correct amounts with GLA, EPA prevents the unwanted increase in circulating AA levels observed with intake of GLA alone \(^{13}\). Efficas Care\(^{\text{TM}}\) was designed to provide precise concentrations and ratios of these key dietary n-6 and n-3 fatty acids whose consumption results in the inhibition of the synthesis of the biologically active leukotrienes by inflammatory cells without increasing circulating AA levels.

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**Figure 4. Dietary GLA reduces leukotriene synthesis.**
Figure 5. The medical food Efficas Care™, containing a proprietary mixture of GLA and EPA, reduces leukotrienes and avoids arachidonic acid accumulation.
Clinical Trials with Efficas Care™

Six clinical studies with 228 participants demonstrated the efficacy and/or safety of Efficas Care. Additionally, one open label in-home use test which included 473 participants has demonstrated the improvements achieved in quality of life.

- **Study 1**: A 21-day, diet controlled, outpatient, open label trial in 16 healthy subjects conducted at Wake Forest University School of Medicine. Fatty acid levels, leukotriene levels and safety/tolerability were assessed. The results demonstrated that the use of the ingredients in Efficas Care decreased leukotriene production.

- **Study 2**: A 21-day, outpatient, open label trial in 30 healthy subjects conducted at Wake Forest University School of Medicine. Fatty acid levels, leukotriene levels, pharmacokinetics and safety/tolerability were assessed. Determined the ratios and concentrations of fatty acids in Efficas Care.

- **Study 3**: A 14-day, single-center, randomized, double-blind, placebo-controlled, parallel-group escalating-intake inpatient clinical trial in healthy adults conducted in a Phase I unit. Patient population included: Thirty non-smoking, healthy male and female subjects aged between 18 and 45 years and within 15% of ideal body weight participated in the study. Fatty acid levels, leukotriene levels and safety/tolerability were assessed. The trial was designed to determine the optimal amount of fatty acids required to reduce leukotriene levels and to confirm safety and efficacy of the formulation.

- **Study 4**: A 28-day, single-center, randomized, double-blind, placebo-controlled, parallel-group prospective efficacy clinical trial in patients with mild to moderate asthma conducted at Wake Forest University School of Medicine. Patient population included 43 adult patients age 15 to 65 years old. All patients had a diagnosis of asthma for at least one year and controlled their symptoms with beta-agonists and/or theophylline only. They also had a positive result on the methacholine challenge test as indicated by a PC20 of <8mg/ml, and a FEV1 > 70% of the predicted value. No patient could have taken inhaled or systemic steroids for ≥4 weeks before study enrollment. Fatty acid levels, leukotriene levels and safety/tolerability were assessed (Surette et al., 2003b). Preliminary assessment of quality of life impact was also made (Surette et al., manuscript in preparation). This study demonstrated that Efficas Care decreased leukotriene production in 75% of asthmatics.

- **Study 5**: A multi-center pediatric pharmacokinetics trial conducted to determine the optimal intake for pediatric populations. Population consisted of 24 healthy children aged to 6 to 11 and 12 to 17. (Efficas, unpublished)

- **Study 6**: A 28-day, two-center, randomized, double-blind, placebo-controlled, parallel-group prospective study was conducted in adult subjects with allergic asthma, allergic rhinitis or allergic eczema. Population consisted of males and females aged 18 – 65 years. Fatty acid level, leukotriene levels, quality of life and safety/tolerability were assessed. (Efficas, unpublished)

- **Consumer Study**: A 28-day, nation-wide, open label test of the impact of medical food Efficas Care on Quality of Life in consumer populations with Asthma, Allergic Rhinitis and Atopic Dermatitis. Population consisted of 473 adults age 22 to 55 years old. The study objective was to evaluate the impact on quality of life by using self-administered QOL instruments. There were no dietary or medications restrictions during the test period. The study period encompassed the summer allergy season.
Summary of Results in Clinical Trials

In initial studies carried out in a General Clinical Research Center, normal healthy subjects consumed oils containing the fatty acids in Efficas Care™. Baseline plasma fatty acids and stimulated whole blood leukotriene production were measured and the subjects’ diets were then supplemented daily with GLA. Following the three week supplementation period, these parameters were measured again. The capacity to synthesize leukotrienes was significantly decreased within 2 weeks when compared to baseline levels (Fig. 6). Following a 2-week washout period during which the subjects ceased supplementation, the capacity to synthesize leukotrienes returned to baseline levels.

*Figure 6. Biosynthesis of leukotriene B₄ in stimulated whole blood from subjects consuming GLA.*

In individuals consuming GLA alone, the decrease in leukotriene synthesis was accompanied by a marked increase in the plasma AA concentrations (Table 1). However, when subjects were provided with the combination of GLA and EPA found in Efficas Care™, plasma AA concentrations were unchanged from baseline.

*Table 1. Fatty acid concentrations (μmol/L) measured in plasma isolated from healthy subjects at baseline and three weeks after daily consumption of GLA or GLA + EPA (mean ± standard error).*

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>GLA Baseline</th>
<th>GLA Week 3</th>
<th>GLA+EPA Baseline</th>
<th>GLA+EPA Week 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>378±48</td>
<td>572±66*</td>
<td>455±66</td>
<td>493±78</td>
</tr>
<tr>
<td>GLA</td>
<td>35±5</td>
<td>47±4</td>
<td>32±4</td>
<td>59±4*</td>
</tr>
<tr>
<td>DGLA</td>
<td>115±12</td>
<td>227±14*</td>
<td>132±10</td>
<td>165±12*</td>
</tr>
<tr>
<td>EPA</td>
<td>22±11</td>
<td>15±3</td>
<td>25±9</td>
<td>76±9*</td>
</tr>
</tbody>
</table>

*Significantly different compared to baseline determined by one-way ANOVA (p<0.05).*
Two placebo-controlled trials were conducted to measure the efficacy of 10g/day of Efficas Care™ in decreasing leukotriene synthesis in both normal healthy subjects and in patients with mild to moderate Asthma (FEV₁ >70% predicted) [18,19]. Tables 2 and 3 show that the addition of Efficas Care™ to the diet results in a significant decrease in the capacity for leukotriene biosynthesis in both groups of asthmatics compared to placebo.

Table 2. Whole blood leukotrienes in healthy subjects consuming placebo or Efficas Care™ daily for 2 weeks.

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng*ml⁻¹ *10⁶ PMN)</td>
<td>(ng*ml⁻¹ *10⁶ PMN)</td>
</tr>
<tr>
<td></td>
<td>Mean  SD  Min  Max</td>
<td>Mean  SD  Min  Max</td>
</tr>
<tr>
<td>Placebo</td>
<td>22.2  7.3  12.6  33.4</td>
<td>26.0  16.2  17.3  65.7</td>
</tr>
<tr>
<td>10g Efficas Care™</td>
<td>19.8  4.8  13.1  31.1</td>
<td>15.7*  6.0  4.1  25.6</td>
</tr>
</tbody>
</table>

*Significantly different compared to Placebo determined by ANCOVA, p<0.03.
SD = standard deviation; PMN = polymorphonuclear neutrophils.

Table 3. Whole blood leukotrienes in asthmatic subjects consuming placebo or Efficas Care™ daily for 4 weeks.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>10g Efficas Care™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>Week 4</td>
</tr>
<tr>
<td>LTB₄ (ng*ml⁻¹ *10⁶ PMN)</td>
<td>18.8±3.9</td>
</tr>
</tbody>
</table>

Values represent mean ± SE. *significantly different compared to placebo determined by ANCOVA (p<0.05).

Pharmacokinetic data have also been analyzed with Efficas Care™ to determine adult and pediatric intakes. Serial blood samples were collected following the consumption of 10g of Efficas Care™ in adults and following consumption of 4g of Efficas Care™ in asthmatic children ages 6-11 years. Figure 7 shows that the consumption of 4g of Efficas Care™ in children ages 6-11 years produces maximum concentration (Cmax) and Area Under the Curve (AUC) values for plasma concentrations of GLA and EPA during the 24-hour post-consumption period which are comparable to those obtained with the adults consuming 10g of Efficas Care™.
Figure 7. Gammalinolenic acid and eicosapentaenoic acid concentrations in plasma of adult and pediatric subjects following a single consumption of 10g or 4g of Efficas Care™, respectively.

A number of clinical parameters were also monitored to evaluate the safety of these dietary management strategies. The supplementation of diets with 10g of Efficas Care™ per day for up to 4 weeks had no effect on circulating triglycerides, LDL cholesterol, HDL cholesterol, vital signs, clinical chemistry parameters, hematology, blood pressure or platelet aggregation compared to baseline values or compared to values obtained in subjects administered placebo containing olive oil (Table 4).

Table 4. Safety profile of Efficas Care™ compared to placebo.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet Aggregation</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical Chemistry</td>
<td>NS</td>
</tr>
<tr>
<td>Hematology Evaluations</td>
<td>NS</td>
</tr>
<tr>
<td>Blood Pressure (mm Hg)</td>
<td>NS</td>
</tr>
<tr>
<td>Heart Rate</td>
<td>NS</td>
</tr>
<tr>
<td>EKG</td>
<td>NS</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS= No significant difference from Placebo in subjects administered the recommended daily amount.
Asthma and Allergy Management and Quality of Life Improvements

An open-label test to evaluate the impact of Efficas Care on the quality of life in people with asthma or allergic rhinitis was conducted nationwide during the summer allergy season. The participants added Efficas Care to their daily diet while continuing to use their asthma or allergy medications. Quality of Life assessments were made using the validated MiniAQLQ, ACQ and MiniRQLQ questionnaires.

Results in Asthma. Overall, 71% of study participants reported an improvement in Quality of Life during the open label study. This change is of the same magnitude demonstrated in a placebo controlled study, where 72% of subjects with asthma taking Efficas Care versus 37.5% of subjects taking placebo reported improved quality of life after 4 weeks (Surette et al., manuscript in preparation).

Study participants reported a 44% mean improvement in Quality of Life from baseline after 4 weeks of taking Efficas Care. The Quality of Life improvements attained were evident within 28 days, were statistically significant, and of meaningful magnitude.

Asthma sufferers reported:
• reduced wheezing and shortness of breath
• improved sleep
• significant reductions in rescue bronchodilator use
• an increased ability to participate in physical activities

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mini AQLQ*</th>
<th>Mini AQLQ*</th>
<th>QOL Mean Improvement from Baseline</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>3.6</td>
<td>5.2</td>
<td>44%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Mini AQLQ Asthma scale 1-7 with higher numbers indicating higher QOL (21)

<table>
<thead>
<tr>
<th>Condition</th>
<th>ACQ*</th>
<th>ACQ*</th>
<th>QOL Mean Improvement from Baseline</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthma</td>
<td>3.2</td>
<td>2.2</td>
<td>31%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* ACQ Asthma Control Questionnaire scale is 0-6 with lower numbers indicating greater asthma control (22). Subjects did not determine FEV₁ in this study.
**Results in Allergy.** Overall, study participants reported a 34% mean improvement in Quality of Life from baseline after taking Efficas Care for 4 weeks. The Quality of Life improvements attained by the study participants were evident within 28 days, were statistically significant, and of meaningful magnitude.

Allergy sufferers reported:
- reduced allergy symptoms
- reduced allergy-related daytime fatigue
- an improved quality of life

<table>
<thead>
<tr>
<th>Condition</th>
<th>Mini RQLQ* Mean Score Day 1</th>
<th>Mini RQLQ* Mean Score Day 28</th>
<th>QOL Mean Improvement from baseline</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic Rhinitis</td>
<td>3.8</td>
<td>2.5</td>
<td>34%</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

* Mini RQLQ Allergy scale 0-6 with lower numbers indicating higher QOL (23)
REFERENCES


