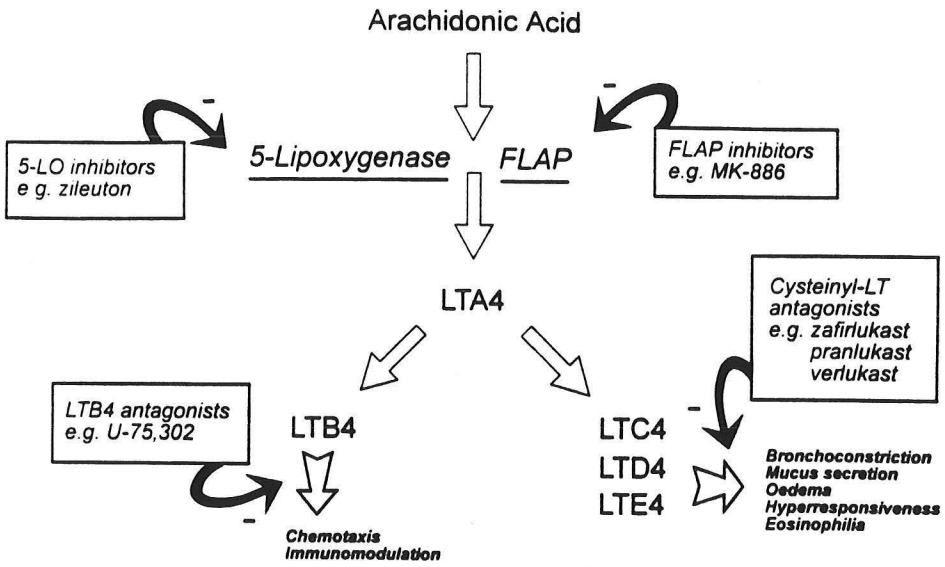
ASTHMA MANAGEMENT IN THE NEXT MILLENNIUM: A NEW DIMENSION

Role of Leukotriene Modifiers



UT Southwestern Medical Center
Internal Medicine Grand Rounds
January 29, 1998

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Areas of Interest:

Currently, Dr. Gruchalla's primary areas of interest are asthma and antibiotic drug allergy. She is Principal Investigator of a multicenter, inner-city pediatric asthma study that will evaluate two interventions. The first intervention is a communication/physician feedback program that will provide physicians with up-to-date information on the clinical status of the patient, medication use, and health care utilization. The second intervention is an extensive environmental intervention that will be implemented in the homes of the asthmatic children randomized to that intervention. The efficacy and cost-effectiveness of the individual components will be analyzed in the 1000 children who are to be evaluated.

Dr. Gruchalla also has an interest in the area of antibiotic drug allergy, specifically sulfonamide drug allergy. Her laboratory has been investigating human immune responses to sulfamethoxazole as a model system to test a series of hypotheses about haptenation and immunopathologic reactions to haptens in humans.

This is to acknowledge that Rebecca Gruchalla has disclosed no financial interests or other relationships with commercial concerns related directly or indirectly to this program.

INTRODUCTION

Asthma is a chronic inflammatory lung disease that affects 14 to 15 million persons in the United States alone. It is the most common chronic disease of childhood, affecting approximately 4.8 million children [3, 21]. Asthmatics have more than 100 million days of restricted activity and they have more than 470,000 annual hospitalizations. Even more striking is the fact that more than 5,000 people die from asthma each year [21].

It is now well-established that asthma is a disease that involves chronic airway inflammation. No longer is it thought to be simply a disease of episodic bronchial smooth muscle constriction. In light of this newly-acquired knowledge, our management strategies are changing. As outlined in the recently-published *Guidelines for the Diagnosis and Management of Asthma:Expert Panel Report 2* [39], effective asthma management must include comprehensive pharmacologic therapy that is aimed at reversing and preventing asthma-associated inflammation. While quick-relief bronchodilators are still required to treat asthma symptoms and exacerbations, long-term anti-inflammatory control medications are the mainstay of asthma treatment. Currently, the most potent and effective medications in this category are the corticosteroids, and, these agents, in their inhaled form, should be used regularly by all asthmatics with persistent disease [39].

Despite the effectiveness of inhaled glucocorticoids, side effects do occur, especially at high doses. In addition, in some patients, these agents have limited efficacy. Therefore, in light of these limitations, investigators have been stimulated to discover new approaches to asthma therapy. Therefore, over the last few years, asthma research has focused on the development of agents that can, more effectively, inhibit the actions of specific inflammatory mediators.

The leukotrienes (LTs) are a family of lipid mediators that have pro-inflammatory properties. They are derived from arachidonic acid and evidence is accumulating that supports an important role for these mediators in asthma as well as in other inflammatory diseases [135]. For this reason, over the last few years, research has focused on the development of both leukotriene antagonists and inhibitors. Currently, two are on the market in the United States as anti-asthmatic medications. The purpose of this review is to discuss the role of leukotrienes in asthma and to summarize the results of the clinical experience with leukotriene modifying agents in asthmatic patients.

HISTORY

In 1925, Joseph Harkavy, a research fellow at the Institute of Pharmacology in Leiden, discovered a substance in the sputum of asthmatic patients that could elicit spasms in isolated muscle strips. Subsequently, in a follow-up study in the United States, he again isolated a smooth muscle-stimulating substance in sputum extracts from asthmatic individuals. This substance generated either one of two characteristic patterns: progressive increasing contractions or violent spasm along with contraction. While this substance appeared to be similar to histamine, it was thought to be distinct [127]. In 1940, Kellaway and Trethewie [73] demonstrated conclusively that the contractions observed by Harkavy were not produced by histamine and they coined the term "slow-reacting, substance of anaphylaxis"

(SRS-A) to describe this newly-defined mediator.

During the 1930's and 1940's, biogenic amines were "hot" research interests for many investigators. Von Euler at the Karolinska Institute in Stockholm observed that the seminal fluid substance responsible for uterine muscle contraction and relaxation was different from all known active compounds. Von Euler named this substance prostaglandin because of its location in both the prostate and vesicular glands. Subsequently, prostaglandins were isolated from various tissues and body fluids and were found to cause both bronchoconstriction and vasodilation. Von Euler's work opened the door to a new era of research into the chemical mediators responsible for inflammation.

In 1960, Brocklehurst [12] observed and reported that lung segments obtained from an asthmatic individual released SRS-A upon allergen exposure. This finding suggested that SRS-A was involved in the development of asthmatic symptoms since it was able to cause more prolonged muscle contraction than any other smooth-muscle constrictors, including histamine. Along with these studies, others, using animal models, also demonstrated that SRS-A was a potent bronchoconstrictor [36]. Because of its probable role in the pathogenesis of asthma, the race was on to determine the structure of SRS-A.

In the late 1970's, Samuelsson [123] won the race. He identified the component molecules of SRS-A as being the cysteinyl leukotrienes C_4, D_4, and E_4. In addition, he described the bronchoconstrictor, chemotactic, and pro-inflammatory properties of the leukotrienes. Thus, it was becoming clear that these molecules played a central role in asthma, allergy and inflammation. Because of his great contribution, Samuelsson was awarded the Nobel Prize for Physiology or Medicine along with Sune Bergstrom and Sir John Vane in 1982.

PATHOPHYSIOLOGY OF ASTHMA

Clinically, asthma is characterized by bronchoconstriction, wheezing, breathlessness with exertion, cough, increased mucous production, blood and tissue eosinophilia and nonspecific airway hyperreactivity. The disease affects both large and small airways, but not the alveoli. While there are complex events that occur that lead to the development of asthma, the structural changes that are seen with this disease result from chronic inflammation. Principally, the bronchi and bronchioles are involved with extensive denudation of airway epithelium and the formation of markedly hyperplastic goblet cells. The basement membrane is thickened due to the deposition of sub-basement membrane collagen and the lamina propria is infiltrated with lymphocytes, eosinophils, and neutrophils [70, 72]. The smooth muscle is contracted and hyperplastic and the submucous glands are hyperplastic and actively produce mucus. There is an increase in mast cells in the airway and many of these are degranulated. The airway lumen often is filled with mucus, edema fluid, eosinophils, inspissated mucus plugs, Charcot-Leyden crystals and Curshchmann [27] spirals which are casts of the airways formed by the exudate.

Reduction in small airway diameter is the key pathophysiologic event responsible for the clinical manifestations of asthma. The causes of airway obstruction are multiple and include: airway wall edema, increased luminal secretions, cellular infiltration of the airway wall

and bronchial smooth muscle contraction [70]. Airway resistance is increased making expiration difficult. The result is a reduction in forced expiratory volumes (FEV_1) and flow rates as well as hyperinflation of the lung and air trapping.

Smooth muscle contraction. In light of the fact that airway obstruction reverses within minutes after treatment with β-adrenergic agonists, it appears that smooth muscle contraction plays a significant role in airway obstruction in asthma. The mediators responsible for this contraction are predominantly derived from mast cells and include: histamine, bradykinin, leukotrienes LTC_4, LTD_4, and LTE_4, prostaglandins $PGF_{2\alpha}$ and PGD_2, thromboxane A_2 and platelet-activating factor (PAF).

The biologic effects of histamine are wide-ranging and are mediated through the activation of three receptor subtypes, termed H_1, H_2, and H_3. Activation of histamine via its H_1 receptor results in airway smooth muscle contraction, increased vascular permeability and prostaglandin and thromboxane production. Histamine also initiates vagally-mediated reflex parasympathetic actions, causes venule dilation and is responsible for increased vascular permeability. The fact that atropine-like agents cause bronchodilation in some asthmatics suggests that histamine-mediated parasympathetic stimulation may contribute to the bronchoconstriction seen in asthma. However, it is not thought that histamine plays a major role in the pathophysiology of the bronchospasm seen in asthma since little, if any, improvement occurs with antihistamine therapy.

The role for prostaglandins in asthma also is not well-defined. It is known that $PGF_{2\alpha}$, PGD_2, PGG_2 , and thromboxane A_2 can cause airway bronchoconstriction. However, since aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) (agents known to reduce prostaglandin production) fail to improve airway function in asthmatics, it is thought that prostaglandins do not play a key role in the pathophysiology of airway obstruction in asthma.

In contrast to histamine and the prostaglandins, the leukotrienes appear to play a significant role in asthma-associated bronchoconstriction. Moreover, the leukotrienes have a more sustained mechanism of action. The fact that leukotriene receptor antagonists produce both acute and chronic bronchodilation suggests that these chemical mediators are, at least partially, responsible for the bronchoconstriction associated with asthma.

Edema. Mucosal airway edema results from the leakage of serum proteins into the interstitial space due to increased capillary permeability. Increased permeability is caused by several mast cell-derived mediators including: histamine, PGE_2, LTC_4, LTD_4, PAF and bradykinin. The importance of airway edema in asthma was revealed by Dunnill [38] in 1960. Upon performing pathologic examinations of asthmatic lung tissue he found that the most striking abnormality was the marked mucosal edema that was present. More recently, mucosal edema has been visualized by bronchoscopy after airway allergen challenge[7, 70].

Inflammation. Upon examining the mucosa of asthmatics who have died in status asthmaticus, a mixed cellular infiltrate that consists of eosinophils, neutrophils, macrophages, CD4+ T lymphocytes, mast cells, and plasma cells is observed. In addition, the airway lumen is filled with secretions rich in eosinophils (and their products), neutrophils, and epithelial cell clumps. It is thought that the eosinophil and neutrophil infiltrates result from the release or

generation of factors from activated mast cells. These include histamine, chemotactic factors and arachidonic acid metabolites [70]. In addition, the mast cell also generates newly synthesized cytokines that can elicit cellular infiltrates hours after the initial antigen-induced event [90]. This late asthmatic response is characterized microscopically by the influx of inflammatory cells, and functionally by a decline in pulmonary function and an increase in airway hyperresponsiveness.

The early asthmatic response occurs within minutes after allergen exposure. During this short time interval, mast cells degranulate releasing mediators, such as histamine, PGD_2 and tryptase, that are detectable in bronchoalveolar lavage fluid [95] and that lead to increased vascular permeability and subsequent edema. These mediators, along with newly-generated cytokines, cause the upregulation of cell adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), both of which enable eosinophils to attach to the pulmonary endothelium and extravasate into the lung [137]. Release of eosinophil-derived proteins leads to epithelial denudation, mucus secretion and airway irritability. It has been demonstrated in monkey models of asthma, that if ICAM-1 expression is prevented after allergen challenge, eosinophilia and airway hyperresponsiveness do not occur [137].

Mucus hypersecretion. Patients who have died from asthma are found to have diffuse collections of mucus and mucus plugs, both of which appear to contribute markedly to airway obstruction. Increased mucus production occurs due to both hyperplasia of submucosal glands and increased numbers of epithelial goblet cells. In addition, inflammatory mediators act on submucosal glands to produce mucus. Those that are thought to be important in mucus secretion include: LTD_4, LTC_4, $PGF_{2\alpha}$, PGD_2, PGI_2, PGE_1 and PGA_2. Cholinergic, adrenergic, and sensory neuropeptides also stimulate mucus secretion as well [126].

Airway hyperresponsiveness. In the presence of various irritating stimuli, asthmatic airways demonstrate an exaggerated nonspecific airway reactivity. These stimuli include: cold air, exercise, chemicals, laughing, and coughing. In the laboratory, provocations are elicited with histamine, methacholine, $PGF_{2\alpha}$, cold air hyperventilation and ultrasonic mist [25]. Airway hyperresponsiveness is present in all asthmatics and in some individuals with chronic bronchitis and allergic rhinitis. It has been shown that asthmatics who have not wheezed for years may continue to have persistent abnormal airway reactivity. In addition, there is a correlation between the degree of airway hyperresponsiveness and disease severity [70]. Airway hyperresponsiveness may return to normal if allergens are avoided, immunotherapy is initiated or anti-inflammatory medications are used regularly [69]. Over the last decade, both airway hyperresponsiveness and airway inflammation have become the primary targets of asthma therapy.

LEUKOTRIENE SYNTHESIS

Before embarking upon a discussion of novel asthma treatments that inhibit the actions of the leukotrienes, it is important to understand the biochemistry of the leukotrienes themselves. The leukotrienes are derived from arachidonic acid, a ubiquitous 20-carbon fatty acid that is often found esterified in the *sn*-2 position of cell membrane phospholipids [135]. Leukotrienes, along with prostaglandins, thromboxanes, and lipoxins, are members of a larger

group of molecules known as eicosanoids [123]. Importantly, leukotrienes are not stored in a preformed state. They are synthesized de novo after trauma, infection, and inflammation [54].

Upon activation of effector cells, phospholipase A_2 is translocated from the cytosol to the cell membrane and is activated resulting in the cleavage of arachidonic acid from membrane phospholipids. Once released, arachidonic acid may undergo oxygenation or it may be reincorporated into cell membrane phospholipids by reacylation. While there are several pathways by which arachidonic acid subsequently may be metabolized, this review will focus only upon the 5-lipoxygenase pathway (Figure 1).

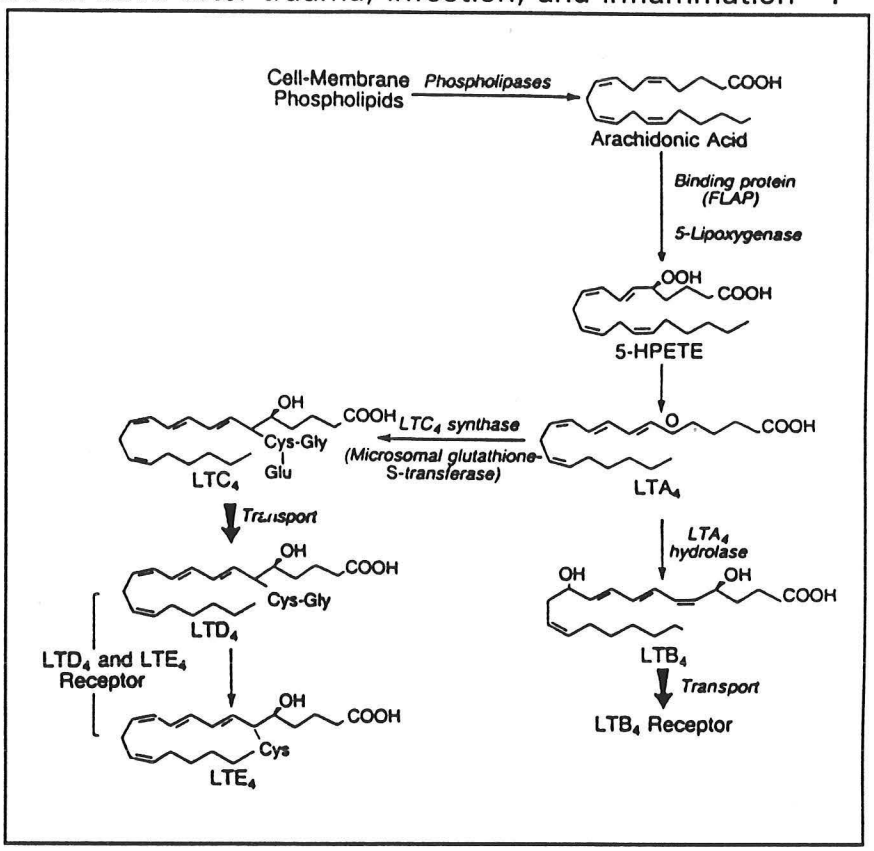

Figure 1. 5-Lipoxygenase pathway to leukotriene generation[93].

Arachidonic acid is converted to 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) by a catalytic complex consisting of 5-lipoxygenase and 5-lipoxygenase activating protein (FLAP) [103, 107]. This same enzyme complex then converts HPETE toLTA_4. Subsequently, LTA_4 may be converted into LTB_4 by LTA_4 hydrolase. Alternatively, in the presence of LTC_4 synthase [83], LTA_4 is conjugated to reduced glutathione to form LTC_4 [91]. LTC_4 is then exported from the cytosol to the extracellular microenvironment where γ-glutamyltranspeptidase cleaves the glutamic acid moiety yielding LTD_4 [92]. LTE_4, the final product, is then formed upon cleavage of the glycine moiety from LTD_4 [114]. LTC_4, LTD_4, and LTE_4 are known as the cysteinyl leukotrienes and together these molecules comprise the biologic material previously known as the slow reacting substance of anaphylaxis (SRS-A).

The sites of leukotriene synthesis are dependent upon the cellular distribution of the enzymes responsible for their formation. Since 5-lipoxygenase is found only in cells of the myeloid lineage, LTA_4 formation is limited to these cells only [118]. In contrast to 5-lipoxygenase, the enzymes farther down the arachidonic acid cascade have a more wide distribution. Therefore, while LTA_4 production is limited to a few cell types, the enzymes responsible for its metabolism are much more widely distributed. In addition, the fact that LTA_4 is exported once synthesized enables a much broader range of cells to act as leukotriene secretors [54].

The predominant myeloid cells that have 5-lipoxygenase activity are mast cells [124], eosinophils [138], macrophages [117], monocytes, basophils [118] and B lymphocytes [65]. Even

amongst these cells, there is much variation in the types and amounts of leukotrienes that are secreted [54]. Monocytes and macrophages have been shown to release both LTB_4 and LTD_4 after both immunologic and nonimmunologic stimulation [41, 50, 139]. In contrast, the other myeloid cells described above produce significant quantities of either LTB_4 or LTC_4, but not both. Both eosinophils [66, 138] and mast cells [98] predominantly produce LTC_4 after stimulation while the principal 5-lipoxygenase product of neutrophils [53] and basophils [128] is LTB_4.

While 5-lipoxygenase has limited cellular distribution, LTA_4 hydrolase and LTC_4 synthase are found in a variety of cell types. This broadened enzyme distribution, in addition to the fact that LTA_4 is actively secreted from the cells that synthesize it [28], enable leukotriene production to be amplified in inflammatory sites [54]. Thus, while endothelial cells and platelets lack 5-lipoxygenase, both contain LTC_4 synthase and therefore are able to produce LTC_4 from neutrophil-synthesized LTA_4 [40, 99]. Erythrocytes too lack 5-lipoxygenase, but they do contain LTA_4 hydrolase and thus can form LTB_4 from neutrophil-derived LTA_4 [44].

LEUKOTRIENE RECEPTORS

The receptors for LTB_4 and the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4 are distinct. For LTB_4, there exists two separate binding sites, one of low affinity and one of high affinity, on the surface of neutrophils [48, 49, 94]. These receptors are G-coupled and are members of the rhodopsin-like receptor superfamily.

At the present time, there exists controversy regarding the number and specificity of receptors for the cysteinyl leukotrienes [54]. In the lungs of both rats [116] and guinea pigs [55], a highly selective LTC_4 receptor has been identified. However, a receptor of this type has not been identified in human lung tissue [15]. In humans, it has been found that the cysteinyl leukotrienes mediate their biological activity via a recently-described specific receptor termed the cysteinyl leukotriene receptor type 1 ($CysLT_1$) [26]. Previously, this receptor was known as the leukotriene D_4 receptor and it too is a member of the rhodopsin-like superfamily. Its predominant location is smooth muscle cells [52]. Since LTD_4 receptor antagonists inhibit the contractile activity of both LTC_4 and LTD_4 it is thought that both of these molecules interact with a single common LTD_4 receptor [15, 16, 6]. An alternative hypothesis is that LTC_4 activates the LTD_4 receptor after its conversion to LTD_4. LTE_4 acts as a partial agonist of the LTD_4 receptor [54]. Neither the $CysLT_1$ receptor nor a second cysteinyl leukotriene receptor ($CysLT_2$) that has been identified has been cloned.

ROLE OF LEUKOTRIENES IN ASTHMA

The cysteinyl leukotrienes play a major role in both hypersensitivity and inflammatory conditions including asthma. Predominantly, they act upon smooth muscle and the vasculature and in the lung they are the major mediators of bronchoconstriction. The leukotrienes appear to be more potent and more sustained mediators of contraction of smooth muscle than are arachidonic acid cyclooxygenase products. Upon stimulating asthmatic lung tissue with allergen, the timecourse and the severity of the bronchospasm noted is attributable to the large amount of LTC_4, LTD_4, and LTE_4 that are released. Bronchospasm provoked in this way is unaffected by pretreatment with antihistamines or inhibitors of cyclooxygenase, but it is diminished by inhibitors of the 5-lipoxygenase pathway [31]. The

cysteinyl leukotrienes are 100- to over 1000-fold more potent than histamine in causing contraction of human and guinea pig lung parenchymal strips [37] and the bronchospasm that is produced is more prolonged than that produced by histamine [56]. When the cysteinyl leukotrienes are administered by inhalation to either healthy individuals or those with asthma all three are capable of producing bronchoconstriction, with LTD_4 being the most potent of the three mediators. In addition, asthmatic airways have been shown to be more responsive to the contractile effects of LTD_4 than the airways from normal individuals, but there does appear to be wide inter-individual variability [4, 22].

Individuals with asthma have increased nonspecific airway reactivity compared to nonasthmatic individuals. In other words, little stimulus is required to produce a bronchoconstrictive response in asthmatic patients and the more hyperresponsive the airways, the more severe the asthma [121] and the more medication required to control symptoms [67]. While few studies have been performed to determine the role played by leukotrienes in causing airway hyperresponsiveness, some supportive data does exist. A recent study by Fischer and colleagues [43] showed that regular treatment of asthmatics with a 5-lipoxygenase inhibitor for thirteen weeks improved airway hyperresponsiveness to cold air for up to ten days following the completion of therapy. Thus, the positive effect noted was of much longer duration than the expected duration of action of the enzyme inhibitor drug used for treatment. These findings are consistent with the hypothesis that leukotrienes do contribute to airway hyperresponsiveness in asthma and that inhibition of their synthesis may lead to decreased airway hyperreactivity, possibly through reducing airway inflammation [111]. The only other asthma medications known to have a beneficial effect beyond their known duration of action are the glucocorticosteroids [68].

The cysteinyl leukotrienes are known to have profound effects on the vasculature. In addition to causing vasoconstriction of vascular smooth muscle, these mediators also cause increased vascular permeability. Piacentini and Kaliner [115] found that when cysteinyl leukotrienes were applied topically to hamster cheek pouches, there was an increased extravasation of markers such as Evans Blue dye. In addition, in humans, it has been shown by several investigators [18, 130] that intradermal application of LTC_4, LTD_4 and LTE_4 leads to the generation of a wheal and flare reaction. This increased vascular permeability induced by leukotrienes is thought to be important in asthma pathogenesis for two reasons. It allows proinflammatory cells to migrate into sites of inflammation and it causes plasma leakage into the airway wall and lumen resulting in reduced airway caliber and increased bronchial hyperresponsiveness [115].

As stated previously, airway inflammation plays a key role in asthma pathogenesis and, numerous studies have demonstrated that even mild asthmatics possess both mast cells and activated eosinophils in their airway wall and lumen [9, 75]. That cysteinyl leukotrienes contribute to the cellular infiltration noted has been demonstrated in several studies. Diamant and colleagues [35] demonstrated that eosinophils are increased in number in induced sputum samples from asthmatic patients after inhalation of LTD_4. In addition, it has been shown that inhalation of LTE_4 by asthmatics leads to increased eosinophils in airway biopsies [81].

Unlike the cysteinyl leukotrienes, the importance of LTB_4 in asthma pathogenesis is not known. LTB_4 is a potent neutrophil chemoattractant in human lungs and these cells are

thought to play a role in both fatal [133] and nocturnal asthma [102]. LTB_4 also is a chemoattractant for eosinophils [125], it induces T-lymphocyte production of IL-5 [140] and it potentiates the effects of IL-4 on IgE production [141]. The fact that this leukotriene has been found in the bronchoalveolar lavage (BAL) fluid of asthmatics [84] suggests that this mediator, like the cysteinyl leukotrienes may play a role in asthma pathogenesis. Other primary functions of the leukotrienes are outlined in Table 1.

Table 1. Primary Leukotriene Functions [135]

Mediator	Activity
$LTC_4/D_4/E_4$	Smooth muscle contraction Bronchoconstriction Stimulation of airway mucus and electrolyte secretion Dilation and increased permeability of microvasculature Constriction of coronary/cerebral arteries Depression of myocardial contractility Stimulation of gastric acid secretion
LTE_4	Induction of airway hyperreactivity
LTC_4	Initiation of LH release
LTB_4	Elicitation of leukocyte chemotaxis and activation Increased leukocyte adherence to endothelium Suppression of T lymphocyte function Enhancement of NK cell activity Stimulation of keratinocyte proliferation

MEASUREMENT OF LEUKOTRIENES IN BIOLOGICAL FLUIDS

The identification of leukotrienes in biological fluids has been difficult since they are present in nanomolar and picomolar concentrations. In addition, the leukotrienes have a very short half-life *in vivo* and they are susceptible to oxidative degradation during sample preparation [106]. Another difficulty lies in the fact that these mediators are artificially generated easily and they may be released from blood leukocytes during blood sampling [33]. For this reason, leukotriene analysis of blood samples is of little use clinically. However, unlike blood sample analysis, it has been demonstrated that production of LTB_4 by isolated and stimulated white blood cells provides useful information regarding the role played by this mediator in various disease states [104, 105].

For determination of systemic cysteinyl leukotriene production, analysis of urinary metabolites may be performed. It has been shown that after radiolabeled LTC_4 is administered to humans [^{3}H]LTE_4 is the main urinary metabolite [113]. In patients with asthma, LTE_4 excretion has been shown to be elevated after allergen challenge [77] and after aspirin

challenge of aspirin-sensitive asthmatics [77]. It is also increased in patients with nocturnal asthma [10].

The presence of leukotrienes can be determined quantitatively using various analytic techniques: bioassays, HPLC, RIA, enzyme immunoassays, chromatography and mass spectrometry [106]. The preferred method for unequivocal identification is gas chromatography-mass spectrometry [108]. In addition to identifying LTE_4 in urine, the cysteinyl leukotrienes have been demonstrated in lavage fluid of asthmatics, as well, using these methods [84]. The fact that these mediators are elevated in patients with asthma, along with their known pharmacologic effects, make them a natural target for therapeutic intervention.

LEUKOTRIENE MODIFYING DRUGS: EARLY DEVELOPMENT

In order to interfere with the actions of the leukotrienes, in theory, any of the enzymes in their biosynthetic pathway may be targeted for inhibition. However, to date, only one enzyme, 5-lipoxygenase, has been selectively inhibited successfully[20]. In addition, several FLAP inhibitors that prevent arachidonic acid from binding to the FLAP molecule, have been developed as well. Both of these classes of drugs interfere with leukotriene biosynthesis and thus, block the formation of both the cysteinyl leukotrienes and LTB_4. In addition to these agents, another class of drugs exist that block the activity of the leukotrienes at the receptor level. Competitive cysteinyl leukotriene receptor antagonists block the activity of LTC_4, LTD_4, and LTE_4 at the *CysLT*$_1$ receptor and LTB_4 may be competitively inhibited by LTB_4 antagonists acting at its receptor. Figure 2 demonstrates the sites of action of the four classes of leukotriene modifying drugs and Table 2 provides the status of the various antileukotrienes that are or have been studied in humans.

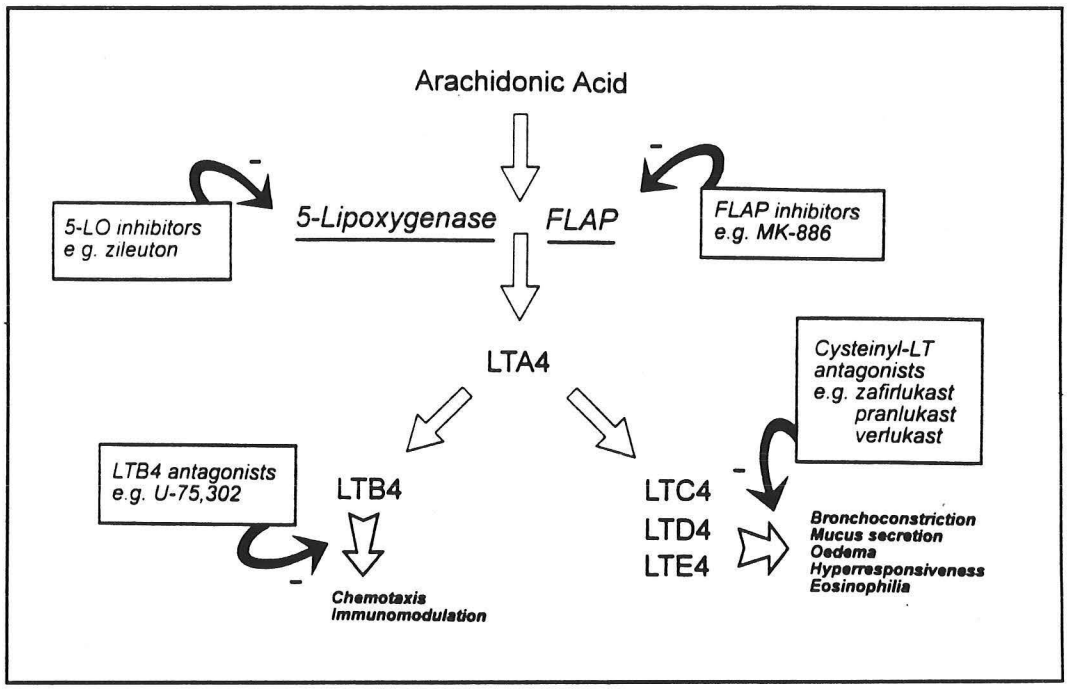

Figure 2. The four groups of antiasthma drugs directed against LT synthesis and activity[56].

Table 2. Antileukotrienes in Human Disease [111]

DRUG	DRUG CLASS	CURRENT STATUS
Zileuton	5-lipoxygenase inhibitor	Available in the United States
Genleuton	5-lipoxygenase inhibitor	Withdrawn from development
BAYx1005	FLAP antagonist	Withdrawn from development
MK-886	FLAP antagonist	Withdrawn from development
LY171883	*CysLT*$_1$ antagonist	Withdrawn from development
MK-571	*CysLT*$_1$ antagonist	Withdrawn from development
Cinalukast	*CysLT*$_1$ antagonist	Withdrawn from development
Montelukast	*CysLT*$_1$ antagonist	In phase III clinical trials
Pranlukast	*CysLT*$_1$ antagonist	Available in Japan; In phase III clinical trials in the United States
Verlukast	*CysLT*$_1$ antagonist	Withdrawn from development
Zafirlukast	*CysLT*$_1$ antagonist	Available in the United States and several European countries

Cysteinyl Leukotriene Receptor Antagonists. One of the first receptor antagonists developed was FPL-55712 in the late 1970's [2]. This agent was shown to reduce the smooth muscle contraction induced in guinea-pig trachea and human bronchial tissue after *in vitro* antigen application. In the last 20 years, since this initial antagonist was identified, hundreds of cysteinyl leukotriene antagonists have been developed [136], but, of these, less than twenty have undergone clinical evaluation.

The discovery of the cysteinyl leukotriene receptor antagonists helped to elucidate the important role played by the leukotrienes as mediators of asthma. A common initial stage in clinical evaluation was to evaluate the effect of leukotriene antagonists on LTD_4-induced bronchoconstriction in normal volunteers or individuals with mild to moderate asthma. Early studies revealed that the LTD_4 dose-response curve was shifted to the right when individuals were pretreated with various investigational leukotriene antagonists [13]. Most of the early agents that were developed were structural analogs of FPL-55712 and, thus, all contained a common hydroxyacetophenone moiety linked to an acidic group. While each of these early hydroxyacetophenone antagonists demonstrated variable inhibition of LTD_4-induced smooth muscle contraction *in vitro*, none of these agents were efficacious in humans. They caused only minimal effects on the early asthmatic response to inhaled allergen in sensitized subjects and they had no effect on the late asthmatic response or on airway hyperreactivity [56]. Studies that were conducted in chronic asthma also were disappointing. In a large study conducted by Cloud and colleagues [24], tomelukast (LY171883) produced only a 7% improvement in FEV_1 and only minor reductions in β-agonist use. It is generally believed that the disappointing results that were seen with these first generation antagonists were due to a combination of low potency and poor specificity [56]. The low potency seen with these agents existed because of their weak receptor binding affinity. None caused greater than a 6-fold shift in the dose-response curve for inhaled LTD_4-induced bronchoconstriction in humans [13].

While the early receptor antagonists were not clinically effective, their discovery was an impetus to further research in this area. A major advance occurred with the development of MK-571, a quinolyl carboxylate LTD_4 antagonist. This agent was found to have an affinity

for the LTD_4 receptor comparable to the natural LTD_4 ligand. In asthmatic patients, a plasma concentration of 0.55 ug/mL caused a mean rightward shift in the dose-response curve (for inhaled LTD_4-induced bronchoconstriction) of at least 44-fold. At a mean plasma concentration of 10 ug/mL (280 mg dose), the shift was increased to at least 83-fold [74]. MK-571 significantly inhibited both the early and late asthmatic responses [74] and it markedly inhibited exercise-associated bronchospasm as well [100]. In a six-week placebo-controlled chronic asthma trial, MK-571 was given at a dose of 75 mg tid po for two weeks followed by 140 mg tid po for four weeks [101]. At the completion of the trial, FEV1 was improved 8-14% and both asthma symptoms and β-agonist use were reduced by 30%. While these results confirmed the therapeutic benefit of leukotriene inhibitors in chronic asthma, the clinical development of this agent was suspended when it was discovered that it caused an increase in peroxisomal enzymes in mice, a feature that has been linked to the possible occurrence of hepatic tumors in humans [13].

Further studies of the enantiomers of racemic MK-571 revealed that peroxisomal proliferation was associated totally with the *S* enantiomer of this compound [51]. The *R*-enantiomer, verlukast (MK-679), lacked this property and was chosen, therefore, for further study. It was found to improve lung function in moderately severe asthmatics by 13% [85] and it caused a 4.4-fold shift in the dose-response curve to inhaled aspirin in aspirin-sensitive asthmatics [30]. In addition, a single oral 825 mg dose produced a marked bronchodilator response in a group of aspirin sensitive asthmatics in the absence of aspirin challenge [29]. The fact that this improvement was demonstrated in individuals who also were taking inhaled corticosteroids suggests that leukotriene production is unaffected by corticosteroids. Also, these findings suggest that leukotriene antagonists may add extra benefit beyond that provided by corticosteroids in aspirin-sensitive asthmatics and that leukotrienes are involved in the pathogenesis of asthma of this type [13, 29]. While this agent also appeared to be as beneficial as MK-571 in a chronic asthma trial, its development was halted due to the fact that 5% of the patients studied developed liver function abnormalities [45].

Despite the problems with the early agents, there are several potent, selective cysteinyl leukotriene receptor antagonists that either have made it to market or that are about to be launched. Zafirlukast is one of these drugs. It was recently approved in the United States, it is active both orally and by inhalation, and it appears to be one of the most potent leukotriene antagonists that has been developed. Pranlukast (ONO-1078), another orally active agent, was the first cysteinyl leukotriene to be marketed and used in clinical practice [47]. It became available in Japan in 1995, but it has not yet been marketed in the United States. Several other agents are at various stages of clinical development. Of these, montelukast (MK-0476) has been the most extensively evaluated and it is to be marketed soon in the United States. The clinical efficacy of each of the agents that are available for use in the United States will be discussed in subsequent sections.

5-Lipoxygenase Inhibitors. An alternative method of inhibiting leukotriene activity in asthma is to block their synthesis using agents that inhibit either 5-lipoxygenase or FLAP. Unlike the cysteinyl leukotriene antagonists, the 5-lipoxygenase/FLAP inhibitors block both the synthesis of the cysteinyl leukotrienes as well as LTB_4.

5-lipoxygenase is a nonheme iron-containing enzyme that is responsible for the

dioxygenation of arachidonic acid in the leukotriene synthesis pathway of arachidonic acid metabolism. While several inhibitors of this enzyme have been developed, progress has been challenged by the complex nature of the reactions catalyzed by this enzyme along with the fact that this protein is unstable when it is purified. To date, it remains unclear exactly how the inhibitors of this enzyme function. Some are thought to complex with iron in the active site of the enzyme while others are thought to cause inhibition by an antioxidant mechanism.

The early 5-lipoxygenase inhibitors were nonselective, they had problems with toxicity and they lacked oral bioavailability. The first agents that were developed were the hydroxamate 5-lipoxygenase inhibitors that interacted with the catalytically important iron moiety [13]. Many inhibitors of this type were identified that had potent *in vitro* inhibitory activity. Unfortunately, *in vivo* studies of these agents yielded disappointing results. A major limitation was that, *in vivo,* the hydroxamate was rapidly hydrolyzed to an inactive carboxylate compound [132]. Subsequently, key structural modifications were made that yielded agents with improved *in vivo* activity. However, despite these modifications, studies performed in rats and dogs revealed that, due to rapid glucuronidation, the half-lives of these agents were only about one hour. Clinical investigation of these inhibitors in humans was suspended due to the accumulation of metabolites that resulted from extensive *in vivo* metabolism. Thus, because of the limited pharmacokinetic properties of the hydroxamic acid inhibitors in humans, they were not considered feasible clinical 5-lipoxygenase inhibitors [13].

The search was on to discover alternative novel inhibitors that were less susceptible to metabolism. It resulted in the identification of a new class of agents, the *N*-hydroxyurea series of 5-lipoxygenase inhibitors [19]. These drugs had *in vitro* 5-lipoxygenase inhibitory activity comparable to the hydroxamate compounds, but, unlike the earlier agents, they had significantly reduced glucuronidation rates. Despite the fact that hundreds of *N*-hydroxyureas have been studied, only one, zileuton (A-64077), was chosen to be evaluated clinically. Subsequently, this drug has been shown to be an effective orally-active, selective 5-lipoxygenase inhibitor in man [14]. Studies demonstrating the clinical effectiveness of this agent in asthma will be discussed in a subsequent section.

FLAP Inhibitors. As stated above, leukotriene synthesis depends upon the interaction of 5-lipoxygenase with FLAP. The mechanism of cooperativity between these two molecules is not completely known. FLAP is thought either to be a membrane-docking protein for 5-lipoxygenase or an arachidonic acid-presenting protein. The initial FLAP-inhibitor drug that was discovered and that was used in clinical evaluations was MK-886. This drug was shown to reduce the early asthmatic response to allergen by 58% and the late response by 44%. Urinary production of LTE_4 was markedly reduced as well [46]. Subsequently, a second generation FLAP inhibitor, MK-0591, was developed that had even more potent FLAP binding activity and leukotriene inhibitory activity than MK-886. In one study in which MK-0591 was give to eight asthmatic men prior to allergen challenge, the drug blocked leukotriene synthesis *in vivo* as demonstrated by an almost complete (98%) inhibition of ionophore-stimulated blood cell synthesis of LTB_4 *ex vivo* and by an 87% inhibition of urinary LTE_4 excretion. In addition, the early response to allergen was inhibited by 79% and the late response by 39% [34]. Despite these promising results, none of the FLAP inhibitors have yet been marketed.

EFFECT OF ZAFIRLUKAST IN ACUTE CHALLENGE STUDIES

In September, 1996, zafirlukast (Accolate®) or ICI 204,219 was approved by the United States Food and Drug Administration. It is a very potent leukotriene receptor antagonist in that it produces a 100-fold rightward shift in the dose-response curve to inhaled LTD_4. The following section will discuss the experience of this agent in acute challenge studies and clinical asthma.

Allergen challenge. Inhalation of environmental allergens by sensitized patients results in an early asthmatic response that is characterized by acute bronchospasm that usually resolves within two hours. In a large number of these patients, the early response is followed by a second episode of bronchospasm, termed the "late-phase response", that occurs approximately three to four hours after inhalation and that lasts as long as 24 hours [110]. The late phase response is associated with increased airway hyperresponsiveness that may last days to weeks. Also, it is accompanied by migration of inflammatory cells into the airway and by air way wall edema.

Drugs that affect the bronchoconstriction induced by allergen challenge classically are examined for their ability to block early phase reactions, late phase reactions and/or bronchial hyperreactivity as measured by methacholine or histamine challenges. Many of the early leukotriene receptor antagonists had no effect on the late phase asthmatic reaction and they were minimally effective in clinical studies. In contrast to these early agents, zafirlukast was shown to have potent activity both *in vitro* and *in vivo* [76].

One of the first double-blind, placebo-controlled clinical studies that demonstrated the beneficial effects of zafirlukast on allergen-induced bronchoconstriction and airway hyperreactivity was performed by Taylor and colleagues [134] in 1991. Ten atopic subjects with mild asthma were selected on the basis of the demonstration of an immediate drop in FEV_1 of at least 15% after allergen challenge and eight of these individuals completed the study. Patients ingested a single 40 mg dose of zafirlukast or matched placebo. Two hours later they underwent an aerosolized allergen challenge. FEV_1 was measured every ten minutes after allergen challenge for one hour and then every twenty minutes for another hour. Thereafter, FEV_1 was determined every thirty minutes for an additional four hours. After placebo, inhalation of allergen caused a maximum fall in FEV_1 of 32.4%, ten minutes after allergen challenge. In contrast, the

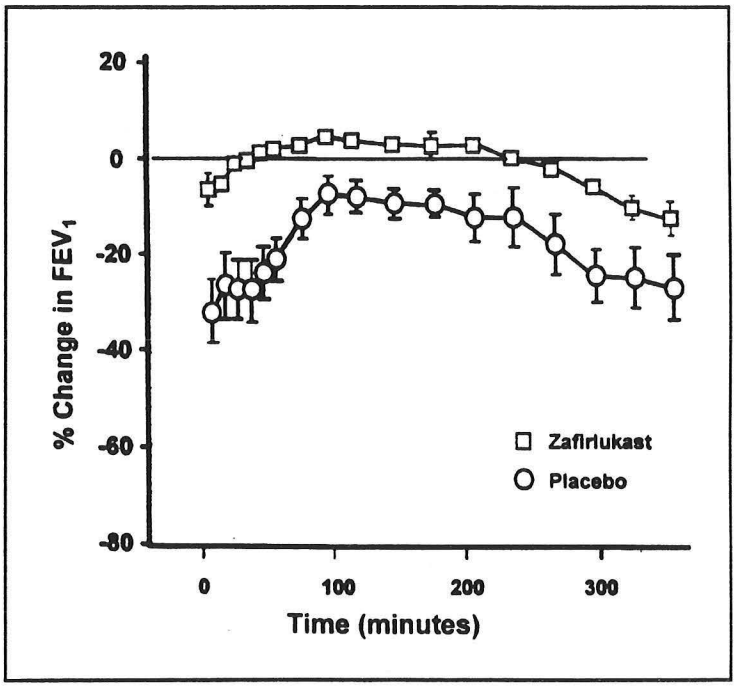

Figure 3. Effect of Zafirlukast on allergen-induced bronchoconstriction [134]

maximum fall in FEV_1 noted with zafirlukast was only 6.3%, ten minutes after allergen challenge (Figure 3). The allergen-induced late phase response was attenuated as well but not to the same extent as the early response. In addition, there was suppression of the allergen-induced increase in nonspecific bronchial hyperreactivity.

A subsequent study revealed that an even greater shift in the antigen dose-response curve could be achieved if the inhaled route of delivery was used as opposed to the oral route [109]. These results suggested that the inhaled route of administration may provide a higher local concentration of active drug in the airway than does the oral route. Despite this possibility, currently, leukotriene synthesis inhibitors and receptor antagonists are being developed as oral formulations due to the superior duration of action of oral agents, the ability of oral agents to deliver the drug to the lower airway in a reproducible fashion and the preference of an oral form over inhaled formulations by patients [14].

It is thought that cysteinyl leukotrienes produced during the early asthmatic response may play a role in promoting cellular infiltration during the late phase response. A recent study provides evidence in support of this hypothesis. Calhoun and colleagues [17], in a double-blind, placebo-controlled crossover study, assessed the efficacy of oral zafirlukast in altering the cellular inflammatory response to antigen challenge in eleven asthmatic patients. After five days of treatment with either drug or placebo, patients underwent segmental antigen bronchoprovocation (SBP) followed by bronchial alveolar lavage (BAL) immediately and 48 hours after allergen challenge. In comparison to placebo, zafirlukast produced a marked decrease in basophil number, lymphocyte number and histamine concentration 48 hours after SBP (16000 versus 0 cells/mL; 61000 versus 41000 cells/mL; and 9078 versus 6445 pg/mL, respectively; $p<0.01$). Eosinophils showed a trend toward reduction. In addition, there was a significant reduction in superoxide production by alveolar macrophages. These exciting results suggest that cysteinyl leukotriene receptor antagonists are capable of altering cellular infiltration and activation associated with allergen challenge.

Exercise-induced asthma. In addition to having a beneficial effect in allergen-induced asthma, zafirlukast has been shown to inhibit exercise-induced asthma as well. Finnerty and colleagues [42] evaluated the effect of 20 mg of zafirlukast on the bronchoconstrictor response to exercise in eight asthmatic patients in a placebo-controlled, randomized crossover study. Two hours after drug administration, a six minute treadmill exercise test was performed followed by an assessment in change in FEV_1 over 30 minutes postexercise. The mean timecourse of exercise-induced bronchoconstriction following administration of placebo and zafirlukast is shown in Figure 4. As shown, the mean

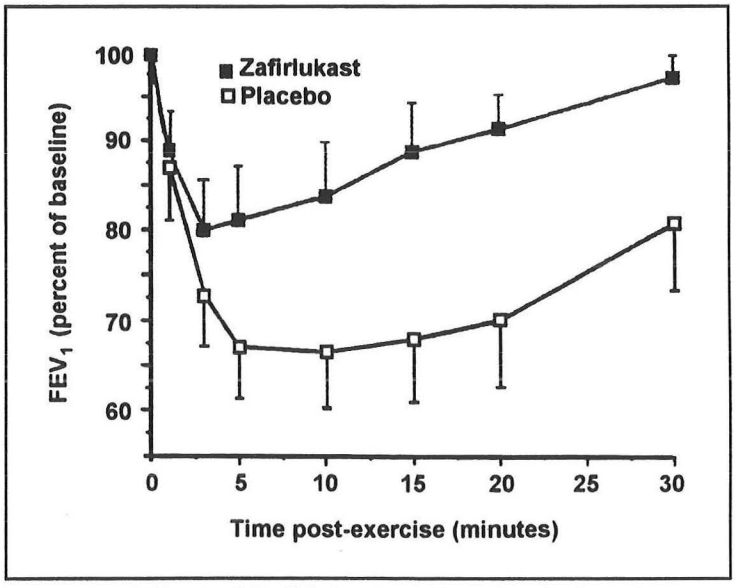

Figure 4. Effect of Zafirlukast on Exercise-induced Bronchoconstriction [42].

maximum percentage fall in FEV_1 following exercise in the zafirlukast group was markedly less than that demonstrated by the placebo group. In seven of the eight individuals studied, zafirlukast caused a marked reduction of the maximum bronchoconstriction response exhibited and it caused almost total inhibition of the response in three of these individuals.

More recently, it has been shown that cinalukast, a potent receptor antagonist, causes an even greater reduction in exercise-induced bronchoconstriction and one that is more long-lasting than that seen with zafirlukast. Exercise-induced bronchoconstriction was decreased by more than 80% in asthmatic patients and this effect lasted for more than eight hours after drug administration [5]. This agent is not available for clinical use, however.

EFFECT OF ZAFIRLUKAST IN CLINICAL ASTHMA

While there are numerous reports of the effectiveness of leukotriene receptor antagonists in asthma induced in the laboratory setting, there are fewer examples of the beneficial effect of these agents in clinical asthma. In 1991, Hui and Barnes [58] published the first report of the effect of zafirlukast in chronic asthma. The ten patients evaluated in the double-blind, placebo-controlled crossover study had a baseline FEV_1 that was 50-80% of predicted and all had at least a 15% increase in baseline FEV_1 following inhalation of the bronchodilator, salbutamol. Before each of the two study days, which occurred at least a week apart, inhaled bronchodilators were held for at least six hours and oral theophylline was held for at least 24 hours. On both study days, baseline FEV_1 was similar. Nine of the ten subjects were taking inhaled corticosteroids and these agents were not discontinued during the study period. After receiving either placebo or 40 mg zafirlukast, FEV_1 was measured every thirty minutes for four hours. On the day of active treatment FEV_1 increased significantly from baseline compared with the placebo treatment day. A significant increase was noted at one hour and it persisted during the four-hour study period. Despite the fact that this study was small and that it demonstrated only an 8% improvement in mean baseline FEV_1 following zafirlukast treatment, its findings suggested that there is a persistent generation of cysteinyl leukotrienes in patients with chronic asthma even in the face of regular inhaled corticosteroid treatment.

More recently, several studies have been conducted in which the treatment periods with zafirlukast have been much more prolonged. A key, and often quoted study, was published by Spector and colleagues [131] in 1994. In this multicenter, double-blind, placebo-controlled study, 276 subjects with mild to moderate asthma were randomized to treatment for six weeks with total daily doses of zafirlukast of 10 mg, 20 mg, 40 mg or placebo if they had a baseline FEV_1 between 40 and 75% of predicted (in the absence of bronchodilator therapy) and a daytime asthma score of >10 (range 0-21) for

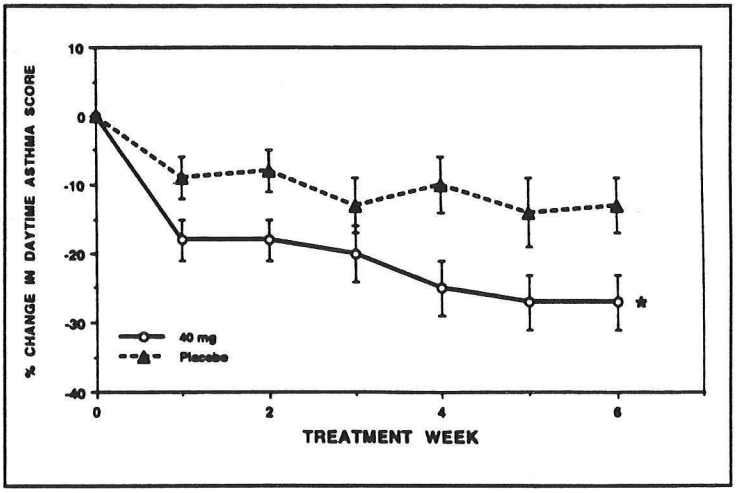

Figure 5. Effect of zafirlukast on daytime asthma score [131]

seven consecutive days. None of these patients were taking inhaled corticosteroids. Symptom scores, pulmonary function tests and rescue medication use was evaluated each week during the treatment period. It was found that the 40 mg dose of zafirlukast was more effective than placebo in reducing nighttime awakenings, first morning asthma symptoms, daytime asthma score (Figure 5) and albuterol use. It was also more effective than placebo in increasing peak expiratory flow rates (PEFR) and FEV_1. Compared with baseline values, patients receiving 40 mg of zafirlukast experienced a 46% decrease in night-time awakenings, a 30% decrease in albuterol use and a 26% improvement in daytime asthma symptoms. In addition, increases in FEV_1 were more pronounced in those subjects who had a baseline FEV_1 below 45%, a finding that suggests that zafirlukast may be more effective in individuals who have a greater potential for improved lung function.

More recently, the long-term efficacy of zafirlukast was evaluated in a thirteen-week, double-blind, placebo-controlled trial in patients with mild to moderate asthma [97]. Seven hundred sixty-two patients were enrolled in the trial and each were taking as needed β-agonists only, each had a cumulative daytime asthma score of 8 or more per week and each had an FEV_1 of 55% or greater of predicted. It was found that the onset of action of zafirlukast was within the first week of therapy and improvements were noted in pulmonary function, asthma symptoms and β-agonist use. These improvements were maintained throughout the study. Forty milligrams of zafirlukast resulted in a 20% decrease in nighttime awakenings, a 29% decrease in morning asthma symptoms, a 27% decrease in daily asthma symptoms and a 22% decrease in β-agonist use. There was also a 7% increase in both FEV_1 and morning PEFR over baseline. The improvements in daytime symptoms, nocturnal awakenings, β-agonist use and pulmonary function seen in the zafirlukast-treated group were significant compared to the values of the placebo-treated group.

In summary, the data suggest that zafirlukast is a highly selective and potent leukotriene receptor antagonist that appears to be effective in the treatment of patients with mild to moderate asthma. Its place in current asthma management guidelines and its safety profile will be discussed in subsequent sections.

EFFECT OF ZILEUTON IN ACUTE CHALLENGE STUDIES

In spring of 1997, zileuton (Zyflo®) or A-64077 was approved by the United States Food and Drug Administration. As stated previously, this agent was the first selective, orally active 5-lipoxygenase inhibitor to demonstrate effective leukotriene inhibition in man [14]. The following section will discuss the experience of this agent in acute challenge studies.

Allergen challenge. In an initial allergen challenge study, no improvement was noted in allergen-induced asthma despite the fact that marked 5-lipoxygenase inhibition could be demonstrated [57]. More recently, the results obtained in lung challenge studies in ragweed-sensitive patients were more promising. Kane and colleagues [71] found that patients given zileuton 600 mg orally qid for seven days were protected against the effects of segmental allergen challenge. Both eosinophil migration into BAL fluid and capillary permeability were decreased in treated patients. In addition, the urinary increase in LTE_4 resulting from allergen challenge was decreased by 86% in these individuals.

Cold air challenge. In 1990, Israel and colleagues [60] demonstrated the effects of zileuton on asthma induced by cold, dry air. In a randomized, double-blind, placebo-controlled, crossover study, the effects of zileuton (800 mg) on the bronchoconstriction induced by hyperventilation of cold air was evaluated in thirteen cold-air-sensitive asthmatic patients. Each of these patients was known to respond to cold air challenge with at least a 20% fall in FEV_1 and the baseline mean FEV_1 of the group was 81% of predicted. In the six weeks prior to the study, none of the patients had used oral or inhaled corticosteroids nor had they had an upper respiratory infection. To verify that zileuton indeed was effectively blocking the 5-lipoxygenase enzyme, *ex vivo* LTB_4 production was used as a marker of 5-lipoxygenase activity. Blood samples of patients taken three hours after zileuton administration (immediately prior to cold air challenge) and activated with ionophore demonstrated a 74% reduction in LTB_4 production compared to that seen with blood samples taken before treatment. While there was no significant difference between the mean FEV_1 three hours after placebo and the mean FEV_1 three hours after study drug, zileuton did increase the tolerance of these asthmatics to the hyperventilation of cold, dry air by 47%. In other words, the provocative dose of cold air that was required to cause a 10% fall in FEV_1 ($PD_{10}FEV_1$) was increased by 47% after treatment with zileuton. This level of benefit was greater than that reported for cromolyn sodium [86].

Aspirin challenge. Aspirin-sensitive asthmatics develop bronchoconstriction and, in many cases, naso-ocular, gastrointestinal and/or dermal reactions after ingestion of aspirin (ASA) or nonsteroidal anti-inflammatory compounds [122]. It has been hypothesized that inhibition of cyclooxygenase by ASA may lead to upregulation of 5-lipoxygenase activity with resultant increased formation of leukotriene products in these patients[87]. In support of this hypothesis, it has been found that, in ASA sensitive asthmatics, ASA ingestion leads to increased urinary LTE_4 levels compared to placebo ingestion. In contrast, LTE_4 levels are unaffected in non-ASA sensitive asthmatics after ASA ingestion [23].

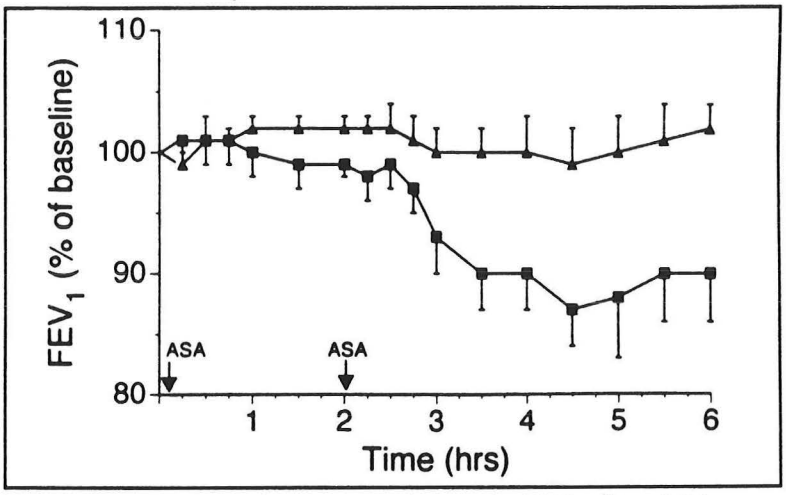

Figure 6. Effect of zileuton on change in FEV_1 after ASA challenge. Triangles: zileuton; squares: placebo [62].

An important study that demonstrated the importance of 5-lipoxygenase products as mediators of the bronchoconstriction and associated reactions seen after ASA ingestion in ASA sensitive asthmatics was published by Israel and colleagues [62] in 1993. In this study, the effects of zileuton was evaluated in a group of eight asthmatic patients that had known ASA sensitivity and LTE_4 hyperexcretion. The subjects were randomized in a double-blind, crossover trial to examine the effects of zileuton (600 mg po qid for six to eight days) versus placebo on the response to ASA challenge. As shown in Figure 6, zileuton significantly inhibited the fall in FEV_1 demonstrated after ASA challenge. In the patients who received placebo, ASA challenge caused a 18.6% decrease in baseline FEV_1 while in those who

received zileuton the ASA-induced decrease was only 4.4%. Zileuton also prevented the nasal, gastrointestinal and dermal responses associated with ASA administration. In addition, zileuton reduced the mean maximal urinary LTE_4 levels seen after ASA challenge by 68%.

More recently, Dahlen and colleagues [32] demonstrated in a crossover study of 40 ASA-sensitive asthmatics on inhaled glucocorticords that add-on treatment with zileuton, 600 mg qid po for six weeks, caused significant bronchodilation and decreased nasal symptoms. These results suggest that 5-lipoxygenase inhibitors may produce additional clinical benefit when added to glucocorticoid treatment.

EFFECT OF ZILEUTON IN CLINICAL ASTHMA

There are several randomized, controlled trials that have been conducted with zileuton to determine its effectiveness in clinical asthma. One of the first of these was performed by Israel and colleagues [61] and published in 1993. In this randomized, double-blind, placebo-controlled, multicenter study, 139 asthmatic patients, each of whom had a baseline FEV_1 of 40% to 75% of predicted and each of whom were not receiving inhaled or oral glucocorticoids, received either zileuton, 600 mg po qid, 800 mg po bid, or placebo for four weeks. While all groups had a slight initial improvement in mean FEV_1 after enrollment, only those who received zileuton showed statistical improvement compared with the dummy lead-in period by the fourth week of the study. The subjects who received 2.4 g/d of zileuton demonstrated a 0.32 L improvement, a 13.4% increase, in mean FEV_1 at four weeks compared with a 0.05 L increase in patients taking placebo Ffigure 7). In addition, symptoms and frequency of β-agonist use decreased with zileuton, 2.4 g/d. These results indicated that 5-lipoxygenase inhibition could improve airway function, could cause decreased symptoms and could cause decreased β-agonist use in patients with asthma. Therefore, this study was pivotal in that it provided the first evidence that 5-lipoxygenase inhibitors may have an important role in the treatment of chronic asthma.

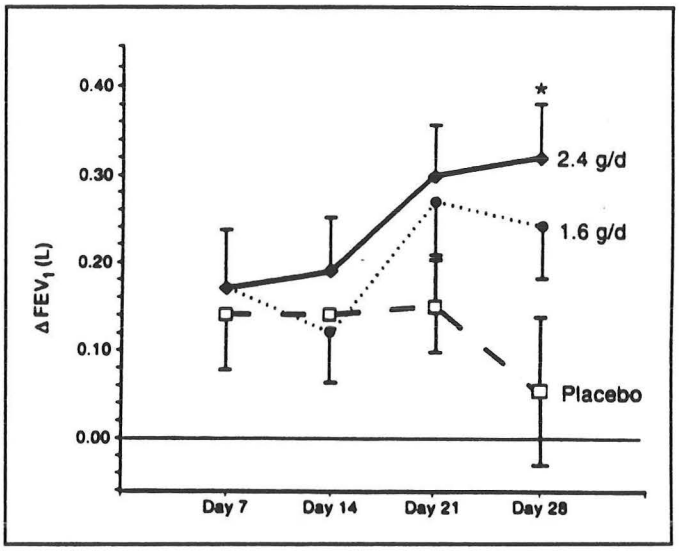

Figure 7. Change in forced expiratory volume after four weeks of administration of zileuton or placebo [61]

More recently, two additional studies have demonstrated similar results. Following their initial study in 1993, Israel and colleagues [64] performed a subsequent randomized, double-blind, parallel-group study in which mild to moderate asthmatics were evaluated following treatment with zileuton for three months. Four hundred and one patients with mild to moderate asthma, whose only treatment was inhaled β-agonists, received either zileuton (600 mg qid or 400 mg qid) or placebo for thirteen weeks. As in the previous study, zileutin treatment caused significant improvement in the FEV_1 compared to placebo treatment (15.7% versus 7.7%) over the trial period. In addition, β-agonist use was significantly decreased and quality of life significantly increased in the group treated with 2.4 g/d of zileuton.

Liu and colleagues [96] demonstrated similar findings. In this recently published six-month multicenter, double-blind, parallel-group, placebo-controlled trial, 373 mild to moderate asthmatic patients were treated with either zileuton (600 mg qid or 400 mg qid) or placebo for six months. Patients in both zileuton groups had significantly increased FEV_1 values as compared to the placebo group by day eight of the study. On day 36, FEV_1 improvement was 16% and 12% above baseline for patients treated with zileuton 600 mg qid and 400 mg zileuton qid, respectively, compared to an improvement of only 6% for the placebo group. In the group that received the higher dose of zileuton, morning peak expiratory flow rate improved by 7% to 10%; daytime and nocturnal symptoms decreased by 37% and 31%, respectively; β-agonist use decreased by 31%; and the proportion of patients who required rescue steroid medications was reduced by 62%. Importantly, in both of the above studies FEV_1 did not deteriorate significantly during the study periods. Therefore, the results obtained in these trials extend previous findings that demonstrated that patients do not develop a tolerance to the effects of 5-lipoxygenase inhibitors.

In summary, data from the above three studies suggest that zileuton is a potent 5-lipoxygenase inhibitor that is effective in the treatment of patients with mild to moderate asthma. Its place in current asthma management guidelines, its safety profile and its potential steroid sparing effect will be discussed in subsequent sections.

MONTELUKAST, A NEW CYSTEINYL LEUKOTRIENE RECEPTOR ANTAGONIST ON THE "LAUNCHPAD"

Within the next month or two, a new cysteinyl receptor antagonist, montelukast (Singulair®) will be marketed in the United States. This compound resulted from efforts to find structurally diverse agents that had greater potency than other leukotriene antagonists but, even more importantly, that did not have effects on peroxisomal enzyme proliferation and other toxic liver effects [80]. This second-generation quinoline compound is the most potent of the available agents and it induces the most lasting blockade of LTD_4-induced bronchoconstriction of any agents developed to date [13]. After asthmatics received a single 40 mg oral dose, a greater than 50-fold shift in the LTD_4 dose-response curve persisted

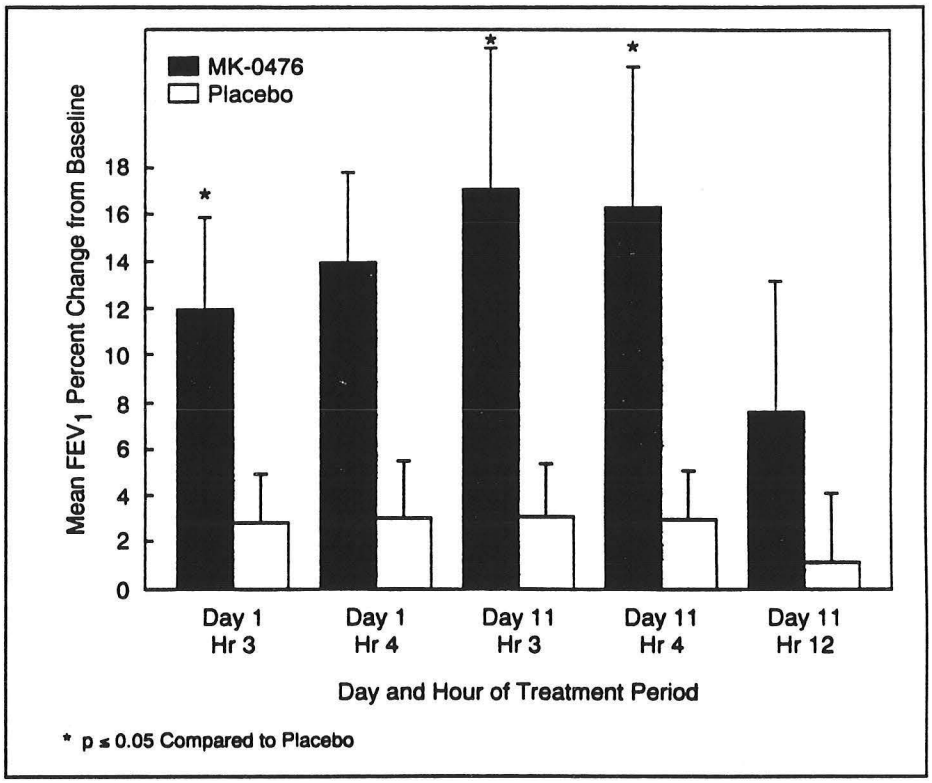

Figure 8. Increase in FEV_1 a percentage improved from the day one predosing baseline value [119].

for twenty four hours [11] and, in another study, a single 100 mg dose caused a 10-12% increase in baseline FEV_1 in moderate asthmatics throughout the nine hours in which they were evaluated [129].

More recently, in a double-blind, placebo-controlled, crossover study, 29 patients with asthma with FEV_1 values between 50% to 80% were treated with montelukast, 200 mg po tid, for ten days. Fifteen of these individuals received concomitant treatment with inhaled corticosteroids also. Compared with placebo, montelukast caused marked improvement in FEV_1 on days 1 and 11 (the days on which spirometry was performed) compared to placebo three and four hours after dosing (Figure 8). In addition, compared to placebo, significant improvements also were noted in daytime asthma symptom scores and as-needed β-agonist use. As importantly, it was demonstrated that the benefits seen with montelukast occurred irrespective of the presence of concomitant treatment with inhaled steroids. These results suggest that inhibiting the actions of leukotrienes may complement corticosteroid therapy in asthma treatment [119]. More recent studies have demonstrated the effectiveness of montelukast in both exercise- [89] and ASA-induced asthma [78]. In addition, it has been shown to be effective in chronic asthma over a three month period [120]

STEROID SPARING EFFECT OF LEUKOTRIENE MODIFIERS

To date, only a few studies have been performed to determine whether the addition of a leukotriene modifying agent permits reductions in dosages of inhaled corticosteroids in patients with asthma. Bateman and colleagues [8] evaluated the effect of adding zafirlukast, 20 mg bid, on inhaled steroid dosage. Three hundred fifty nine patients with mild, stable asthma who were being treated with 400-750 ug/day inhaled corticosteroids were randomized to receive either zafirlukast or placebo. It was found that the difference in outcomes measured between the placebo and treatment groups did not reach statistical significance. Groups did not differ in regards to daytime symptoms, daily β-agonist use, or morning peak flow values. In another study of patients who were on higher doses of inhaled steroids (800-2000 ug/day), similar results were reported [82]. While these results suggest that there is no beneficial effect of zafirlukast in reducing inhaled steroid dosages, the authors felt that the study designs were not capable of showing a difference in steroid sparing effect between zafirlukast and placebo [59].

More promising results have been obtained with zileuton and montelukast. Israel and colleagues [63] analyzed the requirement for steroid treatment in a double blind study designed to evaluate the clinical efficacy of thirteen weeks of treatment with zileuton in patients with moderate asthma. Four hundred and one patients who had an FEV_1 of 40%-80% of predicted and who had reversible bronchoconstriction were randomized to receive placebo, zileuton, 400 mg qid, or zileuton, 600 mg qid. During the study, patients were treated with as-needed β-agonists alone. Inhaled corticosteroids, cromolyn and nedocromil were not allowed, but oral corticosteroids were administered if patients experienced an asthma exacerbation. Upon analyzing corticosteroid use based upon entry FEV_1, a marked steroid sparing effect was found in those patients who had an FEV_1 less than 50% of predicted. Thus, these results reveal that 5-lipoxygenase inhibition decreases the need for acute steroid use in more severe asthmatics. In addition to this finding, a more recent study by O'Connor and colleagues [112] showed that the addition of zileuton to low doses of inhaled beclomethasone was as effective

as higher doses of beclomethasone alone in controlling asthma.

Leff and colleagues [88] determined whether or not montelukast would allow inhaled corticosteroids to be tapered in clinically-stable asthmatics in a double-blind, randomized, parallel study. Two hundred twenty six adult asthmatics who had a baseline FEV_1 >70% of predicted entered into a single-blind, six-week evaluation in which inhaled corticosteroids were tapered. Those who remained stable, but who required moderate to high doses of inhaled corticosteroids were randomized to receive either 10 mg montelukast or placebo for twelve weeks. A composite clinical score was calculated [79], and based upon the score, the inhaled steroid dose was tapered, maintained or increased. Compared to placebo, montelukast allowed steroids to be tapered to an extent that was statistically significant. At the end of the study, the mean last tolerated dose of corticosteroids was 727 ug in the placebo group versus only 526 ug in the montelukast group. These results suggest that montelukast may allow clinically-significant tapering of inhaled corticosteroids in patients who require moderate to high doses of inhaled corticosteroids.

PRACTICAL DIFFERENCES REGARDING ZILEUTON AND ZAFIRLUKAST [1, 142]

- Neither zileuton nor zafirlukast are indicated for the reversal of acute bronchospasm. However, therapy with both drugs can be continued during acute asthma exacerbations.
- Zafirlukast is dosed twice a day while zileuton is dosed four times a day.
- The bioavailability of zafirlukast may be decreased when taken with food. Patients should take zafirlukast at least one hour before or two hours after meals. The bioavailability of zileuton is not affected by food.
- Both zileuton and zafirlukast are approved for use in adults and children ages 12 and older.
- Zafirlukast inhibits the cytochrome P450 2C9 isoenzyme system and due to this inhibition there is a significant increase in the mean half-life (+36%) of co-administered warfarin. No formal drug interaction studies have been performed with other drugs known to be metabolized by the cytochrome P450 2C9 isoenzyme (eg, tolbutamide, phenytoin, carbamazepine). However, it is recommended that "care be exercised" when zafirlukast is coadministered with these agents.
- Coadministration of zafirlukast with terfenadine causes a decrease in the mean serum concentration of zafirlukast. No effect of zafirlukast on terfenadine plasma concentrations or ECG parameters has been demonstrated.
- Coadministration of zafirlukast with aspirin causes a 45% increase in zafirlukast plasma levels; Coadministration of zafirlukast with theophylline causes a 30% decrease in zafirlukast plasma levels with no effect on serum theophylline levels. Coadministration of zafirlukast with erythromycin causes a 40% decrease in zafirlukast plasma levels.
- When administered together, zileuton decreases the clearance rates of theophylline, warfarin, propranolol and terfenadine.
- Most frequent adverse reactions reported with zileuton are: headache, unspecified pain, abdominal pain, asthenia, accidental injury, dyspepsia, nausea and myalgia. Dyspepsia was the only reaction that was significantly more common in patients taking zileuton compared to those taking placebo.
- Most frequent adverse reactions reported with zafirlukast are: headache, infection,

nausea, diarrhea, generalized pain, asthenia and abdominal pain. None of these reactions were significantly more common in patients taking zafirlukast compared to those taking placebo.

- **Elevations of one or more liver function tests may occur during zileuton therapy. For this reason, it is recommended that hepatic transaminases be measured before treatment, once-a-month for the first three months of treatment and then every two to three months for the remainder of the first year. Subsequently, measurements should be made periodically thereafter. If clinical signs of liver dysfunction develop or if transaminases rise greater than 5 times the upper limits of normal, zileuton should be discontinued and transaminase levels followed until they normalize.**
- **An FDA advisory was released on July 23, 1997 regarding a possible drug side effect associated with zafirlukast. The FDA learned of six asthma patients who developed Churg-Strauss Syndrome while taking zafirlukast. These reported cases occurred in patients whose steroidal asthma medications were being gradually lowered or discontinued during zafirlukast treatment. Despite this possible association, the FDA currently believes that the benefits of this drug outweigh any of its known or potential risks.**

POSITION OF LEUKOTRIENE MODIFIERS IN ASTHMA MANAGEMENT GUIDELINES

As stated in the recently published *Guidelines for the Diagnosis and Management of Asthma: Expert Panel Report 2* [39], anti-inflammatory therapy is the key component in the pharmacologic management of asthma. At the present time, glucocorticoids are the most effective and most commonly used anti-inflammatory agents that are used for asthma treatment. While the mode of action of these agents is complex, their efficacy probably is due to the fact that they act upon multiple inflammatory processes. For the most part, glucocorticoids are well-tolerated when inhaled. However, side effects may occur, especially at high doses.

It is unlikely that the new leukotriene modifying agents will replace inhaled corticosteroid therapy in the management of chronic asthma. However, the studies described in this review do show that antileukotriene drugs cause improvement in a variety of asthma-associated parameters. At this time, it is unclear how these drugs will fit into asthma management schemes. In order to make this determination, comparative data that describes the efficacy of these drugs in relation to that of the inhaled corticosteroids is required.

From the studies that have been published, it does appear that the antileukotrienes may play an important role in patients with certain specific types of asthma. In those asthmatic individuals who are aspirin-sensitive, studies reveal that these agents effectively block aspirin-induced bronchospasm [62]. Thus, these agents should be considered in the management of aspirin-sensitive asthmatics if there is no contraindication.

Another place for the antileukotrienes may be in the management of the patient with mild, persistent asthma as has been recommended in the new *Expert Panel Report* [39]. The recommended treatment for these individuals includes daily anti-inflammatory therapy, either inhaled corticosteroids or cromolyn or nedocromil. Possibly, the introduction of a leukotriene modifier may allow inhaled steroid dosages to be lowered.

SUMMARY

The antileukotrienes offer a new dimension to asthma management. It has been shown that these agents effectively inhibit the actions of the leukotrienes and that they have a beneficial effect in chronic asthma as well as in asthma that has been induced. Thus, these findings suggest that leukotrienes play an important role as mediators of the asthmatic response. However, we now must await the results of studies that are in progress to determine exactly where these therapeutic agents will be positioned in asthma treatment protocols in the future.

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