5-Lipoxygenase Inhibitors for the Treatment of COPD*

Stephen Kilfeather, PhD

The potential for leukotrienes as mediators to target in the development of novel therapies for diseases such as COPD is underscored by their inflammatory behavior and the capacity of leukotriene receptor antagonists and synthesis inhibitors to reduce inflammatory responses when administered in vivo. Airway neutrophilia in COPD patients is believed to be a contributing source of inflammation and is associated with airway remodeling. The presence of neutrophils is mediated in part by leukotriene B4 (LTB4), and the capacity for LTB4 alone to replicate many aspects of neutrophilic inflammation has provided the focus of drug development toward its specific antagonism. More recently, the potential involvement of the monocyte-macrophage lineage in the etiology of COPD has received growing attention as a target for leukotriene inhibition. The future avenues for exploration of leukotriene inhibition could have been expanded by the realization that 5-lipoxygenase activity is primarily located at the nuclear membrane and that there are differing cell surface receptors to LTB4. The success of compounds under development in this and other anti-inflammatory classes, however, depends as much on the evolution of clinical studies designed to test the “proof of concept” in efficacy through the examination of surrogate markers or physiologic readouts of changes in lung function.

**Key words:** antagonist; COPD; 5-lipoxygenase; 5-lipoxygenase inhibitor; leukotriene; leukotriene B4; neutrophil

**Abbreviations:** AA = arachidonic acid; BLTR = leukotriene B4 receptor; COX = cyclooxygenase; FLAP = 5-lipoxygenase activating protein; IL = interleukin; 5-LO = 5-lipoxygenase; LTB4 = leukotriene B4; PPAR = peroxisome proliferator activating receptor

The rationale for targeting leukotrienes in the development of therapeutic agents for inflammatory disease is derived from their inflammatory cellular sources and activities. This thesis is endorsed by the capacity of leukotriene receptor antagonists and synthesis inhibitors to reduce certain inflammatory responses involving neutrophils when administered in vivo. The development of inhibitors of leukotrienes has evolved from the concept that these mediators are synthesized at the cell surface, and that their actions are mediated through cell surface receptors. It is now established that leukotriene production occurs at the nuclear membrane and that there are leukotriene receptors within different cellular compartments. There has been success in the development of inhibitors of leukotriene synthesis through the inhibition of 5-lipoxygenase (5-LO) and 5-LO activating protein (FLAP), and through antagonism of leukotriene B4 (LTB4) and cysteinyl leukotriene receptors (Fig 1). In the development of a therapy for the treatment of COPD, however, there may be an additional benefit derived from reduced specificity and the targeting of other aspects of the arachidonic acid (AA) cascade such as through the inhibition of cyclooxygenase (COX).

The early identification of sputum neutrophilia in COPD set the focus of attention on this cell type as a potential contributing source of inflammation and change in airway structure in COPD patients. LTB4 mediates elements of neutrophilic inflammation, and neutrophils produce LTB4 in sufficient quantities to induce LTB4-mediated inflammatory responses. LTB4 is chemotactic for neutrophils and is involved in their activation through paracrine and autocrine activity. Levels of LTB4 in the sputum of patients with COPD reach sufficient levels to occupy a significant proportion of neutrophil cell surface receptors and to induce chemotaxis. The inhibition of LTB4, therefore, has provided a potential route for the interruption of neutrophilic inflammation in COPD.

More recently, the potential involvement of the monocyte-macrophage lineage in the etiology of COPD and emphysema has received attention, including the potential for cells of this lineage as targets for leukotriene inhibition. The relatively low levels of neutrophils in the lower airways raises the possibility that neutrophilic inflammation in COPD patients could be more concentrated in the upper airways, while macrophage-derived mediators and proteases could provide a more predominant activity in the peripheral airways and alveolar region.

The investigation of the nuclear membrane site of 5-LO activity and the existence of differing cell surface receptors and nuclear receptors could extend the future avenues for exploration of leukotriene inhibition. The inhibition of

---

*From the Institute for Cardiovascular and Respiratory Pharmaceutical Development, University of Sunderland, Tyne & Wear, Sunderland, UK.

Correspondence to: Stephen Kilfeather, PhD, Institute for Cardiovascular and Respiratory Pharmaceutical Development, University of Sunderland, Tyne & Wear, Sunderland, SR1 3SD, United Kingdom; e-mail: Skilfeather1CRPD@aol.com

Figure 1. Current inhibition of leukotriene activity. HPETE = 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid; LTC4 = leukotriene C4; LTD4 = leukotriene D4; LTE4 = leukotriene E4.
noncysteinyl leukotrienes such as LTB₄ will not provide a route for bronchodilatation. The success of treatment with compounds under development in antileukotriene and other anti-inflammatory cell classes in COPD patients depends, therefore, on the capacity of early-stage clinical studies to test the “proof of concept” in efficacy through the examination of surrogate markers of airway inflammation or lung function.

The impetus for the exploration of leukotrienes, particularly LTB₄ in COPD patients, is derived from the consistent level of sputum neutrophilia in patients with this condition. The levels of neutrophilia have a wide range among patients (ie, < 5% to > 90%) and this range has supported the consensus view that neutrophils provide a significant contribution to the development of chronic inflammation and airway remodeling in COPD. The presence of neutrophilia is not consistent in BAL fluid or in the lower airway wall, and this raises the possibility that neutrophil-mediated inflammation could be more significant to the etiology of the symptomatic features of COPD that are observed in the upper airways. The macrophage provides an alternative candidate cell as the main promoter of inflammation in the lower airways and alveolar regions in COPD patients, and has received more attention recently as a potential target for development of agents to modulate the inflammatory and structural changes in COPD. Macrophages are also a source of LTB₄, and their behavior is influenced by leukotrienes. As discussed below, LTB₄ appears to be involved in macrophage phagocytosis of Klebsiella. Agents that inhibit leukotriene activation, therefore, may reduce macrophage activities throughout the airways and may reduce the effects on neutrophil activation and migration.

The capacity for leukotrienes, particularly LTB₄, to amplify neutrophil activity⁴ has supported the drive to develop compounds with an anti-inflammatory activity that is mediated through the inhibition of leukotrienes and thereby underlines neutrophil activity in inflammatory conditions. The potential efficacy of LTB₄ antagonists against neutrophilic aspects of airway inflammation in humans has been observed through reductions in the number of airway neutrophils following antigen challenge in asthmatic patients² (Table 1). Currently, there are long-acting and potent LTB₄ receptor antagonists that have shown efficacy against inflammation and neutrophilia in primates.³ This class of compound has also shown efficacy in models of other inflammatory conditions, including arthritis.⁴ Members of the 5-LO inhibitor, FLAP inhibitor, and dual 5-LO/COX inhibitor classes, together with LTB₄ receptor antagonists have all demonstrated the inhibition of neutrophil influx and tissue edema when administered orally to animals. In the case of the synthesis inhibitors, this often has been associated with a reduction in tissue LTB₄ levels and LTB₄ synthesis ex vivo from circulating neutrophils.

The understanding of AA metabolism has been advanced considerably by the recognition that 5-LO synthesis occurs at the nuclear membrane⁵ (Fig 2) and that leukotrienes are, therefore, likely to exert autocrine effects in this region in addition to actions through cell surface receptors. An example of such an autocrine action at the nucleus could be the support of neutrophil survival by LTB₄.⁶

In addition to the leukotriene B₄ receptor (BLTR) at the plasma membrane, two other receptors have been reported with 30 to 45% homology to BLTR and without affinity for established BLTR antagonists.⁷,⁸ This implies an additional scope for the design of LTB₄ receptor antagonists (Fig 2). Furthermore, the concept of a nuclear action of LTB₄ is strengthened by the finding that this agonist is a ligand for the nuclear peroxisome proliferator-activating receptor (PPAR)‑α.⁹ This receptor is related to the PPAR-γ receptor, which is a receptor to 15-deoxy-D₁₂,14-prostaglandin J₂, a metabolite of prostaglandin D₂.¹⁰ In isolated cell studies, agonists of these receptors exert effects that are similar to those of corticosteroids, resulting in the reduction of levels of interleukin (IL)-2, IL-6, IL-8, tumor necrosis factor-α, and matrix metalloproteinases. This has led to the concept that cellular physiologic responses to LTB₄ could represent an integration of proinflammatory and anti-inflammatory actions are mediated by the cell surface receptors and nuclear receptors, respectively (Fig 2). In this context, compounds that interact with both cell surface and nuclear receptors have been developed, displaying differential agonist and antagonist properties at each receptor subtype¹¹ (Table 2). It is noteworthy, however, that agonists to PPAR-α and PPAR-γ, when administered in vivo, have been found to cause the elevation of circulating tumor necrosis factor-α levels, implying further complexity to the potential regulation of leukotriene involvement in inflammation when targeting PPAR receptors.

### Table 1—BLTR Antagonist LY293111 vs Placebo in Asthmatic Patients and the Effect on Content of BAL Fluid After Antigen Challenge

<table>
<thead>
<tr>
<th>Variable</th>
<th>LY293111</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophil cell counts</td>
<td>0.04</td>
<td>0.09 million/mL</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>3.5</td>
<td>16.0 ng/mL</td>
</tr>
<tr>
<td>LTB₄</td>
<td>2.2</td>
<td>4.6 pg/mL</td>
</tr>
</tbody>
</table>

*Eosinophils, macrophages, and lymphocytes in BAL fluid did not differ between treatments. Adapted from Evans et al.⁵*
The inhibition of 5-LO alone or through a dual 5-LO/COX inhibitor would provide the inhibition of a wider range of mediators with the retention of 12-lipoxygenase and 15-lipoxigenase products. The blanket removal of mediators, including lipoxins, does not facilitate specificity or provide the refinement of receptor antagonism to distinguish among LTb4 receptor subtypes but may have additional benefits in application toward comorbidity within COPD. COPD patients often have ischemic heart disease, possibly in relation to an invariable smoking history, and some already may be taking nonsteroidal anti-inflammatory products for this condition and/or arthritis. Both 5-LO inhibitors and BLTR antagonists have been considered as potential treatments in the latter conditions. The comorbidity in COPD patients could, therefore, present an argument for decreased selectivity in the development of leukotriene inhibitors for the treatment of COPD and could indicate the potential for dual 5-LO/COX inhibitors.

A potential problem in the development of a class of compounds aimed at reducing neutrophilic inflammation is the reduction of neutrophil-dependent host defense. Corticosteroid agents are used to accelerate the resolution of inflammation during exacerbations of COPD. This apparent reassurance for the use of anti-inflammatory compounds in treating COPD patients should be viewed with the awareness that neutrophilia can result from oral corticosteroid administration and that corticosteroids promote neutrophil survival. Therefore, while corticosteroids have been found to reduce neutrophil accumulation under certain conditions, they may potentially elevate certain aspects of neutrophil defense. LTb4 contributes to neutrophil survival and BLTR antagonism reverses neutrophil survival responses. LTb4 receptor antagonism could, therefore, target aspects of neutrophilic inflammation that are insensitive to corticosteroids, and the combined effect of corticosteroid and LTb4 inhibitor on host defense has not been fully explored.

Several studies have been conducted in 5-LO knockout mice to test their response to a range of infections and to sensitization to ovalbumin. Overall, the mice showed reduced neutrophil responses to infection, but survival was not impaired to a significant degree, apart from infections involving Klebsiella pneumoniae. Further studies revealed that macrophages from genetically 5-LO-deficient mice show impaired phagocytosis of K pneumoniae, which can be restored by the addition of LTb4. This may imply caution under certain circumstances in the use of 5-LO inhibitors.

5-LO knockout mice also showed reduced responses following attempts to sensitize them to ovalbumin, and we have observed a reduction in the capacity to sensitize mice to ovalbumin when treating them during the sensitization period with FLAP inhibition or cysteinyl leukotriene receptor antagonists. These findings are consistent with the influence of 5-LO products over the development of certain immune responses. In the context of COPD, several reports appeared a decade ago concerning the capacity for LTb4 to influence lymphocyte differentiation, and the findings demonstrated consistently an up-regulation of the CD8 suppressor lymphocyte phenotype. This may be of relevance to the cause of increased numbers of CD8+ cells that are observed in the airways of COPD patients, but a direct link between elevated LTb4 levels and their involvement in the up-regulation of CD8 cells in COPD patients would be difficult to establish.

It appears that the inhibition of 5-LO will exert effects on both immune and inflammatory responses in COPD patients, and this may be of benefit in reducing the acute inflammatory responses, in which those responses are excessive, even if the progression of the disease is not slowed. An examination of the capacity of these classes of compounds to influence exacerbation frequency, duration, or intensity in COPD patients is required to generate confidence that the inhibition of leukotrienes will underwrite excessive rather than crucial defenses.

There are potentially unseen hurdles to the development of leukotriene inhibitors as anti-inflammatory agents in COPD patients. For example, certain 5-LO inhibitors appear to require glutathione for optimal activity, and this raises the potential for a loss of potency of this class of agents in treating COPD patients, in whom glutathione levels are compromised under conditions of oxidative stress that are likely to be encountered in cells in COPD patients.

One of the largest obstacles to the successful development of classes of anti-inflammatory compounds that have no bronchodilator action and none of the profound immuno-modulatory effects of corticosteroids is the difficulty in generating studies that will adequately test drug efficacy. In COPD treatment, the clinical development of such classes of compounds is dependant on the requirement to see an early sign of proof of concept before engaging in more protracted trials. A proof-of-concept study often will involve an examination of effects on a surrogate marker that is not guaranteed to produce a quick readout, such as effects on neutrophilia or neutrophil activation. It is noteworthy that the reductions in airway neutrophils observed by Evans et al due to LY293111, a BLTR antagonist, following antigen challenge in asthmatic patients were significant but were achieved from a very low pretreatment baseline compared with the level of sputum neutrophilia observed in COPD patients. In addition, the correct mode of assessment of indexes of neutrophil activity has not been confirmed. Figure 3 illustrates in a group of patients with COPD the diminishing effect of changes in absolute sputum cell number on the proportion of cell population occupied by neutrophils in sputum at high neutrophil concentrations. The implication from this observation is that treatment-induced reductions in cell content from high pretreatment baseline levels (ie, > 70%
neutrophilia) may not be observed if only percentages of neutrophils are measured. The measurement of absolute cell numbers in the examination of effects of leukotriene inhibition or other anti-inflammatory strategies on neutrophilia may be more difficult to measure accurately compared to the measurement of neutrophils as a percentage of total cell population, but may be necessary to see efficacy.

In addition to the practical aspects of the examination of surrogate markers in the assessment of drug efficacy in the treatment of COPD patients, it is not clear how the benefits of antileukotriene therapy will be manifested in patients with this condition and, therefore, what indexes should be examined clinically. It could be argued that agents acting primarily on neutrophils may exert their actions on the symptoms derived from the upper airways, such as sputum production, if the neutrophilia is mainly a feature of the upper airways. The consequences of leukotriene inhibition on macrophage behavior, however, as well as the effects on other aspects of COPD in the lower airways and alveolar regions also could emerge. This potential oversimplification does not take into account the communications among cell types or the dramatic up-regulation and change of inflammatory events during COPD exacerbations and their resolution. There are potential benefits of antileukotriene agents as with other anti-inflammatory agents during exacerbation periods, but the complexity of the events, including the need for host defense at these times, precludes accurate predictions. Similarly, beyond exacerbations, benefits may be derived through a range of effects that are integrated systemically or physiologically into indexes of quality of life such as an effect on exercise tolerance, rather than through observations of changes in baseline lung function.

In conclusion, the complexity of leukotriene regulation of cell behavior and, therefore, the scope for the redesign of 5-LO inhibitors and leukotriene receptor antagonists will make for a further evolution of this route of anti-inflammatory development. The main hurdles to the future establishment of these compounds in the treatment of COPD appear to be the potential for undermining crucial aspects of host defense responses and the capacity to adequately test the efficacy of the compounds in early clinical development stages.

**References**


**Figure 3.** Relationship between sputum neutrophil content in absolute terms and percentages in COPD patients.