PROTECTIVE EFFECT OF H₁ AND CYSLT₁ ANTAGONISTS
ON ALLERGEN INDUCED AIRWAY RESPONSES IN ATOPIC ASTHMA

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ABSTRACT

Background

The mechanism by which allergies trigger asthma occurs through the interaction of antigen, IgE and the F_{c}R_{1} receptor on mast cells resulting in the release of mediators that exert their effects on various surrounding tissues causing bronchoconstriction, plasma exudation and mucus hypersecretion. The response is usually maximal within 30 minutes and resolves spontaneously within two hours. At least half of the individuals who exhibit this so called “early response” also manifest a “late response” which is a subsequent episode of bronchoconstriction that is usually maximal around six hours following exposure and involves airway inflammation.

Montelukast has proven efficacious in the management of asthma and desloratadine is effective in the treatment of allergic rhinitis and chronic idiopathic urticaria. Since the early response involves the actions of multiple mediators, including histamine and the leukotrienes, the question of whether concurrent mediator blockade would be superior to either agent alone was raised. Additionally, the recent evidence supporting anti-inflammatory activity for these agents suggested potential efficacy against the late airway response.

Methods

Two double-blind, randomized, placebo-controlled, 4-way crossover allergen inhalation challenge investigations were conducted in twenty (10 per investigation) mild atopic asthmatics. The early response investigation involved the administration of either 5 mg desloratadine, 10 mg montelukast, the combination, or placebo (Vitamin B_{1}) at 26 hours and 2 hours prior to allergen inhalation. The late response investigation involved single dose administration of each agent, alone or in combination, 2 hours prior to allergen inhalation. Measurements of changes in airway responsiveness and inflammation were also conducted.
Results

The early response was significantly inhibited by montelukast and the combination. Desloratadine did not differ from placebo. The late response was significantly decreased by desloratadine and montelukast and completely blocked with the combination. Desloratadine decreased sputum eosinophils at 7 hours, montelukast at 24 hours, and the combination at both time points. Airway responsiveness to methacholine trended lower with montelukast and the combination. Montelukast was the only treatment to significantly decrease exhaled nitric oxide levels.

Conclusion

The combination of desloratadine and montelukast provides inhibition that is superior to both monotherapies on the early and the late airway responses to inhaled allergen in people with mild atopic asthma.
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LIST OF ABBREVIATIONS

5-HPETE – 5-hydroperoxyeicosatetraenoic acid
AC – adenylyl cyclase
ADHD – attention deficit hyperactivity disorder
AHR – airway hyperresponsiveness
AMP – adenosine 5’-monophosphate
ANOVA – analysis of variance
anti-CD3+ – CD 3 positive cell antibody
anti-CD11a – cluster of differentiation 11a antibody
anti-CD40 – cluster of differentiation 40 antibody
anti-IgE – immunoglobulin E antibody
anti-TNFα - tumor necrosis factor alpha antibody
ATS – American Thoracic Society
AUC – area under the curve
B – budesonide
B cell – B lymphocyte
BAL – bronchoalveolar lavage
BALB/c – laboratory inbred murine model for animal experimentation
Bcl-2 – B cell lymphoma cell line
C57BL/6 – laboratory inbred murine model for animal experimentation
CCR3 – chemokine receptor 3
CIU – chronic idiopathic urticaria
CNS – central nervous system
COS-7 – African green monkey cell line
CysLT1 – cysteinyl leukotriene receptor subtype 1
CysLT2 – cysteinyl leukotriene receptor subtype 2
DAG – diacyl glycerol
DAR – dual asthmatic response
DF – dermatophagoides farinae
DP – dermatophagoides pteronyssinus
EAR – early asthmatic response
ECP – eosinophil cationic protein
EIB – exercise induced bronchoconstriction
FeNO – fraction of exhaled nitric oxide
EOS - eosinophil
F - flonase
F_{\text{eRI}} – high affinity IgE receptor
FDA – United States Food and Drug Administration
FEV$_1$ – forced expiratory volume during the first second of exhalation
GABA – gamma amino butyric acid
GINA – Global Initiative for Asthma
GMCSF – granulocyte macrophage colony stimulating factor
H$_1$ – histamine receptor subtype 1
H$_2$ – histamine receptor subtype 2
H$_3$ – histamine receptor subtype 3
H$_4$ – histamine receptor subtype 4
HDM – house dust mite
HUVEC – human umbilical vein endothelial cells
ICAM – intercellular adhesion molecule
ICS – inhaled corticosteroid
IFN$\gamma$ - interferon gamma
IgE – immunoglobulin E
IgG – immunoglobulin G
IL – interleukin
IP$_3$ – inositol 1,4,5 trisphosphate
LAR – late asthmatic response
LFA – lymphocyte function associated antigen
LPS – lipopolysaccharide
LSD – least squared difference
LTB$_4$ – leukotriene B 4
LTC₄ – leukotriene C₄
LTD₄ – leukotriene D₄
LTE₄ – leukotriene E₄
LTRA – leukotriene receptor antagonist
M₁ – muscarinic receptor subtype 1
MAO-B – monoamine oxidase subtype B
MBP – major basic protein
MCP-1 – monocyte chemoattractant protein - 1
MEP – mepyramine
MET - metiamide
MIP – macrophage inhibitory protein
MMP – matrix metalloproteinase
MPC₂₀ – concentration of methacholine that causes a 20 % decrease in FEV₁
NFκB – nuclear factor kappa B
NHLBI – United States National Heart Lung and Blood Institute
OVA – ovalbumin
PAF – platelet activating factor
PBMC – peripheral blood mononuclear cell
PC₂₀ – concentration of allergen that causes a 20% decrease in FEV₁
PD – pharmacodynamic
PDGF – platelet derived growth factor
PEFR – peak expiratory flow rate
PGD₂ – prostaglandin D subtype 2
PK – pharmacokinetic
PLC – phospholipase C
PKA – protein kinase A
RANTES – regulated upon activation, normal T cell expressed and secreted
S- salbutamol
SCG – sodium cromoglycate
sIL-4R – soluble interleukin 4 receptor
sICAM – soluble intercellular adhesion molecule
SEM – standard error of the mean
SFRM-B – sulforhodamine-B
SRS – slow reacting substance
SRS-A – slow reacting substance of anaphylaxis
T cell – T lymphocyte
Th₁ – T lymphocyte helper cell subtype 1
Th₂ – T lymphocyte helper cell subtype 2
THP-1 – human acute monocytic leukemia cell line
TGFβ - transforming growth factor beta
TNFα - tumour necrosis factor alpha
TPD – Therapeutic Products Directorate
VCAM – vascular cell adhesion molecule
WHO – World Health Organization
1.0 INTRODUCTION

1.1 ASTHMA

1.1.1 Definition

A strict definition for asthma has been difficult to establish due to the heterogeneity of the disorder and the lack of a complete understanding of the events leading to the changes in airway physiology (i.e., hyperresponsiveness), pathology (i.e., inflammation) and structure (i.e., tissue remodeling). The Global Initiative for Asthma (GINA), a partnership between the World Health Organization (WHO) and the United States National Heart Lung and Blood Institute (NHLBI, Bethesda Maryland, USA) provides the following “operational description” [GINA 2008] which remains controversial with noted limitations [Hargreave and Nair, 2009]:

“Asthma is a chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread, but variable, airflow obstruction within the lung that is often reversible either spontaneously or with treatment.”

1.1.2 Epidemiology

The prevalence of asthma continues to increase worldwide. In 2005, the WHO reported an estimated 300 million individuals are affected by asthma and this is expected to increase by approximately 34% by 2025. The WHO also reported 255,000 deaths due to asthma in 2005 and expects a 10% increase in mortality over the next 10 years. Disease morbidity poses a huge burden to society both in terms of health care costs including emergency room visits, physician visits and drug costs, as well as school absenteeism and lost work days; this amounts to 6 billion dollars annually in the United States alone.
Various risk factors have been identified and likely contribute to the asthma phenotype. Host factors such as genetic predispositions to atopy and airway hyperresponsiveness, as well as gender and obesity influence disease development. Environmental factors include agents to which individuals have a sensitivity (e.g., seasonal pollens and animal dander), tobacco smoke and air pollution which influence disease manifestation.

1.1.3 Atopic Asthma

Atopic asthma (a.k.a. allergic asthma or extrinsic asthma) describes a subpopulation of individuals that experience a worsening of their asthma upon exposure to allergens to which they are sensitized. Atopic asthma is believed to account for more than 50% of adult asthma sufferers [WHO, 2003]. Common triggering antigens include seasonal pollens (e.g., grass, trees and weeds), house dust mite and domestic animals (e.g., cat and horse) and multiple sensitivities are usually present. Atopy and asthma are not absolute co-morbidities; all individuals with asthma do not have allergies and all individuals with allergies do not have asthma.

1.2 AIRWAY RESPONSES IN ATOPIC ASTHMA

1.2.1 Early and late asthmatic responses (Figure 1.1)

In the research setting, a standardized allergen challenge model has been developed to induce airway responses following allergen exposure allowing for the investigation of disease mechanism(s) and therapeutic efficacy [Boulet et al., 2007; Hendeles and Harman, 1997]. Almost immediately following aerosolized (i.e., inhaled) administration of an allergen to which an individual is sensitized, the acute or early asthmatic response (EAR) develops. The response is quantified by measuring changes in airflow and a positive result has been arbitrarily defined as a $\geq 20\%$ (or 15%) decrease in the amount of air forcefully exhaled during the first second of expiration following a full inspiration (forced expiratory volume in one second, FEV$_1$). This reversible episode of airflow obstruction, which is usually maximal at 10 to 30
minutes post exposure, resolves within 3 hours of exposure, either spontaneously or with treatment. Many individuals (50 to 75%) who develop an EAR will also develop a subsequent episode of
Figure 1.1: Changes in FEV₁ during the early and the late asthmatic responses.

Grass inhalation results in a decrease in the FEV₁ of approximately 30%. The induced airflow obstruction is allowed to recover without treatment and returns to near baseline within three hours of allergen inhalation. A subsequent decrease in FEV₁ develops over the next 4 hours in the absence of re-exposure to allergen. Saline challenge data is represented by open circles. Grass challenge data is represented by closed circles. EAR, early asthmatic response. LAR, late asthmatic response. FEV₁, volume of air forcefully exhaled during the first second of exhalation.
reversible airflow obstruction termed the late asthmatic response (LAR) arbitrarily defined as a
decrease in FEV$_1$ of $\geq$ 15\%. This delayed response develops over the 4 to 5 hours following
resolution of the EAR, is usually maximal at 6 to 7 hours post inhalation, and is associated with
inflammation, notably eosinophil recruitment, increased levels of exhaled nitric oxide, and
increased airway hyperresponsiveness to direct acting stimuli (e.g., methacholine).

### 1.2.2 Early asthmatic response - mechanism of action (Figure 1.2)

The EAR is an immunological Type I hypersensitivity reaction triggered
by the interaction of allergen with allergen specific immunoglobulin E (IgE) which binds with
high affinity to FceRI receptors on mast cells leading to mast cell degranulation and the release
of various newly synthesized lipid derived mediators (e.g., cysteinyl leukotrienes) as well as
preformed mediators (e.g., histamine) the physiological actions of which include
bronchoconstriction, vasodilation, increased vascular permeability and mucus hypersecretion.
IgE mediated mast cell activation also leads to the release of a variety of other mediators that
function in immune regulation and inflammation, some of which are preformed (e.g., tumor
necrosis factor alpha, TNF-\(\alpha\)) and some of which are produced within hours of activation (e.g.,
numerous interleukins, monocyte chemoattractant protein - 1 (MCP-1), macrophage inhibitory
protein – 1\(\alpha\) (MIP-1\(\alpha\)). Activated mast cells are also a source of various cytokines, chemokines
and growth factors which likely contribute to the development of the LAR and airway
inflammation.
Figure 1.2: Schematic of cellular events surrounding the early allergic/asthmatic response. Inhaled allergen will stimulate the release of preformed mediators and lipid derived mediators by crosslinking allergen specific IgE molecules bound to the high affinity IgE receptor (FceRI) on mast cells. Mediators include histamine and the cysteinyl leukotrienes (cys-LT’s) the actions of which include bronchoconstriction, vasodilation, increased vascular permeability and mucus hypersecretion. Mast cell activation also results in the synthesis and release of other mediators (cytokines, chemokines and growth factors) that function in leukocyte recruitment and likely contribute to the late allergic/asthmatic response. Modified from Galli, et al., [2008].
1.2.3 Late asthmatic response - mechanism of action (Figure 1.3)

The mechanism of action of the late asthmatic response is not well defined. Assuredly the response is a concerted action of numerous cells and mediators, some of which likely contribute to the recurrent airway narrowing including the cysteinyl leukotrienes and histamine, some of which propagate the inflammation (e.g., interleukins 4 and 13; IL-4, IL-13) and some of which lead to chronic remodeling (e.g., matrix metalloproteinases, MMP’s, and major basic protein, MBP). Our current understanding is that the LAR is driven by a T helper type 2 (Th2) cell inflammatory response predominantly orchestrated by the effects of Th2 cytokines (i.e., IL-4, IL-5 and IL-13) including the recruitment and maturation of eosinophils as well as B cell immunoglobulin isotype switching and the production of IgE. Recent reports however have dissociated the eosinophil from such a controlling role and we are looking to better understand the regulatory influence of other cells (e.g., basophils and dendritic cells) and other mediators (e.g., histamine, cysteiny1 leukotrienes) on the development of the LAR.
Figure 1.3: Cells and mediators involved in the late allergic/asthmatic response.

Various cells and mediators likely contribute to the LAR. Lymphocytes (notably Th2 lymphocytes), leukocytes (predominantly eosinophils) and the interleukins 4, 5 and 13 (IL-4, IL-5 and IL-13) are strongly implicated in the development of the LAR. CysLT’s, cysteinyll leukotrienes, GMCSF, granulocyte macrophage colony stimulating factor; RANTES, regulated upon activation, normal T cell expressed and secreted; PDGF, platelet derived growth factor; TGFβ, transforming growth factor beta; TNFα, tumour necrosis factor alpha; INFγ, interferon gamma; MCP-1, monocytes chemoattractant protein – 1. Modified from Chung and Adcock [2001].
1.2.4 Sequelae of the late asthmatic response

In addition to the reversible airflow obstruction that occurs in individuals that have an EAR with subsequent LAR, or dual asthmatic response (DAR), we also observe inflammation, changes in airway responsiveness to direct stimuli (e.g., methacholine, histamine) and increases in the level of exhaled nitric oxide. Th2 driven leukocyte recruitment, eosinophils in particular, has been repeatedly documented by various methods including bronchoalveolar lavage (BAL), biopsy and sputum analysis [Beasley et al., 1989; Aalbers et al., 1993; Pin et al., 1992; Fahy et al., 1994]. Cysteinyl leukotrienes have also been shown to increase in sputum following allergen challenge [MacFarlane, et al., 2000]. The method of sputum induction and analysis is now a common procedure included in many allergen inhalation challenge protocols and provides valuable information about cellular events without the invasiveness of lavage or biopsy.

In addition to, or perhaps as a consequence of, inflammation, the airway also becomes hyperresponsive to direct stimuli (e.g., methacholine, histamine) following allergen exposure. Allergen induced increases in airway hyperresponsiveness have been documented as early as 3 hours post exposure [Durham et al., 1988] and may remain for at least 7 days in some individuals [Cockcroft and Murdock, 1987]. The mechanisms leading to the increase in airway hyperresponsiveness are poorly understood and likely multifactorial [Cockcroft and Davis, 2006].

Levels of exhaled nitric oxide (FeNO) have also been shown to increase following allergen exposure [Kharitonov et al, 1995]. Measurement of FeNO is another non-invasive and relatively simple procedure for assessing airway inflammation, eosinophilic inflammation in particular. Limitations of this technique are related to cost, both initially and for general preventative maintenance and the uncertainty surrounding the sensitivity and specificity of the test. Nonetheless, the measurement of FeNO is being incorporated into many clinical research study designs to assess airway inflammation pre and post allergen exposure with and without treatment.
1.2.5 **Summary of airway responses in asthma**

Allergen exposure in individuals with atopic asthma results in the development of an EAR and, in many, the subsequent development of an LAR. In those who exhibit a DAR there is associated recruitment of inflammatory cells, changes in airway responsiveness to direct stimuli and an increase in FeNO, all of which can be induced and assessed under controlled conditions in a research setting which is an attractive model for studying the effects of novel therapeutic interventions.

1.3 **PHARMACOLOGY OF ASTHMA**

1.3.1 **General**

Current guidelines recommend a hierarchy of therapeutic options, the choice of which depends upon the current level of asthma control. The concept of control has largely replaced that of severity since an individual who is well controlled with proper treatment may indeed have severe disease. Similarly, an individual receiving suboptimal treatment (*i.e.*, poorly controlled) may have apparent severe disease; however, an individual who is extremely tolerant of airway obstruction may seem well controlled. It should be appreciated therefore that treatment is not necessarily static, and that periodic re-assessment with both subjective and objective (*i.e.* spirometry) data is required to avoid suboptimal or unnecessary therapeutic intervention. In addition, the heterogeneity and various phenotypes of asthma (*e.g.*, aspirin sensitive, atopic, chronic persistent, seasonal, etc.) make treatment choices and routine re-evaluations rather important. The role of the patient with respect to disease awareness and therapeutic compliance cannot be overstated when assessing the level of asthma control and treatment efficacy.
1.3.2 Available treatments

Most available asthma treatments fall into two broad categories defined as controller medications and rescue medications. Rescue medications provide quick relief of bronchoconstriction and include the $\beta_2$ receptor agonists salbutamol and terbutaline. Controller medications target airway inflammation and include the “gold standard” inhaled glucocorticosteroids (e.g., budesonide and fluticasone), as well as mast cell stabilizers (e.g., cromones), leukotriene receptor antagonists (e.g., montelukast and zafirlukast), 5-lipoxygenase enzyme inhibitors (e.g., zileuton), phosphodiesterase inhibitors (e.g., theophylline) and anti-IgE (omalizumab). More recently, the combination of an inhaled glucocorticosteroid with a long acting $\beta_2$ receptor agonist in a single inhaler has become available (e.g., budesonide/formoterol and fluticasone/salmeterol). Occasionally systemic steroid (i.e., prednisone) and anti-viral therapies may be indicated and annual vaccinations can be beneficial in certain individuals.

In spite of the many currently available treatments which are very effective at controlling and managing asthma in the majority of patients, none have been shown to be disease modifying or curative. Furthermore, excessive use of some treatments, conventional $\beta_2$ agonists for example, may result in an increase in responsiveness to allergen, and in tolerance to its bronchoprotective effects against direct stimuli [Cockcroft et al, 1993] as well as contribute to inflammation [Gordon et al, 2003]. Additionally, high dose or long term chronic dosing with inhaled corticosteroid can increase the risk of undesirable side effects, especially in the young and old [Dahl 2006]. There is therefore an ongoing need to develop alternative therapies which would ideally alter the development, course or manifestation of the disorder and/or decrease the potential untoward effects of the currently available controller and rescue medications.

1.3.3 Investigational therapies

Over the last twenty years anti-asthma treatments have undergone few changes. For the most part, new treatments are the result of modifications of existing therapies or the development of new delivery devices. For example, bronchodilators are now “long
acting” extending the duration of action to twelve hours (e.g., salmeterol and formoterol) instead of the four to six hour duration of the short acting bronchodilators (e.g., salbutamol). Many of the available therapies are delivered via dry powder inhalers as well as the conventional aerosol delivery systems. These patterns of change are continuing as evidenced by recent investigations of “ultra” long acting beta agonists capable of bronchodilating the airway for up to 24 hours [Beeh et al, 2007; Brookman et al, 2007] and bronchoprotecting the airway for up to 32 hours [O’Byrne et al, 2009] that, in some cases, are being delivered by inhaler devices designed to provide superior deposition [Dalby et al, 2004; Watts et al, 2008]. With the exception of ciclesonide, a prodrug design corticosteroid that is converted to its active component in the lung, inhaled anti-inflammatory treatments have seen little progress. The most significant therapeutic development in the last 10 years was the introduction of a new class of drug, the leukotriene modifiers which include the leukotriene receptor antagonists (e.g., montelukast and zafirlukast) as well as the 5-lipoxygenase enzyme inhibitor, zileuton. These agents offer no immediate relief of bronchoconstriction and as such are recognized as controller therapy. Leukotriene receptor antagonists have proven particularly beneficial as add on therapies to currently available bronchodilator or anti-inflammatory treatments when control is suboptimal. Leukotriene receptor antagonists are also useful in controlling exercise induced bronchoconstriction and have shown benefit for those with aspirin induced asthma [Blake, 1999; Currie and McLaughlin, 2006].

Recent research has deviated from the traditional therapeutic strategies by targeting specific mediators, signaling molecules and proteins that are released, activated or up-regulated following allergen exposure [O’Byrne, 2006]. In addition to the release of cysteinyl leukotrienes and histamine, mast cell degranulation results in the release of proteoglycans (e.g., heparin), proteases (e.g., tryptase), non-cysteinyl leukotriene eicosanoids (e.g., PGD₂ and LTB₄) and numerous cytokines (e.g., IL-3, IL-5). The recent assessment of a heparin derivative devoid of anti-coagulant activity failed to produce statistically significant reductions of the EAR, LAR or late sequelae in a proof of concept study in humans. This was perhaps disappointing given the positive pre-clinical data in sheep [Ahmed et al, 2000] but a trend toward a reduction of the response was evident following this single dose investigation suggesting multiple and/or higher doses may be necessary for efficacy [Duong et al, 2008]. An antisense oligonucleotide targeting
CCR3 and the common beta chain of IL-3, IL-5 and GM-CSF were found to protect against the EAR, decrease sputum eosinophilia, and suppress the allergen induced increase in CCR3 and beta chain mRNA [Gauvreau et al, 2008]. An interesting finding from this investigation however was the lack of efficacy on the LAR perhaps providing clinical evidence that eosinophil recruitment may not be a major player in the LAR as initially believed. It is possible however, that the negative results may be related to the dose, dosing sequence, dosing mechanism or biological effect. Investigations of inhaled anti-sense oligonucleotide therapies for the treatment of asthma are in their infancy. Other targets include adhesion molecules that play a role in inflammatory cell recruitment and therefore, at least theoretically, blocking these proteins would suppress the LAR. Efalizumab, an anti-CD11a, IgG1 monoclonal antibody targeting lymphocyte function associated antigen-1 (LFA-1) demonstrated a significant decrease in inflammatory cell recruitment but also failed to inhibit the EAR or the LAR [Gauvreau et al, 2003]. LFA-1 is involved in cellular adhesion and leukocyte recruitment and it was not surprising that a significant decrease in inflammatory cells was achieved. However, the results provide additional clinical evidence that inhibiting inflammatory cell recruitment does not correlate with a reduction in the LAR. Investigations of a nebulized soluble IL-4 receptor (sIL-4R) produced positive data in moderate and/or severe asthma preventing the loss of asthma control during steroid withdrawal [Borish et al, 1999; Borish et al, 2001] and promising preclinical data has shown an IL-4 vaccine, administered prior to ovalbumin sensitization, to be very effective in a murine model of allergic asthma decreasing inflammatory cell influx, preventing the formation of ovalbumin specific IgE and inhibiting the increase in airway responsiveness to methacholine five weeks after vaccination [Ma et al, 2007]. A recombinant human interleukin-4 variant, which competitively inhibits the binding of both IL-4 and IL-13 with IL-4Rα, significantly decreased the LAR and baseline FeNO but had no effect on the allergen induced increase in AHR to methacholine or adenosine monophosphate [Wenzel et al, 2007]. Unfortunately, the effect on inflammatory cell influx was not evaluable. These effects were seen after four weeks of treatment with either 25 mg/day administered subcutaneously or 60 mg/bid via nebulization. The authors reported no change in the average percent fall in FEV1 during the EAR (0 to 2 h post challenge) but did not report on the AUC or maximal decrease in FEV1. Mepolizumab, a humanized anti-IL-5 molecule has been shown to decrease blood and sputum eosinophils following allergen inhalation but failed to inhibit the LAR [Leckie et al, 2000]. Similar results
were obtained with a recombinant human IL-12 molecule [Bryan et al, 2000]. In severe corticosteroid dependent and refractory asthma, etanercept, an anti-TNFα fusion protein, improved asthma control, lung function and airway responsiveness to direct stimuli [Howarth et al, 2005; Berry et al, 2006]. Other monoclonal antibodies targeting IL-9 and IL-13 are in development and undergoing early clinical testing.

The information gained from these investigations challenges our current understanding of the role of inflammation in asthma following allergen exposure in individuals with mild atopic asthma. Specifically, the lack of efficacy on the LAR despite an inhibitory effect on inflammatory cell influx suggests that inflammation may not be as causally related, at least with respect to the LAR, as we currently surmise. In addition to the ever difficult issue of moving from animal to human models, we must consider how the dose, delivery, subject characteristics/disease heterogeneity and method of assessment of these agents undergoing early development might be influencing the outcome before drawing any conclusions.

The reality of bringing these types of drugs to market for routine asthma management seems somewhat unlikely for many reasons including cost and mode of delivery. However, those individuals whose asthma is uncontrolled by currently available treatments may potentially benefit from these types of biological agents.

1.4 PHARMACOLOGY OF THE EAR AND LAR (Table 1.1)

1.4.1 Beta2 agonists

Beta2 agonists (e.g., salbutamol) are bronchodilator agents that relax airway smooth muscle by increasing levels of cAMP. These agents are also referred to as rescue agents or relievers. In the research setting, pre-administration of an inhaled short acting β2 agonist results in an increase in the amount of allergen administered to cause a 20% fall in FEV1 by nearly 4 doubling doses or 16 fold [Cockcroft et al., 1993]. These agents are therefore very effective in blocking the early response and this effect is often described as “functional antagonism”. For this reason, short acting bronchodilators are withheld for their duration of
action prior to EAR investigations and similar rationale pertains to long acting \( \beta_2 \) agonists (e.g., salmeterol). The longer duration of action results in the inhibition of both the early and late responses as well as the associated increase in airway hyperresponsiveness. The main mechanism of the inhibition is believed to be the result of functional antagonism at the level of the airway smooth muscle. However, terbutaline has been shown to shift the dose response curve to adenosine 5’-monophosphate (AMP) nearly five doubling dilutions implicating mast cell stabilization as an additional mechanism [O’Connor et al., 1994]. Clinically, these agents serve to reverse acute bronchoconstriction, induced by allergen exposure or other triggers (e.g., exercise). Similarly, the LAR inhibition seen with long acting \( \beta_2 \) agonist use is likely an apparent inhibition resulting from a masking of the response by functional antagonism rather than a prevention of the response via anti-inflammatory mechanisms.

### 1.4.2 Inhaled glucocorticosteroids

Inhaled glucocorticosteroids (e.g., beclomethasone, fluticasone) also block airway responses to inhaled allergen. Chronic, stable dosing will partially block the early response [Cockcroft et al., 1995] and a single dose administered before [Pepys et al., 1974; Cockcroft and Murdock, 1987] or after [Cockcroft et al., 1993] the EAR will prevent the LAR. Interestingly, the inhibitory effects on the EAR and LAR, afforded by one week of stable dosing are diminished within 12 hours of drug withdrawal [Subbarao et al., 2005]. In isolated early response investigations, inhaled steroids are often administered after the challenge as a safety measure to prevent the LAR from occurring. The majority of protocols require inhaled (or systemic) steroids be withheld for at least four weeks prior to enrollment to avoid confounding the anti-inflammatory efficacy of the agent under investigation. The effects of corticosteroids are potentially numerous and result from the trans-activation (i.e., induction) of anti-inflammatory genes and related proteins or trans-repression (i.e., inhibition) of inflammatory genes and related proteins. The result for example, may be an increase in lipocortins which inhibit the activity of phospholipase A\(_2\) and therefore the production of arachidonic acid metabolites or conversely a decrease in the synthesis of pro-inflammatory mediators such as GM-CSF, IL-4, IL-5 and IL-13. [van der Velden, 1998].
1.4.3 Cromones (Mast cell stabilizers)

Cromones, including sodium cromoglycate and nedocromil sodium, are nonspecific chloride channel blockers that alter the function of many cells including mast cells and eosinophils. As such, these agents effectively suppress the early and late airway responses as well as the increase in allergen-induced airway hyperresponsiveness [Hendeles et al., 1995; Cockcroft and Murdock, 1987]. These agents, although rarely used in Canada, would need to be withheld for their duration of action prior to allergen challenge investigations.

1.4.4 Monoclonal antibodies

To date, only one biologic agent has been approved for use in the treatment of asthma. Omalizumab, a humanized anti-IgE molecule, binds free IgE and prevents the interaction of IgE with its high affinity FceR1 receptor on basophils and mast cells. By blocking the IgE mediated cross-linking of receptors, degranulation and mediator release is prevented. Anti-IgE significantly inhibits the early and the late airway responses as well as the late sequelae [Boulet et al., 1997; Fahy et al., 1997]. Individuals currently treated with anti-IgE therapy would be excluded from allergen challenge investigations for at least two reasons. First, and most important, these individuals would not be well controlled without therapy and second, the treatment would confound the results.

1.4.5 Leukotriene modifiers

These agents include the leukotriene receptor antagonists montelukast, pranlukast and zafirlukast as well as the enzyme (synthesis) inhibitors (e.g., zileuton, Bay x1005 and MK-0591). Montelukast [Diamant et al., 1999; Leigh et al., 2002; Palmqvist et al., 2005], pranlukast [Hamilton et al., 1998] and zafirlukast [Dahlen et al., 1991] have all been shown to partially block both the early and late allergen induced airway responses. Enzyme inhibitors have not evolved as clinically useful treatments in protecting against the EAR and LAR. The leukotriene receptor antagonists are competitive inhibitors of CysLT1 receptors and the enzyme inhibitors block the formation of LTA4 the precursor of LTB4 and the cysteinyl
leukotrienes LTC₄, LTD₄ and LTE₄. These agents must also be withheld prior to allergen challenge investigations.

1.4.6 Histamine H₁ blockers

Antihistamines, more specifically histamine H₁ receptor antagonists have been extensively investigated over the years. These agents produce little, if any, protection against the EAR and the LAR. First generation H₁ receptor antagonists displayed some bronchodilatory properties but produced various side effects due to receptor non-specificity (e.g., antimuscarinic). These agents also readily crossed the blood brain barrier which, in addition to their non-histaminergic effects, discouraged further interest in these drugs as potential therapies in the management of asthma [Holgate and Finnerty, 1989; Simons, 2004]. This was perhaps disappointing in view of the extensive evidence to support a role for histamine as a causative agent of bronchoconstriction [Cockcroft et al., 1977, Boushey et al., 1980] and the similar mechanism of action (i.e., mast cell mediator release) shared by other allergic conditions, such as rhinitis, conjunctivitis and urticaria for which these agents are the drug of choice.

Second generation H₁ antihistamines (e.g., loratadine) do not cause the same untoward effects as first generation antihistamines, and investigations into their potential use in asthma are once again of great interest. Partial inhibition of both early and late airway responses to allergen have been documented with second generation antihistamines [Rafferty et al., 1989; Twentyman et al., 1993; Bentley et al., 1996].
Table 1.1 Effects of various drugs on allergen induced airway responses

<table>
<thead>
<tr>
<th>DRUG TYPE</th>
<th>EFFECT ON EAR</th>
<th>EFFECT ON LAR</th>
<th>EFFECT ON LATE SEQUELAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHORT ACTING β₂ AGONISTS (salbutamol)</td>
<td>INHIBIT</td>
<td>NO CHANGE</td>
<td>NO CHANGE</td>
</tr>
<tr>
<td>LONG ACTING β₂ AGONISTS (salmeterol)</td>
<td>INHIBIT</td>
<td>INHIBIT</td>
<td>NO CHANGE</td>
</tr>
<tr>
<td>REGULAR USE OF SHORT ACTING β₂ AGONIST</td>
<td>AUGMENT</td>
<td>AUGMENT</td>
<td>AUGMENT</td>
</tr>
<tr>
<td>MUSCARINIC ANTAGONIST (ipratropium bromide)</td>
<td>MINOR INHIBITION</td>
<td>MINOR INHIBITION</td>
<td>NO CHANGE</td>
</tr>
<tr>
<td>GLUCOCORTICOSTEROID (beclomethasone dipropionate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SINGLE DOSE AFTER EAR</td>
<td>N/A</td>
<td>INHIBIT</td>
<td>MODERATE ↓ AHR EOS UNKNOWN</td>
</tr>
<tr>
<td>GLUCOCORTICOSTEROID - SINGLE DOSE PRE CHALLENGE</td>
<td>NO CHANGE</td>
<td>INHIBIT</td>
<td>MODERATE INHIBITION</td>
</tr>
<tr>
<td>GLUCOCORTICOSTEROID - CHRONIC STABLE DOSE</td>
<td>MODERATE INHIBITION</td>
<td>INHIBIT</td>
<td>INHIBIT</td>
</tr>
<tr>
<td>THEOPHYLLINE</td>
<td>MINOR INHIBITION</td>
<td>MINOR INHIBITION</td>
<td>NO CHANGE AHR EOS UNKNOWN</td>
</tr>
<tr>
<td>CROMONES (sodium cromoglycate)</td>
<td>MODERATE INHIBITION</td>
<td>MODERATE INHIBITION</td>
<td>MODERATE ↓ AHR EOS UNKNOWN</td>
</tr>
<tr>
<td>ANTI-IGE (omalizumab)</td>
<td>INHIBIT</td>
<td>INHIBIT</td>
<td>INHIBIT</td>
</tr>
<tr>
<td>LTRA (zafirlukast)</td>
<td>MODERATE INHIBITION</td>
<td>MODERATE INHIBITION</td>
<td>MODERATE INHIBITION (BOTH)</td>
</tr>
<tr>
<td>ANTI-HISTAMINE (loratadine)</td>
<td>PARTIAL INHIBITION</td>
<td>VARIABLE INHIBITION</td>
<td>UNKNOWN</td>
</tr>
<tr>
<td>SINGLE DOSE ANTI-HISTAMINE + LTRA</td>
<td>INHIBIT</td>
<td>INHIBIT</td>
<td>TREND TO ↓ AHR ↓ EOS</td>
</tr>
<tr>
<td>(desloratadine + montelukast)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.5 INTRODUCTION SUMMARY

Asthma prevalence is increasing and atopy, the prevalence of which is also increasing [Pawankar et al., 2008], is a contributing factor. The response to allergen exposure in sensitized individuals includes the relatively rapid development of bronchoconstriction, the EAR, and in some, a subsequent episode of bronchoconstriction, the LAR and its associated sequelae of inflammation, increased airway hyperresponsiveness to direct stimuli and increased levels of exhaled nitric oxide. Various therapeutic options are available to relieve bronchoconstriction and control inflammation. Clinical research is focusing on biologics that target various pro-inflammatory molecules or their receptors. These entities, if developed and approved, may benefit a small percentage of individuals with difficult to treat or refractory disease. The leukotriene modifiers are the most recently approved treatment for asthma, and second generation antihistamines, with improved pharmacodynamic and safety profiles, have renewed our interest in the role of histamine and antihistamines in asthma pathogenesis and treatment.

1.6 STUDY RATIONALE

The EAR is a Type I hypersensitivity reaction involving the release of mast cell mediators that include the leukotrienes and histamine. The leukotriene antagonists provide only partial inhibition of the EAR, and minor, variable effects have been shown with first generation antihistamines. Second generation antihistamines are more selective for the histamine H₁ receptor and have fewer side effects than their predecessors which warrant re-investigation of their efficacy in asthma. Inhibition of the EAR should be possible by blocking the effects of mast cell mediators (e.g., histamine and the cysteinyl leukotrienes), and concurrent blockade should be superior to single mediator blockade. We hypothesized that the combination of a leukotriene receptor antagonist (montelukast) with a newer second generation antihistamine (desloratadine) would provide better protection against the EAR by concurrent blockade of the direct effects of histamine and the leukotrienes on their respective airway smooth muscle cell receptors. If this were true, the combination of desloratadine and montelukast may also be effective in blocking the LAR in view of the recent evidence of the anti-inflammatory and immunoregulatory activity of these two therapeutic entities.
2.0 LITERATURE REVIEW

2.1 Histamine

2.1.1 Introduction

Histamine is a biogenic amine first discovered by Sir Henry Dale in the early 1900’s [Holgate and Dahlen, 1997]. The physiologic role of histamine extends over various systems including the central nervous system where it influences wakefulness, motor coordination, memory and learning; the gastrointestinal system where it plays a major role in gastric acid secretion; and the respiratory system where its best known effect is smooth muscle contraction and bronchoconstriction. Histamine also plays a role in immune responses and in the process of inflammation [Schneider et al, 2002; Akdis and Blaser, 2003; Jutel et al, 2006].

2.1.2 Biosynthesis, Storage and Release

Histamine is synthesized from L-histidine by histamine decarboxylase and stored in mast cells and basophils. Histamine is metabolized by N-methyltransferase to N-methyl histamine which is subsequently broken down by monoamine oxidase to N-methyl imidazole acetic acid. An alternative metabolic pathway forms imidazole acetic acid through diamine oxidase activity (Figure 2.1). These metabolites, along with a small percentage of unchanged histamine are excreted in the urine and the levels excreted are sometimes used as a measure of histamine release. There is also evidence that histamine is synthesized “on demand” by cells that have high histamine decarboxylase activity such as dendritic cells and T cells and this characteristic may be important in our understanding of the role of histamine in immune and inflammatory responses.
Figure 2.1 Histamine biosynthesis and metabolism. MAO, monoamine oxidase B
2.1.3 Histamine receptors

Four histamine receptors have now been identified (H\textsubscript{1} through H\textsubscript{4}) all of which are G protein coupled and have various tissue expression. Histamine and histamine metabolites bind to the different receptors with varying affinities. (Table 2.1) The H\textsubscript{1} receptor exists in two states, active and inactive, and expresses constitutive activity. Agonists will signal activation whereas antagonists, or in this case, inverse agonists, stabilize the receptor in an inactive state.

The H\textsubscript{1} receptor is coupled to G\textsubscript{q/11} and the binding of histamine results in the activation of phospholipase C (PLC) and the inositol trisphosphate (IP\textsubscript{3}) diacylglycerol (DAG) intracellular signaling pathway. IP\textsubscript{3} mobilizes intracellular Ca\textsuperscript{2+} resulting in increased kinase activity (e.g., myosin light chain kinase) and protein phosphorylation; DAG stimulates PKC which also leads to protein phosphorylation (e.g., myosin light chain). The overall effect of histamine H\textsubscript{1} receptor activation via this pathway is a reduction in airway caliber and decreased air flow (i.e., bronchoconstriction) as a result of airway smooth muscle contraction. Through this mechanism, the administration of exogenous histamine via inhalation was a useful diagnostic and research tool in respiratory disease before being largely replaced by methacholine, a muscarinic agonist that mimics acetylcholine (i.e., binds to M\textsubscript{2} receptors on airway smooth muscle) and causes bronchoconstriction. The H\textsubscript{1} receptor is expressed on a variety of cells including endothelial, epithelial, leukocytes, lymphocytes, nerve, antigen presenting (e.g., dendritic cells) and smooth muscle.

The H\textsubscript{2} receptor is coupled to the Go\textsubscript{4} protein and signals through the adenylyl cyclase (AC) – cAMP – protein kinase A (PKA) second messenger system. The H\textsubscript{2} receptor shares similar expression patterns with H\textsubscript{1} and its major physiological role, to date, is the stimulation of proton release from parietal cells. H\textsubscript{2} antagonists are widely prescribed for the treatment of gastric ulcers (e.g., ranitidine, cimetidine). There is some evidence that H\textsubscript{2} activation in the lung leads to smooth muscle relaxation but this is not a clinical use.
Histaminergic neurons in the central nervous system express H₃ receptors which regulate histamine synthesis and release through an autoreceptor mechanism. This inhibitory function signals through the Gᵢ/o cAMP pathway. In addition to its autoregulatory function, H₃ receptor activation may also regulate the levels of other neurotransmitters such as serotonin, dopamine and GABA. There is considerable interest in the development of H₃ agonists and antagonists for the treatment of CNS disorders including migraine, obesity, ADHD, Alzheimer’s and epilepsy but there are no currently available treatments.

The most recently described histamine receptor is the H₄ receptor which is highly expressed on leukocytes and in bone marrow. Ligand receptor interactions are coupled to the Gαᵢ/o protein, adenyl cyclase, cAMP pathway. Since the cloning of the gene encoding the human H₄ receptor in 2000, investigations into the role of the H₄ receptor and possible therapeutic implications have received much attention. Our current understanding suggests a major role for histamine acting via the H₄ receptor in immunoregulation and inflammation which implicates agonists or antagonists of the H₄ receptor as possible therapeutic intervention in immune and inflammatory disease. Indeed, early clinical investigations are being conducted in atopic asthma.
<table>
<thead>
<tr>
<th>RECEPTOR SUBTYPE</th>
<th>EXPRESSION</th>
<th>G PROTEIN</th>
<th>PREDOMINANT SIGNALING PATHWAY</th>
<th>PHYSIOLOGICAL EFFECTS</th>
<th>ANTAGONISTS</th>
<th>pKa</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₁</td>
<td>Smooth muscle Endothelial cells CNS</td>
<td>$G_{q/11}$</td>
<td>↑ PLC-IP$_3$/DAG ↑ Ca$^{2+}$</td>
<td>Bronchoconstriction Vascular leakage</td>
<td>Chlorpheniramine (DESLORATADINE)</td>
<td>4.2</td>
</tr>
<tr>
<td>H₂</td>
<td>GI tract Cardiac muscle Mast cells CNS</td>
<td>$G_s$</td>
<td>↑ AC-cAMP-PKA</td>
<td>Gastric acid secretion</td>
<td>Ranitidine</td>
<td>4.3</td>
</tr>
<tr>
<td>H₃</td>
<td>CNS</td>
<td>$G_{i/o}$</td>
<td>↓ AC-cAMP</td>
<td>Autoregulation of presynaptic neurotransmitter release</td>
<td>Thioperamide Clobenpropit</td>
<td>7.8</td>
</tr>
<tr>
<td>H₄</td>
<td>Hematopoietic Cells</td>
<td>$G_{i/o}$</td>
<td>↓ cAMP ↑ Ca$^{2+}$</td>
<td>Immunoregulation Anti-inflammatory</td>
<td>Thioperamide</td>
<td>8.4</td>
</tr>
</tbody>
</table>
2.1.4 Histamine H_{1} receptor antagonists

The effects of inhaled histamine are inhibited by the administration of selective H_{1} blockers. This has been shown to be agent specific in that the extent of inhibition varies with the drug. The greatest inhibition (> 3 doubling doses) is afforded by the second generation antihistamine cetirizine and the least by the first generation antihistamine chlorpheniramine [Wood-Baker and Holgate, 1993]. These differences are probably due to differences in receptor selectivity (i.e., additional anticholinergic, antiserotonergic and antiadrenergic activity). As yet however, antihistamines have found limited clinical use in the treatment of atopic asthma despite the theoretical rationale based on the action of histamine in the acute response following allergen exposure. The minimal, at best, inhibitory effect on allergen-induced airway responses following antihistamine administration supports the rationale that blocking more than one mediator may be required to inhibit a response that results from the action of many mediators. Somewhat puzzling perhaps is that H_{1} blockers are the drug of choice in the treatment of allergic rhinitis and chronic idiopathic urticaria, conditions which share similar pathophysiology with atopic asthma but, unlike atopic asthma, respond relatively well to these agents. Individuals who have concomitant hay fever and asthma have been shown to benefit from anti-histamine treatment. Whether this is a mechanistic phenomenon occurring in the lung as well as the nasal passage or a physiologic reaction whereby treatment of a condition of the upper airway benefits that of the lower airway has not been elucidated but is being investigated [Hellings and Ceuppens, 2004; Jeffrey and Haahtela, 2006].
2.2 Desloratadine

2.2.1 General Information

Desloratadine is the most potent active metabolite of the second generation antihistamine loratadine. Desloratadine was approved by the United States Food and Drug Administration (FDA) in late 2001 for the treatment of allergic rhinitis, conjunctivitis and chronic idiopathic urticaria (CIU) in both children and adults. Premarketing clinical trials and Phase IV clinical trial (i.e., postmarketing) data have established that the drug is safe and well tolerated. Classical undesirable effects of first generation antihistamines (e.g., impaired motor function and altered wakefulness) have not been observed, there is no effect on cardiovascular function, and no concerns regarding drug-food or drug-drug interactions. The recommended dose for adults is 5 mg once a day.

2.2.2 Mechanism of action and PK/PD properties

Desloratadine is a tricyclic selective histamine H₁ receptor antagonist which binds with high affinity to the histamine H₁ receptor (Figure 2.2). Following oral administration plasma concentrations are maximal at about 3 hours and, on average, 85% of the drug is bound to plasma proteins. The half life is approximately 27 hours. Desloratadine undergoes hydroxylation and glucuronidation and its metabolites are excreted in both urine and feces.

![Chemical structure of desloratadine.](image)

Figure 2.2: Chemical structure of desloratadine.
2.2.3 Effects of desloratadine in allergic rhinitis and chronic idiopathic urticaria

The beneficial effects of once daily dosing with desloratadine in allergic rhinitis and chronic idiopathic urticaria (CIU) have been well documented [reviewed by Murdoch et al., 2003]. Significant improvements in total symptom scores, nasal discharge, sneezing and pruritis for example are consistent findings. Decreases in asthma symptom scores and in rescue medication use have also been shown in allergic rhinitis with concomitant asthma, and improvements in FEV$_1$ occur in individuals with resting obstruction (i.e., baseline FEV$_1$ ≤ 80% predicted). Most studies have looked at dosing once a day for ≥ 4 weeks but improvements have been noted for some variables after a single dose and as early as 12 hours. These effects are superior to those seen with other second generation antihistamines such as fexofenadine.

2.2.4 Effects of desloratadine on immune and inflammatory responses

Of significant interest are the emerging data on the potential anti-inflammatory and immunomodulatory effects of anti-histamines, desloratadine in particular. An ex vivo analysis of the effect of desloratadine on IgE mediated and non-IgE mediated LTC$_4$, tryptase and ECP release in nasal polyp tissue from 22 subjects with chronic rhinosinusitis was recently reported [Kowalski et al., 2005]. The authors concluded that the H$_1$ blocker significantly decreased the release of tryptase following Ca$^{2+}$ ionophore and anti-IgE stimulation. The same effect was seen with respect to LTC$_4$ release however inhibition of the IgE mediated release was only observed following the 10 μg/mL stimulation and not the 1 or 100 μg/mL stimulation. Non IgE mediated stimulation of eosinophil cationic protein (ECP) release was reduced by almost 60 % following desloratadine pretreatment. In another ex vivo investigation, Schroeder et al., [2001] looked at the effect of fifteen minutes of pretreatment with desloratadine on histamine, LTC$_4$, IL-4 and IL-13 release from basophils activated by a variety of stimuli. They were able to show a dose dependent inhibition of IL-4 and IL-13 release that was superior to that of LTC$_4$ and histamine release regardless of whether basophil stimulation occurred via IgE dependent or IgE independent mechanisms. At the highest dose of desloratadine (10 μM) IL-4 secretion was inhibited by approximately 80 % whereas that of histamine and LTC$_4$ was
about 60%. The effect on IL-13 release was similar (i.e., approximately 80% inhibition at 10 μM). The authors also showed that the reduction in the amount of IL-4 released may be due to the inhibition of IL-4 mRNA. This was also seen in COS-7 cells where desloratadine, and other H₁ antagonists, inhibited both basal and histamine induced NFκB activity suggesting negative regulation of gene transcription of pro-inflammatory mediators as a possible mechanism [Wu et al., 2004]. Another ex vivo investigation using FcεR₁ cells from peripheral blood, skin and lung tissue (i.e., mast cells and basophils) confirmed the inhibitory effect of desloratadine on histamine, tryptase, LTC₄ and PGD₂ release from these cells [Genovese et al., 1997]. A unique fluorescent methodology using the mast cell engulfing agent sulforhodamine-B (SFRM-B) suggests desloratadine may be exerting its effects through mast cell stabilization [Wang et al., 2005]. Two additional investigations in Balb/c mice have implicated histamine as an important signal during the sensitization phase to allergen as well as at the time of subsequent exposure. Both studies showed that desloratadine decreased Th₂ responses when administered at either time point as evidenced by suppression of IL-4, IL-5 or IL-13 [Bryce et al., 2003; Blumchen et al., 2004].

A recent double blind parallel group investigation in a cohort with rhinitis and asthma compared the efficacy of eight days of 5 mg per day desloratadine with placebo on the response to nasal provocation in 26 grass pollen sensitive individuals. Desloratadine was shown to improve baseline peak nasal inspiratory flow and decrease the number of circulating eosinophils following treatment but prior to nasal challenge [Reinartz et al., 2005]. This suggests a lack of protective efficacy to events occurring after exposure but a beneficial effect on underlying inflammation (i.e., an improvement in baseline nasal airflow obstruction).

These recent investigations provide evidence to suggest that desloratadine may be an effective therapeutic agent in the treatment of airway responses to inhaled allergen by inhibiting the release of bronchoconstricting (e.g., LTC₄ and histamine) and pro-inflammatory (i.e., IL-4, IL-5 and IL13) mediators from cells that are known to contribute to the pathophysiology of atopic asthma and the airway response to allergen exposure (i.e., mast cells and basophils).
2.3 Cysteinyl Leukotrienes

2.3.1 Introduction

In 1938, Charles Kellaway and William Siegmund Feldberg were the first to describe a substance that was produced following antigen challenge that, like histamine, caused smooth muscle contraction. The response however was slower in onset and longer in duration. They termed the substance “slow reacting substance; SRS”. Shortly afterward, experiments by Walter Edwin Brocklehurst led to the terminology “slow reacting substance of anaphylaxis; SRS-A” and finally, in the early 1980’s, it was realized that SRS-A was actually LTC₄, LTD₄ and LTE₄ – the cysteinyl leukotrienes [Holgate and Dahlen, 1997].

2.3.2 Biosynthesis (Figure 2.3)

The cysteiny1 leukotrienes are products of arachidonic acid metabolism. Arachidonic acid is a component of membrane phospholipids of many cell types, including those that play a major role in atopic asthma (e.g., mast cells, eosinophils, basophils). Enzymatic cleavage by phospholipase A₂ releases arachidonic acid from the perinuclear membrane which serves as a substrate for 5 lipooxygenase in the formation of LTA₄ and 5-hydroperoxyeicosatetraenoic acid (5-HPETE). LTA₄ undergoes conjugation with glutathione by LTC₄ synthase to form LTC₄ which is actively transported out of the cell where further enzymatic action forms LTD₄ and subsequently LTE₄. Native LTE₄ and various degradation products can be found in the urine and have been used, for example, as a measure of IgE mediated mast cell degranulation.
Enzymes are shown in blue, products in yellow, essential cofactor in green, and drugs in red. Although the synthesis of leukotrienes B and C probably takes place in close proximity to the nuclear membrane, for clarity they are shown throughout the cytosol. BLT denotes the B leukotriene receptor. An individual cell may produce the cysteiny1 leukotrienes, leukotriene B, or in rare cases both. Reproduced with permission from Drazen et al., [1999]. Copyright © 1999 Massachusetts Medical Society. All rights reserved.
2.3.3 Leukotriene receptors

Two cysteinyl leukotriene receptors, (CysLT\textsubscript{1}, CysLT\textsubscript{2}) have been identified and evidence supporting the existence of a third is emerging. The cysteinyl leukotrienes bind to CysLT\textsubscript{1} and CysLT\textsubscript{2} with varying affinities. LTD\textsubscript{4} > LTC\textsubscript{4} > LTE\textsubscript{4} and LTC\textsubscript{4} = LTD\textsubscript{4} > LTE\textsubscript{4} for CysLT\textsubscript{1} and CysLT\textsubscript{2} respectively. Both receptors are differentially expressed on various cell types and tissues including bronchial smooth muscle, monocytes/macrophages, mast cells, eosinophils and basophils. Their expression has been shown to be upregulated in the presence of various cytokines, including Th\textsubscript{2} generated IL-4, IL-5 and IL-13. The CysLT receptors, like the histamine receptors are coupled to G proteins. CysLT\textsubscript{1} is coupled to Gq/11 and Gi/o and CysLT\textsubscript{2} to Gq/11. Little is understood about subsequent signal transduction mechanisms.

2.3.4 Leukotriene modifiers

2.3.4.1 Enzyme inhibitors

While various enzyme inhibitors have been studied for efficacy in asthma, only one, zileuton, is currently available. Zileuton inhibits 5-lipoxygenase and prevents the synthesis of leukotriene A\textsubscript{4} from arachidonic acid. In the absence of LTA\textsubscript{4} subsequent downstream products for which LTA\textsubscript{4} is a substrate are also downregulated, namely LTB\textsubscript{4} and the cysteinyl leukotrienes LTC\textsubscript{4}, LTD\textsubscript{4} and LTE\textsubscript{4}. There were two identifiable reports in the literature that looked at the effect of zileuton on the response to allergen challenge in atopic asthma. The first looked at a single dose administered 3 hours prior to allergen challenge in nine male atopic asthmatics. The investigators found that 800mg of zileuton failed to significantly alter the EAR, LAR or allergen induced increase in airway responsiveness to methacholine 24 hours post allergen challenge [Hui \textit{et al.}, 1991]. The negative results may have been due to the small sample size or, as shown by Hasday \textit{et al.}, [2000] the subjects studied could all have been low leukotriene producers. In the Hasday paper, there was a significant difference in response to zileuton which was dependent on the magnitude of the increase in leukotrienes produced following allergen challenge. High leukotriene producers had a better
response to zileuton than low producers. Zileuton has been shown to be beneficial with respect to asthma control and exercise induced asthma [Israel et al., 1996; Meltzer et al., 1996].

2.3.4.2 Leukotriene receptor antagonists

Antagonists of cysteinyl leukotriene receptors have quickly been recognized as effective therapeutic agents in asthma management. As with most drug classes, individual drugs vary with respect to potency, selectivity, and safety profile. In that regard, montelukast, as opposed to pranlukast or zafirlukast, has emerged as the frontrunner in this class.

2.4 Montelukast

2.4.1 General Information

In Canada, two leukotriene receptor antagonists are available, montelukast which received approval from Health Canada in 1998, and zafirlukast which received approval in 1997. Both drugs are indicated for use in asthma and have been shown to improve various parameters of lung function such as FEV$_1$ and peak expiratory flow rate (PEFR), as well as decrease the need for rescue medication (e.g., $\beta_2$ agonist), and lower the required dose of inhaled steroid. Montelukast is also indicated for use in aspirin sensitive asthmatics, can be used in individuals who experience exercise induced bronchoconstriction and is effective in treating seasonal allergic rhinitis. Montelukast is well tolerated and has a superior safety profile compared to zafirlukast. The recommended dose for adults is 10 mg once per day.

2.4.2 Mechanism of action and PK/PD properties

Montelukast is a competitive antagonist at the CysLT$_1$ receptor inhibiting the physiological responses normally produced by the interaction of the cysteinyi leukotrienes (LTC$_4$, LTD$_4$ and LTE$_4$) with the CysLT$_1$ receptor. The structure of montelukast is provided in figure 2.4. Peak plasma concentrations occur at approximately 3 hours, the drug is highly bound to plasma proteins (>99 %) and has a half life ranging from 2.7 to 5.5 hours. Montelukast is
extensively metabolized by the cytochrome P450 enzymes CYP 3A4 and 2C9 and is excreted mainly in the bile.

Figure 2.4 Chemical structure of montelukast.

2.4.3 Effects of montelukast in asthma

Current guidelines (GINA 2008) now include leukotriene modifiers as add on therapies in the treatment of asthma in individuals who are poorly controlled on beta agonist monotherapy. Similarly, individuals already on rescue bronchodilator and low dose ICS may increase the dose of ICS or add an LTRA to gain control. These recommendations follow evidence of reduced bronchodilator use and improvement in spirometric parameters (e.g., FEV₁, PEFR), in symptom scores, and in quality of life following LTRA treatment [reviewed by Blake, 1999].

2.4.4 Effects of montelukast on immune and inflammatory responses

The role of LTRA’s as modulators of immune and inflammatory responses is an area of considerable interest. The clinical benefits of montelukast on allergic rhinitis and on the various asthma phenotypes (e.g., chronic persistent, allergen induced, aspirin sensitive etc.) in combination with the inflammatory components of these conditions suggest that leukotrienes, and therefore, leukotriene receptor antagonists are involved in immune and
inflammatory pathways. As with desloratadine, much of our current understanding of the role of montelukast in regulating these responses comes from animal and ex vivo data.

In ovalbumin (OVA) sensitized Brown Norway rats, montelukast administered pre-challenge, was shown to significantly decrease eosinophil recruitment and the number of IL-5 positive cells in both bronchoalveolar lavage (BAL) fluid and lung tissue [Ihaku et al, 1999]. Pretreatment with montelukast for fifteen minutes inhibits platelet activating factor (PAF) induced eosinophil transmigration in human umbilical vein endothelial cells (HUVEC) whether stimulated with granulomonocyte colony stimulating factor (GM-CSF) and/or interleukin 13 (IL-13) or not [Virchow et al., 2001]. In a chronic model of inflammation, OVA sensitized Balb/c mice periodically exposed to OVA over 61 days, montelukast significantly decreased eosinophilia, mucus plugging, smooth muscle hyperplasia and sub-epithelial fibrosis with additional inhibitory effects on the levels of IL-4 and IL-13 [Henderson et al, 2002]. Again in OVA sensitized Balb/c mice (all male), montelukast (3 mg/kg and 10 mg/kg) was shown to inhibit airway eosinophilia and airway hyperresponsiveness (AHR) post challenge. The authors also documented lower levels of IL-5 post challenge in the 10 mg/kg group but not in the 3 mg/kg group and neither group altered eotaxin levels [Eum et al., 2003]. In keeping with high dose effects, 25 mg/kg montelukast in the Balb/c mouse model significantly decreased IL-4, 5, 13 and VCAM-1 in the lung, IL-4 and 5 in BAL, IL-5 and IgE in serum, and BAL eosinophil counts. High dose montelukast did not alter eotaxin levels or eotaxin mRNA expression in this model [Wu et al., 2003]. Conversely, however, both montelukast and pranlukast have been shown to block eotaxin production from human fetal lung fibroblasts primed with IL-13 and activated by LTC₄ [Chibana et al., 2003]. In a murine model of chronic asthma (C57BL/6), 6 mg/kg montelukast for 20 days significantly decreased BAL and tissue eosinophilia, suppressed the allergen induced increase in BAL IL-5 and decreased CysLT₁ receptor expression [Zhang et al., 2004]. T cells selected from healthy and atopic donors have low levels of CysLT receptor expression which, for both CysLT₁ and CysLT₂ receptors, is increased following anti-CD3⁺ stimulation, a response that is blocked by pretreatment with montelukast resulting in T cell death possibly through the up-regulation of apoptotic genes such as p53 and down-regulation of proliferative genes such as Bcl-2 [Spinozzi et al., 2004]. Montelukast may also exert its effects via the downregulation of LPS induced pro-inflammatory mediators such as IL-6, TNF-α and
MCP-1 through the inhibition of NFκB activation as has been shown in THP-1 cells [Maeba et al., 2005]. A recent ex vivo investigation documented an upregulation of CysLT1 receptor expression on B lymphocytes following exposure to combined anti-CD40 and IL-4 which resulted in a subsequent increase in responsiveness to LTD4 as evidenced by elevated intracellular calcium concentrations which ultimately resulted in greater IgE and IgG production and was shown to be completely blocked by montelukast [Lamoureaux et al., 2006]. Eosinophils from healthy donors incubated in the presence of montelukast show a reduction in binding to intercellular adhesion molecule 1 (ICAM-1) in response to LTD4 and a decrease in adhesion in response to IL-5 [Kushiya et al., 2006]. In OVA sensitized guinea pigs, 0.9 mg/kg and 3 mg/kg montelukast administered intragastrically 2 h prior to allergen challenge prevented the decrease in IL-10 and inhibited the increase in NFκB which may explain the documented decrease in both blood and BAL eosinophils at these same doses. A lower dose of 0.3 mg/kg was ineffective on these same parameters [Wu et al., 2006]. Another recent investigation in C57BL/6 showed an inhibitory effect on allergen induced increases in both IL-11 protein and mRNA expression following the administration of montelukast (5 mg/kg/day x 10 days). Similar effects were shown for NFκB [Lee et al., 2007].

Importantly, human model investigations have also documented the anti-inflammatory effects of montelukast. In a four week parallel study of once daily montelukast versus placebo, both sputum and peripheral blood eosinophil counts were significantly decreased in adults with chronic asthma [Pizzichini et al., 1999]. This was reproduced later, in a separate 4 week investigation of once daily montelukast, in mild to moderate asthma. [Minoguchi et al., 2002]. In a 6 week double blind, parallel group study of children aged 6-18 years, montelukast 5 mg/day or 10 mg/day, depending on age, significantly improved symptom scores and percent predicted FEV1 and decreased levels of IL-4, sICAM-1, ECP and peripheral eosinophil counts [Stelmach et al., 2002]. This same group also reported increased IL-10 following 4 weeks of once daily montelukast (5mg/day or 10mg/day) in children aged 4-16 years. This effect was again shown by these authors using peripheral blood mononuclear cells (PBMC) from monoallergic grass pollen and monoallergic dust mite sensitive atopic asthmatics. The effect in the monoallergic grass pollen group was observed only when testing occurred during the grass
pollen season and only when the PBMC were stimulated with the sensitizing allergen [Stelmach et al, 2005].

As discussed in Section 1.4.5 leukotriene receptor antagonists, including montelukast, have proven efficacious in blocking both the EAR and the LAR. The data from the inflammatory and immunomodulatory investigations set out above suggests that the clinical benefit, with respect to the LAR in particular, results from actions that extend beyond direct cysLT₁ receptor antagonism on airway smooth muscle cells and the inhibition of eosinophil recruitment and may result from the down regulation of Th₂ cytokines (i.e., IL-4, IL-5, IL-13) possibly through the inhibition of NFκB.

2.5 Investigations of anti-leukotriene and anti-histamine treatment as monotherapy and combination therapy

2.5.1 In vitro

Reports of tissue investigations into the effect of the combination of an anti-histamine and a leukotriene antagonist on antigen induced contractility date back at least thirty years when it was shown that pretreatment of guinea pig tracheal tissue with diphenhydramine plus FPL-55712, a leukotriene antagonist, decreased antigen induced contractility better than either drug administered alone (Adams and Lichtenstein, 1979). This study also showed that histamine release was initiated within minutes following exposure, was maximal at 10 minutes and produced a response that lasted, on average, 81 minutes. Pretreatment with diphenhydramine was shown to suppress the initial phase of the response. In human bronchial tissue the response was somewhat similar. Maximal histamine release occurred at 20 minutes and produced a prolonged and sustained contraction that, on average, lasted 140 minutes. Diphenhydramine pretreatment was also effective in inhibiting the initial response. In guinea pig, FPL-55712 pretreatment dose dependently decreased the duration of the response but had no effect on the development of the response. In human tissue, if FPL-55712 was administered after the response was initiated, the tissue relaxed and recovery to baseline tension occurred earlier. This was not evident after treatment with anti-histamine. These observations
led to the conclusion that both histamine and the leukotrienes were involved in the contractile response to antigen of guinea pig tracheal tissue and human bronchial tissue. Furthermore, it appeared that histamine was responsible for initiating the response (i.e., contraction) whereas the leukotrienes were predominantly responsible for sustaining the contraction.

Years later, as newer leukotriene receptor antagonists were developed, additional studies again showed, in both guinea pig and human tissues, that the combination of an LTRA (SK&F 104353) with an H\textsubscript{1} blocker (SK&F 93944 a.k.a temalastine or mepyramine) was more effective in blocking antigen induced contractility than either agent alone (Hay et al., 1987). As was documented by Adams and Lichtenstein [1979], the LTRA SK&F 104353 did not affect the initiation of the contraction but partially inhibited the duration of the contraction. Both temalastine and mepyramine inhibited the initial phase of the response in guinea pig tissue but, unlike the earlier study, had no effect on the initiation of the contraction in human bronchial tissue.

The first investigation of the effect of combining anti-leukotrienes with anti-histamines to inhibit IgE stimulated contractions of human bronchi, in the absence of other potential inhibitors (e.g., meclofenamic acid) came from researchers at the Karolinska Institute in Stockholm, Sweden (Bjorck and Dahlen, 1993). This \textit{in vitro} investigation used normal bronchial tissue obtained from individuals undergoing surgery for pulmonary carcinoma and included tissue from both non-asthmatic as well as asthmatic patients. Bronchial strips were placed in organ baths and subjected to increasing concentrations of anti-IgE in the presence and absence of various compounds including an H\textsubscript{1} receptor antagonist (mepyramine) and an H\textsubscript{2} receptor antagonist (metiamide), three leukotriene receptor antagonists (L-648,051, ICI 198,615 and SKF 104353), two leukotriene synthesis inhibitors (MK886 and U-60,257 (a.k.a piriprost)), a thromboxane agonist (U-44,069) and the platelet activating factor antagonist WEB 2086. Their work resulted in numerous observations regarding the effects of these agents on bronchial contractility. First, bronchial relaxation following IgE mediated contraction is minimal with the addition of anti-histamines (H\textsubscript{1} in combination with H\textsubscript{2}) but can reach 80% when pretreated with an LTRA (60% for L-648,051 and 80% for SKF 104353). Combining both histamine (H\textsubscript{1} and H\textsubscript{2}) blockers with L-648,051 resulted in a 100% relaxation after 21 minutes. Second,
pretreatment with the various agents produced a variety of responses ranging from no effect with the combination of mepyramine and metiamide to complete inhibition of IgE mediated bronchial contraction when the anti-histamines were combined with the leukotriene antagonist ICI 198,615 (Table 2.2).

Table 2.2: *In vitro* effects of various drugs and drug combinations on bronchial contractility.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>MEP + MET</th>
<th>L-648,051</th>
<th>FPL 55712</th>
<th>U-60,257</th>
<th>MK-886</th>
<th>Indomethacin</th>
<th>ICI 198,615</th>
<th>ICI 198,615 + MEP + MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFFECT</td>
<td>No effect</td>
<td>~ 50% inhibition</td>
<td>~ 50% inhibition</td>
<td>~ 50% inhibition</td>
<td>~ 70% inhibition</td>
<td>No effect</td>
<td>~ 65% inhibition</td>
<td>Complete inhibition</td>
</tr>
</tbody>
</table>

MEP = mepyramine; MET = metiamide

Third, the Schultz-Dale response (*i.e.*, IgE mediated antigen induced contraction) in tissue from atopic asthmatics (n=2) was completely inhibited when pretreated with ICI 198,615 in combination with the H₁ antagonist mepyramine providing evidence to suggest that blocking the effects of leukotrienes and histamine in atopic asthma should be a useful therapeutic strategy.

A more recent *in vitro* investigation by Ruck *et al* [2001] using a different LTRA (MK-571) and different anti-histamine (chlorpheniramine) supported the work by Bjorck and Dahlen. Cryopreserved human bronchial tissue isolated from lungs donated to the International Institute for the Advancement of Medicine (Scranton, PA, USA) was pretreated for 30 min with either MK-571, chlorpheniramine or the combination and then exposed to anti-human IgE antibody. The combination produced a synergistic (87 %) inhibition of the contraction whereas inhibition with monotherapy was only 15 % following chlorpheniramine treatment and 36 % following MK-571 treatment. Additionally, MK-571 did not affect histamine stimulation, and the anti-histamine did not affect LTD₄ stimulated contractions. Furthermore, neither monotherapy or the combination had any influence on histamine release from passively sensitized human umbilical cord blood mast cells.
2.5.2 *In vivo*

In spite of the positive preclinical *in vitro* data on the effect of combining an H₁ blocker with a leukotriene receptor antagonist on airway contractility, there is relatively little in the literature investigating the clinical effects of such a combination in individuals with atopic asthma. In fact, some of the early pre-market clinical investigations of leukotriene antagonists showed little potential as therapeutic agents in the management of atopic asthma. L-649,923 was one of the first compounds to be clinically investigated as an LTD₄ receptor antagonist. In normal subjects, L-649,923 shifted, to the right, the dose response curve to exogenous LTD₄ 3.8 fold [Barnes *et al.*, 1987]. However, in atopic asthmatics with dual responses to allergen, L-649,923 failed to show any effect on the LAR and produced only a small improvement in the decrease in FEV₁ over the course of the EAR [Britton *et al.*, 1987]. These observations suggest either that L-649,923 is not potent enough to block the effects of endogenous LTD₄, or that LTD₄ is not an integral mediator in the airway response to allergen exposure in atopic asthma. A few years later, a similar compound, L-648,051 administered by inhalation to healthy males, was shown to partially inhibit LTD₄ induced bronchoconstriction and to improve recovery time from bronchoconstriction [Evans *et al.*, 1989]. In atopic asthma patients however, it had a minimal effect on the early response, no effect on the late response and produced no improvement with respect to recovery from antigen induced bronchoconstriction [Rasmussen *et al.*, 1991]. The authors concluded that LTD₄ was involved in the manifestation of the EAR. Despite some of the early equivocal results, a number of LTRA compounds were in development at that time. These included ICI-204,219 (zafirlukast), SR2640, SK&F 104353, MK-571, RG 12525, ONO-1078 (pranlukast), MK-679, Bay x 7195, MK-476 (montelukast), Ro 245913 (cinalukast), and clinical trials were being carried out to assess efficacy, safety and tolerability in a variety of indications including asthma, atopic asthma, exercise induced bronchoconstriction (EIB) and aspirin induced asthma. Trials with ICI-204,219 provided the first solid clinical evidence of the ability of an oral LTRA to alter the airway response to inhaled allergen. The first of three investigations of ICI-204,219 was a placebo controlled crossover design that looked at the effect of a single 40mg dose of ICI-204,219 administered orally 2 hours prior to allergen challenge in 10 asthmatic subjects with allergen induced asthma. Both the EAR (0-2h) and LAR (2-6h) were significantly suppressed following active treatment and the results
were published in the Lancet in 1991. [Taylor et al, 1991]. The second investigation confirmed significant inhibition of the airway responses to inhaled allergen following a single 40mg dose of ICI 204,219 [Findlay et al, 1992] and the third study documented the inhibitory effects of ICI-204,219 on the EAR following a single administration of half the previous dose (i.e., 20mg) [Dahlen et al, 1994]. Similar results were subsequently documented for other agents in this class, including Bay x 7195, pranlukast and montelukast [Boulet et, 1997; Hamilton et al, 1998; Diamant et al, 1999]. We can conclude from these investigations that LTRA’s are efficacious in blocking the airway response to inhaled allergen, but the inhibition is suboptimal, approximately 75 % for the EAR and approximately 50 % for the LAR.

The clinical effects of antihistamine monotherapy on airway responses to inhaled allergen are not so clear. First generation histamine H\textsubscript{1} receptor antagonists such as clemastine (1 mg) and ketotifen (2 mg) have failed to alter the airway response to inhaled allergen in vivo [Cockcroft et al, 1992]. Conversely however, azelastine has been shown to inhibit the EAR FEV\textsubscript{1} AUC by 32.5 % and the LAR FEV\textsubscript{1} AUC by 70.2 % [Rafferty et al, 1989]. Terfenadine has also been shown to inhibit both the mean maximal fall in FEV\textsubscript{1} during the EAR (from 33.2 to 20.3 %) and the mean maximal fall in the peak expiratory flow rate (PEFR) during the LAR (from 22.6 to 15.2 %) [Hamid et al, 1990]. The azelastine and terfenadine investigations may however be limited in that the sample sizes of 5 and 7 are small and the overall power of the study (< 70 %) is less than the generally accepted level of 80 % or higher. It is also unclear, with respect to the terfenadine study, why the LAR would be reported as a change in PEFR versus FEV\textsubscript{1} which is commonly used and, interestingly, was used for the analysis of the EAR. It is worth noting that terfenadine was withdrawn from the market due to cardiac toxicity and replaced by its metabolite fexofenadine. Although not studied in humans, there are data to suggest that fexofenadine modulates T cell function in a murine model of allergen induced airway inflammation and hyperresponsiveness [Gelfand et al, 2002; Gelfand et al, 2003]. Also worth noting is that the inhibitory effect of azelastine on the EAR was later confirmed in a randomized, placebo controlled, double blind study in ten subjects with atopic asthma pretreated for 4 days with azelastine [Twentyman et al, 1993]. Soon after however, the second generation anti-histamine, loratadine, was reported to have no significant effect on either the EAR or the LAR following once daily administration for 3 days [Town and Holgate, 1990].
Negative results have also been shown following oral or inhaled cetirizine [Rafferty et al, 1993] which were later confirmed following 18 days of 30 mg per day cetirizine [de Bruin-Weller et al, 1994].

We can conclude from the literature that from 1979 through 1994 there were limited clinical investigations that produced equivocal results of the effects of CysLT$_1$ and H$_1$ antagonism on the early and late airway responses to inhaled allergen in individuals with atopic asthma. Drug development with both classes has subsequently produced potent and selective compounds that are effective in asthma (e.g., zafirlukast and montelukast) and allergic rhinitis (e.g., loratadine, desloratadine) which may have been the impetus, together with the relatively strong theoretical basis and preclinical data of these newer agents, to revisit the hypothesis of a superior efficacy in the combined treatment of atopic asthma.

The first study to re-examine this hypothesis documented a 74 % reduction in the maximal fall in FEV$_1$ during the EAR and a 48 % reduction in the maximal fall in FEV$_1$ during the LAR following one week of high dose combination therapy using 80 mg bid zafirlukast and 10 mg bid loratadine [Roquet et al, 1997]. Expressed as FEV$_1$ AUC the combination produced an inhibitory effect on the EAR of 75 % and on the LAR of 74 %. The changes for all active treatments, whether expressed as the maximal fall in FEV$_1$ or FEV$_1$ AUC were all significantly different compared to control challenges. Of particular interest however are the comparative results between the monotherapies and the combination. Regardless of the parameter used for assessment, the combination was significantly better than either single treatment during the LAR, but did not produce greater inhibition than that of zafirlukast during the EAR. This was the first clinical evidence to compare the effects of an antihistamine with that of a leukotriene receptor antagonist and assess combined mediator blockade on the response to inhaled allergen in a bronchoprovocation model. The lack of superior combined efficacy on the EAR as well as the significantly different inhibition with combined therapy on the LAR would not have been expected. Surprisingly, in the last eleven years, only one subsequent investigation of combined antihistamine, antileukotriene treatment on the allergen induced EAR and LAR could be identified [Richter et al, 2008]. The authors showed that the combination of one week of clinically recommended doses of azelastine (4 mg bid) and montelukast (10 mg qd)
significantly decreased the maximum fall in FEV\textsubscript{1} during the EAR and LAR compared to either therapy alone. Furthermore, both montelukast and azelastine significantly decreased the EAR and montelukast was superior to azelastine in that regard. Azelastine and montelukast also decreased the LAR but there was no difference between the two. These results are similar to what was shown in the Roquet \textit{et al.}, [1997] study providing further evidence that these types of drugs provide superior efficacy when administered in combination.
3.0 STATEMENT OF OBJECTIVES AND HYPOTHESES

3.1 Early Asthmatic Response Objective

The purpose of the early response investigation was to prospectively assess the effect of the combination of desloratadine and montelukast on the allergen induced early asthmatic response in a cohort of patients with mild atopic asthma.

3.2 Early Asthmatic Response Hypothesis

The hypothesis is that the combination of desloratadine and montelukast provides better protection against the EAR than either drug administered alone.

Null Hypothesis: $H_0$: Placebo = Desloratadine = Montelukast = Combination
Alternative Hypothesis: $H_1$: Placebo < Desloratadine < Montelukast < Combination

3.3 Late Asthmatic Response Objective

The purpose of the late response investigation was to prospectively assess the effect of the combination of desloratadine and montelukast on the allergen induced late asthmatic response and related sequelae.

3.4 Late Asthmatic Response Hypothesis

The hypothesis is that the combination of desloratadine and montelukast provides better protection against the LAR and related sequelae than either drug administered alone in a cohort with mild atopic asthma and DAR following allergen exposure.

Null Hypothesis: $H_0$: Placebo = Desloratadine = Montelukast = Combination
Alternative Hypothesis: $H_1$: Placebo < Desloratadine < Montelukast < Combination
4.0 EFFECT OF COMBINED MONTELUKAST AND DESLORATADINE ON THE EARLY ASTHMATIC RESPONSE TO INHALED ALLERGEN

4.1 RELATIONSHIP TO THESIS

This chapter addresses the objective and hypothesis stated in sections 3.1 and 3.2 respectively. More specifically, this chapter deals with the investigation of the effect of combined montelukast and desloratadine on the early asthmatic response to inhaled allergen. This work was published in the Journal of Allergy and Clinical Immunology (Appendix A).

4.2 ABSTRACT

The early asthmatic response to inhaled allergen results from IgE-mediated release of multiple mast cell mediators, including leukotrienes and histamine; both of which cause bronchoconstriction. Combination therapy directed at blocking the effects of both mediators may protect against the early asthmatic response better than either therapy alone.

We investigated the combination of montelukast and desloratadine on the EAR to inhaled allergen using the standardized allergen challenge model in ten mild atopic asthmatics with a four way cross over, randomized, double blind, placebo controlled design.

Desloratadine did not protect against allergen induced bronchoconstriction whereas montelukast and the combination of montelukast and desloratadine both significantly increased the allergen PC_{20} compared to placebo (p<0.001). Furthermore, the combination of montelukast and desloratadine provided superior protection against allergen induced bronchoconstriction than that of montelukast monotherapy (p=0.02).

We provide evidence that clinical doses (10 mg montelukast and 5 mg desloratadine) administered in combination at 26 hours and 2 hours prior to allergen inhalation challenge significantly and synergistically inhibit the EAR.
4.3 INTRODUCTION

The response of the airways to inhaled allergen in individuals with atopic asthma is an IgE-mediated mast cell degranulation process leading to the early asthmatic response (EAR) which peaks about 15-30 minutes post inhalation and is often followed by a late asthmatic response (LAR) developing 3-8 hours post inhalation. The degranulation process results in the release of multiple mediators, including the leukotrienes and histamine. The EAR is thought to be due to airway smooth muscle contraction, mediated in part by histamine on H1 receptors and leukotrienes on CysLT1 receptors. It follows that blocking the activity of these mediators may prevent bronchoconstriction. Investigations of the effect of H1 blockers on the EAR have produced variable and inconclusive results [Rafferty et al., 1987; Gong et al., 1990; Rafferty et al., 1990; Cockcroft et al., 1992; Bentley et al., 1996]. Leukotriene modifiers have been shown to provide reasonable inhibition of the EAR and LAR but do not completely abolish the response [Dahlen et al., 1991; Taylor and O'Shaughnessy, 1991; Diamant et al., 1995; Hamilton et al., 1997; Hamilton et al., 1998]. The idea that a mechanism involving multiple mediators may require multiple interventions continues to attract interest in the role of combination therapies for the treatment of atopic asthma. Desloratadine is an antihistamine that has not been clinically investigated in atopic asthma and the response to inhaled allergen. Montelukast is effective in partially attenuating the response to inhaled allergen. There are no published data reporting the effect of the combination of these two therapies on allergen induced airway responses.

4.4 METHODS

4.4.1 Subjects

Ten healthy atopic asthmatics aged 18 years or older with a baseline FEV1 ≥ 65% predicted participated in the study (Table 4.1). Subjects had no respiratory infection or allergen exposure for at least four weeks prior to enrolment. The protocol was approved by the University of Saskatchewan Biomedical Ethics Research Board and subjects provided written consent prior to any procedures being conducted. (Appendix B).
Salbutamol (n=10) was withheld prior to testing for at least 8 hours. Fluticasone was used (stable dose for 3 months) by one subject. No subjects used any other asthma therapies or antihistamines.

4.4.2 Study Design

This was a randomized, four way crossover, placebo controlled (thiamine 100 mg) investigation. A study flowchart is included as Appendix C. Matching placebo tablets were unavailable. Subjects were provided with two small brown envelopes containing either two placebo tablets; one desloratadine tablet (5 mg) and one placebo tablet; one montelukast tablet (10 mg) and one placebo tablet; or one desloratadine tablet and one montelukast tablet. Subjects were instructed to ingest the contents, without looking at it, of one envelope 26 hours, and the other 2 hours prior to allergen challenges which were scheduled at least 7 days apart. No subject had previously used desloratadine and only one subject had previously used montelukast. The investigator was blind to the treatments.

4.4.3 Allergen Challenges

Subjects were challenged during the non-pollen season (i.e., December through March) with the allergen that produced the largest response on skin prick testing. Serial 2-fold dilutions were prepared from 1:8 stock solutions diluted with sterile isotonic saline containing 0.4 % phenol. Starting concentrations for inhalation were determined with the use of an algebraic prediction of allergen PC20 using skin test endpoint and airway responsiveness to methacholine [Cockcroft et al., 1997]. Allergen challenges began with the same concentration for each individual on each occasion. Allergens (Western Allergy Services, Victoria, BC; see Table 4.1) were aerosolized via a Wright Nebulizer (Roxon Medi-tech Ltd., Montreal, PQ) calibrated to deliver 0.13 mL/min. Each concentration was inhaled during two minutes of tidal breathing via a mouthpiece and with nose clips in place. Ten minutes after each inhalation was completed, two technically acceptable FEV1 maneuvers were performed sixty seconds apart. Inhalations were continued until the highest post inhalation FEV1 was at least 15 % lower than the highest baseline FEV1 (obtained from three reproducible flow volume loops after a ≥ 20
minute rest period) or until the top concentration had been administered. The FEV$_1$ was repeated at 10 minute intervals until no further decrease was observed [Cockcroft and Murdock, 1987]. The allergen PC$_{20}$ was then calculated algebraically [Cockcroft et al., 1983]. Salbutamol (200 µg) was administered to reverse the acute bronchoconstriction. Fluticasone (500 µg) was administered to prevent the late response and associated increase in airway responsiveness [Cockcroft et al., 1993].

4.4.4 Data Analysis

Baseline FEV$_1$ data and log transformed allergen PC$_{20}$ data were analysed by 2 way ANOVA followed by pairwise comparison (least squared difference, (LSD)) of means if applicable (Statistix Version 7.0, Tallahassee, FL). Power calculations indicated a 98 % power to detect a one doubling concentration difference in allergen PC$_{20}$ in 10 subjects and a 97 % power in 9 subjects.

4.5 RESULTS

All ten subjects completed the study with no adverse events. A post hoc decision to exclude data of one subject (#10) was made based on the observation that the screening allergen challenge and the placebo treatment allergen challenge were not reproducible. The overall significance of the data was not affected.

Mean baseline FEV$_1$ (litres ± SD) measurements following placebo (3.24 ± 0.55), desloratadine (3.25 ± 0.54), montelukast (3.27 ± 0.54) and the combination (3.31 ± 0.57) were not significantly different from each other (ANOVA p = 0.19).

Comparison of geometric mean allergen PC$_{20}$ (units/mL) differences between combination (697), montelukast (338), desloratadine (123) and placebo (104) treatments was highly significant (ANOVA p<0.00001; Figure 4.1). The mean log values (± SEM) following combination, montelukast, desloratadine and placebo treatments were 2.8433 (±0.3253) 2.5295 (± 0.2979), 2.0883 (± 0.2102) and 2.0166 (± 0.2553) respectively. Compared to placebo (LSD
comparison of means) combination therapy and montelukast therapy significantly increased allergen PC20 (p < 0.001 for both). Allergen PC20 with combination therapy was significantly greater than with montelukast alone (p=0.02) and montelukast alone was significantly greater than with desloratadine alone (p<0.002). Desloratadine and placebo treatments produced similar results (p > 0.2). Analysis of individual fold increases in allergen PC20 (combination vs placebo; montelukast vs placebo and desloratadine vs placebo) indicated mean fold increases of 8.9, 4.8 and 1.4 fold or 3.2, 2.3, and 0.49 doubling concentrations respectively.

4.6 DISCUSSION

We have demonstrated that 5 mg of desloratadine administered 26 hours and two hours prior to allergen inhalation does not protect against the EAR. We have also demonstrated that 10 mg of montelukast administered 26 hours and two hours prior to allergen inhalation increases allergen PC20 2.3 doubling concentrations while the combination increases allergen PC20 3.2 doubling concentrations.

Desloratadine is the principal metabolite of the second generation antihistamine loratadine. Although loratadine had previously been shown to be ineffective in decreasing the EAR [Town and Holgate, 1990], desloratadine possesses superior pharmacokinetic and pharmacodynamic properties; has an excellent safety profile; and has been shown to exert anti-inflammatory effects [Murdoch et al., 2003]. The lack of efficacy following desloratadine therapy reported here suggests that desloratadine alone does not protect against the EAR by blocking H1 receptors on airway smooth muscle.

Montelukast is a leukotriene receptor antagonist with proven efficacy in asthma and exercise-induced bronchoconstriction [Blake, 1999] and has been shown to significantly decrease the EAR to inhaled allergen [Diamant et al., 1999; Leigh et al., 2002; Palmqvist et al., 2005]. Our data are consistent with the existing literature on the effect of montelukast on the EAR, although we do show a slightly greater inhibition of the EAR after montelukast when changes in the maximal decrease in FEV1 are equated to changes in doubling concentrations. In our study, montelukast shifts the dose response curve versus placebo more than 2 doubling
concentrations. This is equivalent to a 75 % decrease (versus an average of around 55 % reported previously [Diamant et al., 1999; Leigh et al., 2002; Palmqvist et al., 2005]) in the maximal fall in FEV$_1$.

It is logical to postulate that the EAR to inhaled allergen could be additively or synergistically blocked by combining CysLT$_1$ and H$_1$ receptor antagonist therapies. This effect was demonstrated in vitro more than ten years ago in isolated human bronchi [Bjorck and Dahlen, 1993]. Since then, only two studies investigating the effect of combining an antihistamine with an LTRA on the EAR could be identified. Roquet et al., [1997] investigated the combination of high dose zafirlukast (80 mg bid) and loratadine (10 mg bid) administered for seven days on the EAR and LAR and found that combination therapy was more effective at inhibiting the EAR than either drug alone however, the difference between the inhibition following zafirlukast was not significantly different from the inhibition following combination therapy. A more recent in vitro investigation of the H$_1$ anti-histamine chlorpheniramine and the LTRA MK-571 documented a synergistic effect of the combination versus either drug alone in allergic isolated human bronchi [Ruck et al., 2001]. We could identify no published data describing the effect on allergen induced airway responsiveness following montelukast in combination with an antihistamine.

We provide evidence that the combination of 5 mg of desloratadine with 10 mg of montelukast administered at 26 hours and 2 hours prior to allergen inhalation increases allergen PC$_{20}$ 3.2 doubling concentrations (8.9-fold) versus placebo. This is the first evidence of clinically significant inhibition of the EAR using clinically relevant doses of the combination of an antihistamine and an LTRA that is significantly greater than the inhibition afforded by LTRA monotherapy. Since no effect was observed with desloratadine alone, the significantly greater combined efficacy is difficult to interpret. The magnitude of inhibition is similar to that achieved with 10 mg inhaled sodium cromoglycate (76 %), lower than 200 µg salbutamol (97 %) and substantially greater than single dose (200 µg) beclomethasone dipropionate (< 1 %) reported as maximal decreases in FEV$_1$ [Cockcroft et al., 1987] suggesting mast cell stabilization as a possible mechanism.
There are several methods to assess the EAR including AUC (0-3h), maximal decrease in FEV\(_1\), and allergen PC\(_{20}\). These differences in methodology present difficulties in data comparison and reinforce the issue of method standardization. Efficacy reported as AUC (0-3h) represents changes in the airway that are occurring over 180 minutes, 2/3 of which represents the spontaneous recovery portion of the EAR and 1/3 of which represents the development of and the maximal response to the sensitizing agent. Comparison of AUC (0-3h) data with data reported as changes in the maximal decrease in FEV\(_1\) or allergen PC\(_{20}\), both of which measure changes occurring in the first 60 minutes, is therefore extremely difficult. Reporting the EAR as maximal changes in FEV\(_1\) requires the same dosing regimen and occasionally single dose administration of allergen. Although single dose administration protocols may raise concerns surrounding subject safety, the change in maximal decrease in FEV\(_1\) can, at least in theory, be compared with the algebraic determination of allergen PC\(_{20}\). Assuming a linear dose response, we estimate that a shift of one doubling concentration is approximately equivalent to a 50 % change in the maximal fall in FEV\(_1\); 2 doubling concentrations approximately a 75 % change; 3 doubling concentrations approximately 87.5 % change and so on. Therefore, allergen PC\(_{20}\) reporting provides a more precise discrimination between treatments that generate a \(\geq 50\%\) change in the maximal decrease in FEV\(_1\). The one major limitation to assessing airway response to inhaled allergen using this method is that it cannot be used to assess the LAR. Therefore, the relevance of these data to the clinical features and management of asthma remain to be determined.

We present *in vivo* data supporting a synergistic effect of the combination of montelukast and desloratadine on the early response to inhaled allergen. The mechanism(s) underlying the apparent synergism and the effect of the combination of montelukast and desloratadine on the LAR and related sequelae require further investigation.
### Table 4.1: EAR Study Subject Demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Height (inches)</th>
<th>Baseline FEV&lt;sub&gt;1&lt;/sub&gt; (litres)</th>
<th>Baseline FEV&lt;sub&gt;1&lt;/sub&gt; % Predicted</th>
<th>Allergen used for inhalation</th>
<th>MPC&lt;sub&gt;20&lt;/sub&gt; (mg/mL)</th>
<th>Medications</th>
</tr>
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<tr>
<td>1</td>
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<td>57</td>
<td>66</td>
<td>2.46</td>
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<td>S; prn</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
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<td>3.87</td>
<td>87</td>
<td>HDM&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>S; prn</td>
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<tr>
<td>3</td>
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<td>86</td>
<td>CAT&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>S; prn</td>
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</tr>
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<td>CAT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.75</td>
<td>S; prn</td>
</tr>
</tbody>
</table>

Mean 32 68 3.41 85 2.7

* not included in analysis

S = salbutamol  
MPC<sub>20</sub> = methacholine  
PC<sub>20</sub>  
F = fluticasone propionate

<sup>a</sup>: Grass Mix 10 (40,000 protein nitrogen units/mL)  
<sup>b</sup>: Standard Mites Mixed (10,000 allergy units/mL)  
<sup>c</sup>: Standardized Cat Pelt (10,000 bioequivalent allergy units/mL)
Figure 4.1: Treatment effects on the EAR allergen PC$_{20}$.

Extract units are generically identified as units/mL. Error bars represent standard error of the mean (SEM).
5.0 SINGLE DOSE DESLORATADINE AND MONTELUKAST AND ALLERGEN-INDUCED LATE AIRWAY RESPONSES

5.1 RELATIONSHIP TO THESIS

The following chapter addresses the objective and hypothesis stated in sections 3.3 and 3.4 respectively. More specifically, this chapter describes the rationale, methods and results and discusses the effect of single dose desloratadine and montelukast on the late asthmatic response and its related sequelae to inhaled allergen. This work was published in the European Respiratory Journal. The Abstract is appended. (Appendix D).

5.2 ABSTRACT

Allergen exposure in atopic asthmatics with a dual response results in two independent episodes of reversible airway obstruction termed the early asthmatic response (EAR) and the late asthmatic response (LAR). The LAR coincides with airway eosinophilia, changes in responsiveness to methacholine, and increased levels of exhaled nitric oxide which remain evident even after FEV$_1$ recovery (i.e., 24 hour post exposure). There is evidence that montelukast suppresses the LAR but the inhibition is incomplete. Having shown a synergistic effect of the combination of montelukast and desloratadine on the EAR we hypothesized that the combination may also be effective in protecting against the LAR and related airway changes.

Ten mild atopic asthmatics were enrolled in a 4 way crossover, randomized, double-blind, placebo controlled standardized LAR allergen inhalation challenge study. Treatment arms consisted of a single dose of montelukast (10 mg), desloratadine (5 mg), the combination of montelukast (10 mg) and desloratadine (5 mg) or matching placebo. Treatments were administered two hours prior to allergen challenge.

The mean LAR AUC was significantly decreased for all active treatments compared to placebo (rank order: combination > montelukast = desloratadine > placebo). Desloratadine, montelukast and desloratadine in combination with montelukast also significantly
decreased the EAR AUC compared to placebo (rank order: combination = montelukast > desloratadine > placebo). Sputum eosinophils were significantly reduced by desloratadine monotherapy at 7 hours and by montelukast monotherapy at 24 hours (p < 0.05). Sputum eosinophils were significantly reduced by the combination of desloratadine and montelukast at both 7 hours and 24 hours (p < 0.05). Significantly lower levels of exhaled nitric oxide were observed following montelukast monotherapy only and only at the 24 hour measurement (p < 0.05). We observed no effect following any of the active treatments on the increased responsiveness to methacholine.

Single dose co-administration of desloratadine and montelukast two hours prior to allergen exposure clinically abolished the LAR AUC. Sputum eosinophils were fewer in number following desloratadine and the combination at 7 hours and montelukast and the combination at 24 hours. Neither the combination nor the individual therapies altered changes in responsiveness to methacholine although a trend toward higher methacholine PC_{20} values was evident following montelukast and the combination. Montelukast suppressed the increase in exhaled nitric oxide at the 24 hour time point. No other active treatment was effective in altering exhaled nitric oxide levels.

5.3 INTRODUCTION

The airway response to inhaled allergen is characterized by airflow obstruction that is usually maximal within 20-30 minutes of exposure. This is referred to as the early asthmatic response (EAR) which results from IgE mediated mast cell degranulation, release of stored mediators (e.g., histamine) and newly synthesized mediators (e.g., leukotrienes) which subsequently exert their effects on surrounding tissues causing bronchoconstriction, plasma exudation and mucus hypersecretion. The late asthmatic response (LAR), which occurs in at least 50 % of individuals with positive allergen challenge, is a subsequent episode of airflow obstruction that develops over the 3-8 hours after the EAR has spontaneously resolved. As the LAR develops, and for a limited time after the LAR has resolved, the airway has been invaded with leukocytes (eosinophils in particular), has become hyperresponsive to direct stimuli (e.g.,
methacholine) and produces increased levels of nitric oxide, collectively termed the late sequelae.

The mechanisms of the LAR and related sequelae remain unknown. A predominant role for a Th2 cell and cytokine (IL-4, IL-5, IL-13) profile has been well established although recent data are challenging this ideology. Desloratadine, the most potent metabolite of the second generation antihistamine loratadine and the LTRA drug of choice montelukast synergistically inhibit the EAR following combined use. The magnitude of the EAR influences the LAR within a given subject. Animal and in vitro models have provided evidence to suggest that both these agents possess anti-inflammatory activity including the ability to decrease IL-4, IL-5 and IL-13 levels. These agents may therefore protect against the allergen induced LAR and related sequelae.

5.4 METHODS

5.4.1 Subjects

Potential study participants were either known dual responders or underwent screening allergen challenges to assess eligibility. These individuals were recruited to be study subjects providing the following criteria had been met and baseline demographics are provided in (Table 5.1):

- baseline FEV$_1$ $\geq$ 70 % predicted
- methacholine PC$_{20}$ $\leq$ 16 mg/mL
- positive skin test to a common aeroallergen
- EAR of $\geq$ 20 % fall in FEV$_1$ and LAR of $\geq$ 15 % fall in FEV$_1$
- No respiratory infection or change in allergen exposure for 4 weeks prior to enrolment and throughout the investigation

Salbutamol (n=10) was withheld prior to testing for $\geq$ 6 hours. One subject was using inhaled corticosteroid and one was using nasal corticosteroid, both on stable dose prior to and throughout the study. The protocol was approved by the Ethics Research Boards of each institution and all
subjects provided written consent prior to the conduct of any study related procedures (Appendix E).

5.4.2 Study Design

We conducted a randomized, double-blind, four-way crossover, placebo-controlled, multicenter allergen inhalation challenge investigation. A study flow chart is included as Appendix F. Assessments of exhaled nitric oxide levels were performed pre allergen inhalation and at 4, 7 and 24 hours post allergen inhalation. Airway hyperresponsiveness to methacholine \(i.e.,\) methacholine challenges) were performed 24 hours prior to allergen challenges and 24 hours after allergen challenges. Sputum samples were also collected 24 hours prior to allergen challenges and 7 hours and 24 hours after allergen challenges. All sites used the same standardized methodology for all assessments. The study was registered at www.clinicaltrials.gov under #NCT00424580. The Saskatoon Health Region Pharmacy Research Unit at the Royal University Hospital, Saskatoon, Saskatchewan provided currently available tablets of desloratadine and montelukast encapsulated with lactose filler to produce identical looking treatments. Individual treatments were provided to study participant on Day 1 of each treatment arm in a sealed small brown envelope. Study participants were instructed to ingest the contents of the envelope 2 hours prior to their allergen challenge visits which were scheduled at \(\geq 10\) day intervals.
Figure 5.1: LAR Study Design

eNO, exhaled nitric oxide; MCh, methacholine challenge; SI, sputum induction; EAR, early asthmatic response; LAR, late asthmatic response
5.4.3 Allergen Inhalation Challenges

Serial 2-fold dilutions were prepared from standardized stock allergen (grass, cat and house dust mite (dermatophagoides pteronyssinus and dermatophagoides farinae) and diluted with normal saline. Starting concentrations for inhalations were determined by algebraic prediction of allergen PC\textsubscript{20} using the skin test endpoint and methacholine PC\textsubscript{20} [Cockcroft \textit{et al.}, 2005]. Allergen challenges began with the same concentration and the same number of concentrations were administered, within a given individual, for each allergen challenge (\textit{i.e.}, the same dose of allergen was administered following each treatment.) Allergens were aerosolized via a Wright Nebulizer (Roxon Medi-tech Ltd., Montreal, PQ) calibrated to deliver 0.13mL/min. Each concentration was inhaled over two minutes of tidal breathing via a mouthpiece and with nose clips in place. Ten minutes after each inhalation was completed, two technically acceptable FEV\textsubscript{1} maneuvers were performed sixty seconds apart. Once the EAR was captured, the response remained untreated and the FEV\textsubscript{1} was assessed at various standardized time points, up to 7 hours post allergen inhalation, to capture the LAR [Boulet \textit{et al.}, 2007]. The area under the curve (AUC) for the EAR and LAR were calculated using the trapezoid rule.

5.4.4 Methacholine Challenges

Methacholine challenges were performed 24 hours before and 24 hours after the allergen inhalation challenge using a standardized two minute tidal breathing method [Crapo \textit{et al.}, 1999; Cockcroft \textit{et al.}, 1977]. All methacholine challenges were performed in an identical manner (\textit{i.e.}, same starting concentration) within a given subject. The PC\textsubscript{20} was extrapolated if a concentration of 16 mg/mL or less resulted in a percent fall in FEV\textsubscript{1} of greater than 17 \% but less than 20 \% [Jokic \textit{et al.}, 1998]; and interpolated if the percent fall was > 20\% [Cockcroft \textit{et al.}, 1983]. Salbutamol (200 \mu g) was administered at the completion of each methacholine challenge.

5.4.5 Sputum Collection and Analysis

Sputum was collected 24 hours pre allergen challenge, and 7 and 24 hours
post allergen challenge. Sputum collection and processing was performed using the methodology of Pizzichini and co-workers [Pizzichini et al., 1996]. In brief, subjects inhaled, via mouthpiece, increasing concentrations (3 %, 4 % and 5 %, each for 7 minutes) of hypertonic saline, aerosolized by a high output ultrasonic nebulizer. Collected specimens were immediately refrigerated and processed within 2 hours of collection. Total cell counts were determined using a Neubauer hemocytometer chamber (Hausser Scientific, Horsham, PA) and expressed as the number of cells per milliliter of sputum. Differential cell counts were performed under blinded conditions from cytopspins stained with Diff Quik (Dade Behring, Newark, DE).

5.4.6 Exhaled Nitric Oxide

Exhaled nitric oxide measurements were collected 24 hours pre allergen challenge and 4, 7 and 24 hours post allergen challenge as per ATS recommendations [American Thoracic Society, 1999] using the Aerocrine NIOX system (Aerocrine, Solna, Sweden). Subjects performed an inhalation via a filter/mouthpiece to total lung capacity followed by exhalation at a constant flow rate of 50 mL/sec until the reading was captured. Comparisons were made using the mean of three measurements at each timepoint.

5.4.7 Data Analysis

Two way (subject/treatment) analysis of variance (ANOVA), followed by pair wise comparison of means (least squared difference) if applicable (Statistix Version 7.0, Tallahassee, FL) was used to examine differences in the endpoints under investigation. The study was appropriately powered (>80 %) with ten subjects to detect differences in the primary endpoint (LAR – 50 % inhibition in AUC), and in the secondary endpoints (EAR, allergen induced airway hyperresponsiveness and sputum eosinophil cell counts) [Inman et al., 1995; Gauvreau et al., 1999]. The appropriate sample size to achieve at least 80 % power in detecting a significant change in FeNO is unknown.
5.5 RESULTS

All ten randomized subjects completed the study without incident. Desloratadine, montelukast and the combination all significantly decreased the LAR area under the $\text{FEV}_1$ curve, in a treatment dependent manner (ANOVA $p<0.001$). Desloratadine reduced the response by 43%, montelukast by 71% and the combination completely blocked the response (Figure 5.2). Desloratadine, montelukast and the combination also significantly reduced the mean AUC EAR by 32%, 72% and 100% respectively (Figure 5.3). The inhibition with combination however was not significantly different from that of montelukast alone for the EAR ($p=0.052$). The mean percent fall in $\text{FEV}_1$ at various time points following allergen challenge is shown in Figure 5.4. The doubling dose increase in methacholine $\text{PC}_{20}$ was $0.66\pm0.19$ (mean±SEM) after placebo; $0.82\pm0.26$ after desloratadine; $0.31\pm0.21$ after montelukast and $0.18\pm0.23$ after combination (ANOVA $p = 0.092$; Figure 5.5). The allergen induced increase in FeNO was significantly less after montelukast treatment only and only at the 24 h time point ($p = 0.03$; Figure 5.6). Sputum eosinophils increased 23.2% 7 hours post allergen inhalation in the untreated arm whereas the increase following desloratadine was only 10.5% and after combination only 2.8% ($p < 0.05$). At 24 hours post allergen challenge, the percent increase in sputum eosinophils was 8.8% following montelukast and 4.5% after combination as compared to 16.2% after placebo ($p < 0.05$). The difference between the individual therapies and combination was not significant at either time point (Figure 5.7 and 5.8).

5.6 DISCUSSION

Our \textit{in vivo} investigation provides new insights into the effects of desloratadine, montelukast and the combination in individuals with mild atopic asthma and a dual asthmatic response. Few clinical studies have investigated the effects of combining a leukotriene antagonist with an antihistamine on the airway response to allergen, and none have looked at a single dose. Loratadine, the parent compound of desloratadine, has been shown by Roquet et al., [1997] to significantly decrease the LAR alone and in combination with zafirlukast following one week of high dose therapy (twice the daily recommended dose of both drugs). We have shown that desloratadine, in a single dose, provides the same magnitude of bronchoprotection.
against the LAR as this previous study of higher dose and longer duration. Comparison of the leukotriene antagonists suggests that a single dose of montelukast is also more effective than multiple high dose zafirlukast. We also document a complete inhibition of the LAR with the combination of montelukast and desloratadine whereas the Roquet study showed only a 75% inhibition of the LAR following the combination of zafirlukast and loratadine. Similarly, a more recent investigation using clinically relevant doses of azelastine and montelukast for 1 week also showed less of an effect compared with our results [Richter et al., 2008]. The differences between our study and these two studies may be related to the pharmacokinetic and pharmacodynamic (PK/PD) properties of the therapies, allergen challenge methodologies, study design or may even suggest the development of tachyphylaxis or the onset of tolerance following the longer and higher dosing regimen.

Our first investigation (i.e., Chapter 4) documented a synergistic inhibition of the EAR with combination therapy (8.9 fold increase in allergen PC_{20}) which was significantly better than the effect of desloratadine (1.4 fold increase in allergen PC_{20}) and montelukast (4.8 fold increase in allergen PC_{20}) monotherapy. Compared to placebo, montelukast also inhibited the response but desloratadine, by itself, had no effect. We now document that a single dose of desloratadine, assessed as AUC, significantly inhibits the EAR, as does both a single dose of montelukast and the combination of desloratadine and montelukast, however, the inhibitory effect of LTRA monotherapy and that of the combination on the EAR AUC is not significantly different. The observed differences are almost certainly not related to differences in the dose of active treatment (i.e., the LAR study used half the dose of that used in the isolated EAR study) but are probably related to the duration the response was observed and the difference in the amount of allergen administered. That is, the airway response during the isolated EAR study was measured until the response was maximal and the FEV_{1} was trending upward, perhaps 45 minutes to 1 hour post allergen inhalation. Whereas in the LAR investigation the EAR, assessed as AUC, captured the maximal response as well as the recovery (i.e., 0-3 hours). Comparison of isolated EAR challenge data (i.e., dose shift data) and EAR data (i.e., AUC data) from an LAR study design is therefore difficult to interpret.
Direct H\textsubscript{1} and CysLT\textsubscript{1} receptor antagonism at the level of the airway smooth muscle is an obvious potential mechanism for preventing the bronchoconstriction associated with the LAR by blocking the action of mediators (\textit{i.e.}, histamine and the cysteiny1 leukotrienes) released by recruited inflammatory cells (\textit{e.g.}, eosinophils and basophils). However, the single dose design, pharmacokinetic and pharmacodynamic (PK/PD) properties of the drugs and the response to combination therapy challenge this rationale. To elaborate, the dose of each treatment is taken 2 hours prior to the start of the allergen challenge, the duration of which is, on average, approximately one hour and the FEV\textsubscript{1} is measured at various time points over the next 7 hours. Additional measurements of FeNO, airway hyperresponsiveness to methacholine and sputum eosinophils are also captured up to 24 hours after the challenge. Montelukast has a half life of 2.7 to 5.5 hours. Therefore, 50 – 75% of the drug is potentially cleared at the time the 7 hour measurements are taken yet we still observe significant inhibition of the FEV\textsubscript{1} with montelukast at this time point (Figure 5.3). Conversely, the half life of desloratadine is 27 hours yet, the effect of desloratadine is no different than placebo at 7 hours when assessed as changes in FEV\textsubscript{1}. One possible explanation is that the relative potency of cysteinyl leukotriene induced bronchoconstriction is 1000 fold more potent than that of histamine. If cysLT\textsubscript{1} receptors are unopposed, as would be the case with desloratadine monotherapy, the protective effect of desloratadine may be undetectable. However, this rationale cannot explain the inhibitory effects observed on the LAR AUC or the allergen induces changes in airway responsiveness to methacholine, exhaled nitric oxide levels or sputum eosinophil counts which suggests a more complex mechanism of action than that of direct H\textsubscript{1} and cysLT\textsubscript{1} receptor antagonism.

Both histamine [Thurmond \textit{et al.}, 2008] and the leukotrienes [Busse and Kraft, 2005; Woszczerz \textit{et al.}, 2008; Woszczerz \textit{et al.}, 2008] play a role in leukocyte recruitment and we indeed provide evidence of a decrease in sputum eosinophils with desloratadine and the combination at 7 hours and montelukast and the combination at 24 hours. A trend for increased efficacy with combination therapy is apparent but the difference between the two monotherapies and the combination therapy at the two time points is not significant. Earlier investigations have shown leukotriene antagonists to significantly reduce eosinophil trafficking to the airway following allergen inhalation. Leigh \textit{et al.}, [2002] documented less of an increase in sputum eosinophils following 10 days of 10 mg/day montelukast at both 7 hours and 24 hours post
allergen challenge and Parameswaran et al., [2004] also documented a decrease in sputum eosinophils at 7 hours and 24 hours post allergen inhalation following two weeks of pranlukast. There is however at least one report documenting no change in allergen-induced sputum eosinophilia at 24 hours post allergen challenge following 3 doses of montelukast administered at 36 and 12 hours prior to challenge and 12 hours after challenge [Diamant et al., 1999]. The discrepancies in these data are difficult to explain. We do observe a trend in our data towards an inhibition of sputum eosinophils at 7 hours post allergen challenge with montelukast and one could suggest that the single dose design was the limiting factor since two other studies have shown a significant reduction in 7 hour sputum eosinophils following longer LTRA dosing regimens (10 days and 14 days). However, the single dose explanation for lack of efficacy at 7 hours is difficult to accept given the observed significant reduction in sputum eosinophils at 24 hours. Our study, taken together with the Leigh et al., [2002], Parameswaran et al., [2004], and Diamant et al., [1999] studies appear to offer inconclusive findings on the effects of LTRA’s and sputum eosinophil counts following allergen challenge which warrant further investigation.

To our knowledge, this is the first report of the effect of desloratadine on sputum eosinophil counts following allergen inhalation challenge. We report that a single 5 mg dose of desloratadine decreases the number of sputum eosinophils at 7 hours post inhalation by approximately 50 %. The effect of desloratadine is noted to trend to an even greater degree when combined with montelukast, which is similarly observed at the 24 hours time point in that the significant inhibition of eosinophil recruitment by montelukast is trending even higher with the addition of desloratadine. The mechanism by which these agents, both alone and in combination, are suppressing eosinophil recruitment in vivo requires further investigation.

Increased responsiveness to direct acting stimuli (e.g., methacholine) is another hallmark of the late airway response to allergen. The increase in airway responsiveness following placebo (i.e., decrease in methacholine PC$_{20}$) 24 hours post allergen inhalation was unaffected by all active treatments. This is the first report of the effects of desloratadine on allergen induced changes in methacholine PC$_{20}$ and our results are consistent with previous investigations which have shown no change in airway responsiveness following allergen exposure and pre-treatment with an H$_1$ blocker [Twentyman et al., 1993; Bentley et al., 1996].
The literature surrounding the effect of leukotriene antagonists on allergen induced changes in methacholine responsiveness is controversial. Palmqvist et al., [2005] documented no change in methacholine PC_{20} following 8 days of montelukast monotherapy, conversely however, 10 mg/day of montelukast for 10 days did decrease the response as shown by Leigh et al., [2002] and 300 mg/bid of pranlukast for 2 weeks also prevented the allergen induced increase in airway responsiveness to methacholine as shown by Parameswaran et al., [2004]. Taylor and O’Shaughnessy, [1991] have also documented an inhibitory effect on the allergen induced increase in airway responsiveness to histamine following single dose administration of a leukotriene receptor antagonist (ICI204.219; developed as zafirlukast) given 2 hours prior to allergen challenge. While our investigation is similar to that of Taylor and O’Shaughnessy (i.e., single dose administered 2 hours prior to allergen challenge) direct comparison is again difficult due to the choice of direct acting agent (i.e., histamine versus methacholine) and the differences in the time points that the measurements were made. Most allergen challenge studies assess this parameter using 24 hour pre and post allergen challenge data. In the Taylor and O’Shaughnessy study, measurements were taken approximately 3 hours pre and 7 hours post allergen challenge and the data at these time points is difficult to interpret as being the response to methacholine or as being an apparent response to methacholine due to the changes in airway caliber evoked by the LAR itself.

The relationship between airway inflammation and airway hyperresponsiveness following allergen exposure remains unclear. Our data support a relationship between these parameters in that a reduction in sputum eosinophils parallels a trend towards a significant decrease in airway hyperresponsiveness with montelukast and the combination at the 24 hour time point. Additionally, at this time point, desloratadine did not affect eosinophil recruitment and there was no change in the increase in airway responsiveness to direct stimuli. Although, as mentioned, interpretation of the response to methacholine would have been difficult, it would have been interesting to have measured the methacholine PC_{20} at the 7 hour time point.

With the exception of montelukast, which significantly suppressed the allergen induced increase in FeNO at the 24 hour measurement, there were no treatment effects on this parameter. The amount of exhaled nitric oxide has been shown to increase after allergen
exposure [Duong et al., 2007] and has been reported to correlate with the degree of eosinophilic inflammation. [Jatakanon et al., 1998]. Even though desloratadine reduced eosinophil influx, H1 receptor antagonism did not affect FeNO at 7 hours suggesting the source of FeNO at this time point may not be eosinophilic in nature. Conversely, a reduction in eosinophils following montelukast is indeed associated with a reduction in FeNO at 24 hours. The temporally associated differences may be related to the kinetics of eosinophil activation. It may also be worth noting that FeNO is trending higher following antihistamine and this is perhaps reflected in the apparent antagonistic effect following combination therapy (i.e., effect of combination on FeNO levels is less than the effect of montelukast alone). Nonetheless, relative to bronchial biopsy and bronchial lavage, exhaled nitric oxide is an attractive non-invasive procedure for assessing airway inflammation and therapeutic efficacy when incorporated into the allergen challenge model. The results however must be interpreted with caution as the reported lack of specificity and selectivity of FeNO measurements may limit the usefulness of the data [Pendharkar et al., 2008]. We must also acknowledge that the study may not be appropriately powered to detect a significant change in FeNO.

The biological role of histamine and the leukotrienes in the pathogenesis of asthma is an area of great interest. Many of the cells involved in the inflammatory process and the immune response possess histamine and/or leukotriene receptors which may serve as potential therapeutic targets for altering these responses. In addition to leukocyte recruitment, histamine may have a role in regulating the phenotype of dendritic cells and T cells, [Akdis and Blaser, 2003] direct T cell trafficking [Bryce et al., 2006] and influence cytokine signaling [Schneider et al., 2002]. Whether or not any of these potential mechanisms can explain our results needs to be investigated.

The importance of histamine and the leukotrienes in the EAR via airway smooth muscle contraction has been well documented. Our current investigation provides clinical evidence that these mediators alone, but to a greater extent in combination, are also important in the development and manifestation of the LAR. The clinical relevance of our data is unknown considering the currently available treatments that effectively suppress the LAR which include the long acting β2 agonists (short if used following EAR), single dose inhaled
glucocorticosteroids, combination therapies such as Symbicort® and Advair®, and anti-IgE. Importantly however, there is a subpopulation of individuals with atopic asthma who are treated with only infrequent short acting bronchodilators. Although the relief provided by the use of rescue bronchodilators is beneficial, it can be problematic with overuse. In addition, prophylactic prevention of the LAR may be a preferred option over masking the response with functional antagonism given the suspected role of inflammation on tissue remodeling. It is therefore these individuals who may benefit from combined desloratadine and montelukast therapy when exposure to a triggering allergen is imminent.

In summary, we provide evidence that concurrent, single dose administration of desloratadine and montelukast impressively and significantly blocks both the early and the late airway responses to inhaled allergen in individuals with mild atopic asthma. The mechanisms by which the combination exerts this inhibitory effect and the effect of combined therapy on the late sequelae requires further investigation.
Table 5.1: LAR Study Subject Demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Height (inches)</th>
<th>Baseline FEV$_1$ (litres)</th>
<th>Baseline FEV$_1$ % Predicted</th>
<th>Allergen</th>
<th>** Allergen Dilution</th>
<th>*** MPC$_{20}$ (mg/mL)</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>60</td>
<td>66</td>
<td>2.42</td>
<td>74</td>
<td>grass</td>
<td>1:256</td>
<td>0.56</td>
<td>S</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>24</td>
<td>61</td>
<td>3.29</td>
<td>106</td>
<td>hdm (dp)</td>
<td>1:256</td>
<td>2.5</td>
<td>S</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>30</td>
<td>70</td>
<td>3.41</td>
<td>77</td>
<td>cat</td>
<td>1:256</td>
<td>0.34</td>
<td>S</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>25</td>
<td>71</td>
<td>4.25</td>
<td>91</td>
<td>grass</td>
<td>1:64</td>
<td>1.4</td>
<td>S</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>24</td>
<td>73</td>
<td>3.49</td>
<td>71</td>
<td>grass</td>
<td>1:128</td>
<td>1.7</td>
<td>S B</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>47</td>
<td>66</td>
<td>2.47</td>
<td>84</td>
<td>cat</td>
<td>1:128</td>
<td>7.0</td>
<td>S</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>58</td>
<td>71</td>
<td>3.44</td>
<td>90</td>
<td>cat</td>
<td>1:128</td>
<td>1.3</td>
<td>S</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>26</td>
<td>65</td>
<td>3.75</td>
<td>110</td>
<td>hdm (df)</td>
<td>1:64</td>
<td>11.7</td>
<td>S</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>23</td>
<td>72</td>
<td>5.22</td>
<td>107</td>
<td>cat</td>
<td>1:32</td>
<td>5.3</td>
<td>S</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>44</td>
<td>68</td>
<td>3.72</td>
<td>122</td>
<td>cat</td>
<td>1:4</td>
<td>5.6</td>
<td>S F</td>
</tr>
<tr>
<td>Mean (± SD)</td>
<td></td>
<td></td>
<td></td>
<td>36.1 (14.8)</td>
<td>68.3 (3.8)</td>
<td>3.55 (0.81)</td>
<td>93.2 (17.3)</td>
<td>2.27</td>
<td></td>
</tr>
</tbody>
</table>

Legend: *geometric mean; ** final concentration of allergen administered; ***methacholine

PC$_{20}$; hdm, house dust mite; dp, dermatophagoides pteronyssinus; df, dermatophagoides farinae;
S, salbutamol – prn; B, budesonide – 400mcg/day; F, flonase – 2 squirts/nare/day
Table 5.2: Dose and effects of H<sub>1</sub> blockers and LTRA’s on the airway response to allergen

<table>
<thead>
<tr>
<th>Author</th>
<th>Treatment</th>
<th>Daily Dose</th>
<th>Duration</th>
<th>AUC EAR / max ↓ FEV&lt;sub&gt;1&lt;/sub&gt; (% inhibition)</th>
<th>AUC LAR / max ↓ FEV&lt;sub&gt;1&lt;/sub&gt; (% inhibition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roquet et al., [1997]</td>
<td>loratadine 20 mg</td>
<td>25/31</td>
<td>1 week</td>
<td>20 mg</td>
<td>40/32</td>
</tr>
<tr>
<td></td>
<td>zafirlukast 160 mg</td>
<td>62/62</td>
<td>1 week</td>
<td>160 mg</td>
<td>55/36</td>
</tr>
<tr>
<td></td>
<td>loratadine 20 mg/160 mg</td>
<td>75/74</td>
<td>1 week</td>
<td>20 mg/160 mg</td>
<td>74/48</td>
</tr>
<tr>
<td></td>
<td>loratadine + zafirlukast</td>
<td>1 week</td>
<td>1 week</td>
<td>20 mg/160 mg</td>
<td>74/48</td>
</tr>
<tr>
<td>Richter et al., [2008]</td>
<td>azelastine 8.0 mg</td>
<td>NR/46</td>
<td>1 week</td>
<td>8.0 mg</td>
<td>NR/43</td>
</tr>
<tr>
<td></td>
<td>montelukast 10 mg</td>
<td>NR/76</td>
<td>1 week</td>
<td>10 mg</td>
<td>NR/59</td>
</tr>
<tr>
<td></td>
<td>azelastine 8.0 mg/10 mg</td>
<td>NR/89</td>
<td>1 week</td>
<td>8.0 mg/10 mg</td>
<td>NR/78</td>
</tr>
<tr>
<td></td>
<td>montelukast 10 mg</td>
<td>NR/76</td>
<td>1 week</td>
<td>10 mg</td>
<td>NR/59</td>
</tr>
<tr>
<td>Davis et al., [current study]</td>
<td>desloratadine 5 mg</td>
<td>32/14</td>
<td>single dose</td>
<td>2 hours prior to allergen challenge</td>
<td>100/73</td>
</tr>
<tr>
<td></td>
<td>montelukast 10 mg</td>
<td>72/54</td>
<td>single dose</td>
<td>2 hours prior to allergen challenge</td>
<td>100/88</td>
</tr>
<tr>
<td></td>
<td>desloratadine 5 mg/10 mg</td>
<td>100/73</td>
<td>single dose</td>
<td>2 hours prior to allergen challenge</td>
<td>100/88</td>
</tr>
</tbody>
</table>

NR, not reported
Figure 5.2  Treatment effects on the LAR AUC.

Treatment dependent inhibition of the LAR expressed as mean area under the curve 3 to 7 hours post allergen inhalation. All treatments significantly decreased the LAR. The difference between desloratadine and montelukast was not significant. The combination of desloratadine and montelukast was superior to that of montelukast.
Figure 5.3 Treatment effects on the EAR AUC.

Treatment dependent inhibition of the EAR expressed as mean area under the curve 0 to 3 hours post allergen inhalation. Desloratadine, montelukast and the combination all significantly decreased (p < 0.05) the EAR AUC. The difference between combination therapy and montelukast therapy is not significant (p = 0.052).
Figure 5.4  Treatment effects on the Mean % Fall in FEV₁.

Changes in FEV₁ over the 7 hours post allergen inhalation expressed as the mean percent fall in FEV₁ for each treatment arm. Combination therapy and montelukast monotherapy were superior to that of desloratadine monotherapy and placebo. Combination was not significantly different from montelukast monotherapy and desloratadine monotherapy was not significantly different than placebo. C, combination of montelukast and desloratadine; M, montelukast monotherapy; D, desloratadine monotherapy; P, placebo.
Figure 5.5  Treatment effects on the allergen induced increase in airway hyperresponsiveness to methacholine.

Airway responsiveness to methacholine expressed as the dose shift from 24 hours prior to allergen challenge to 24 hours after allergen challenge. Active treatments did not have an effect on the allergen induced increase in airway responsiveness. A trend is apparent with montelukast and combination therapy.
Figure 5.6  Treatment effects on the allergen induced increase in exhaled nitric oxide.

Mean absolute change in the fraction of exhaled nitric oxide levels 24 hours after allergen inhalation. Montelukast was the only treatment to significantly suppress the allergen induced increase in exhaled nitric oxide (*p<0.05).
Figure 5.7  Treatment effects on allergen induced sputum eosinophilia at 7 hours.

Percent increase in sputum eosinophils 7 hours post allergen inhalation. Pre-treatment with desloratadine and the combination of desloratadine and montelukast significantly decreased eosinophil recruitment at this time point (*p<0.05). P, placebo; D, desloratadine; M, montelukast; D+M, desloratadine in combination with montelukast.
Figure 5.8  Treatment effects on allergen induced sputum eosinophilia at 24 hours.

Percent increase in sputum eosinophils 24 hours post allergen inhalation. Pre-treatment with montelukast and the combination of desloratadine and montelukast significantly decreased eosinophil recruitment at this time point (*p<0.05). P, placebo; D, desloratadine; M, montelukast; D+M, desloratadine in combination with montelukast.
6.0 OVERALL GENERAL DISCUSSION

We have shown that a single dose of the combination of a selective H₁ antagonist with a selective CysLT₁ antagonist provides superior efficacy towards inhibiting the airway responses to inhaled allergen in individuals with mild atopic asthma. Our initial study showed that two clinically relevant doses of desloratadine in combination with two clinically relevant doses of montelukast, administered 24 hours apart and two hours prior to exposure, synergistically inhibited the EAR to inhaled allergen in individuals with mild atopic asthma. Montelukast alone was superior to desloratadine which failed to suppress the response. These data, together with emerging data on the anti-inflammatory effects of both agents, led to the hypothesis that this particular combination would be effective in suppressing the LAR and related sequelae. In that regard, we also showed superior protection against the LAR AUC with the combination versus either drug alone. Additionally, the combination suppressed the allergen induced increase in eosinophil recruitment at both 7 hours and 24 hours; trended toward suppressing the allergen induced increase in airway hyperresponsiveness to direct stimuli; but failed to alter the allergen induced increase in exhaled nitric oxide.

At the time our LAR investigation began, the Roquet et al., [1997] study was the only clinical evidence of the effect of these types of drugs, administered in combination, on the airway response to allergen in atopic asthma. The inhibitory effect of combining a histamine H₁ receptor antagonist with a leukotriene receptor antagonist on the airway response to inhaled allergen has now been confirmed by Richter et al., [2008] and ourselves. Taken together, these three investigations provide a unique opportunity for post hoc observations and discussion regarding the class effects of these agents on the LAR to allergen in mild atopic asthma using single, multiple and high dose administration.

The FEV₁ data of the three LAR investigations is summarized in Table 5.2. The data is also graphically represented in Figure 6.1. Note that a single dose of montelukast and desloratadine, alone or in combination, appears to exert approximately equal effects on the EAR and LAR for a given treatment, although the effect on the LAR is always greater than that on the EAR. This pattern seems to be lost when much higher doses are given over a longer time period.
(i.e., Roquet study) such that the magnitude of the effect on the LAR is more than 40% less than that on the EAR.

Figure 6.1: Comparison of different doses of antihistamine and LTRA administered as monotherapy or as combination therapy on the maximal fall in FEV\textsubscript{1} following allergen inhalation challenge in individuals with mild atopic asthma.

COMBO is either zafirlukast and loratadine, montelukast and azelastine or montelukast and desloratadine corresponding to the dosing regimes of high dose 7 days, clinical dose 7 days and single dose pre challenge respectively. Note that the longer duration and higher dose regimens show a loss of efficacy on the LAR with combo and LTRA treatments compared to the single dose regimen. Circles represent antihistamine; squares represent LTRA and triangles represent combination therapies.
following combined therapy and LTRA monotherapy. When these types of drugs are provided at clinically recommended doses, the effect on the LAR with combination and LTRA monotherapy is again less than that observed for the EAR but the decrease is not as great (~22%). Loss of therapeutic efficacy is often due to the development of tolerance through downregulation of receptors, a general pharmacological principal commonly seen with excessive agonist activity where prolonged signaling results in a counteractive response by the body to turn the signal off by decreasing the number of available receptors. Conversely, a prolonged inhibition of the signal by an antagonist may lead to receptor upregulation and an apparent loss of therapeutic efficacy. There is however, no existing evidence to suggest that montelukast or LTRA’s in general are subject to the development of tolerance. We also observe that this apparent loss of effect does not appear to occur with histamine H1 receptor antagonist monotherapy. Across all three studies, the inhibitory effect of the antihistamine arm on the EAR and LAR was consistent and approximately equal. The reason that this may be occurring with LTRA and not histamine H1 receptor antagonists might be explained by the dual active/inactive conformations of the histamine H1 receptor such that the endogenous state of being in one form or the other does not necessitate a counteractive response. All H1 receptor antagonists are recognized as inverse agonists. As such, these agents produce the reverse effect of histamine, essentially decreasing the constitutive activity in the absence of histamine. In other words, if the H1 receptor exists in two forms, active and inactive, such that the agonist histamine stabilizes the receptor in the active form producing a response, the inverse agonist stabilizes the receptor in the inactive form producing an apparent response in equal magnitude to that of the agonist but in the opposite direction due to inhibition of the constitutive activity. In the presence of both agonist and inverse agonist the system remains “balanced” (Figure 6.2). Theoretically, the administration of antihistamine could therefore result in bronchodilation. The lack of an improvement in baseline FEV1 values in the three studies is likely explained by the sample population and is analogous to the lack of bronchodilator response to inhaled beta agonist (i.e., test of reversibility) in this population. That is, in a sample of individuals with mild atopic asthma who are asymptomatic and have near normal baseline lung function underlying bronchoconstriction is absent and bronchodilator treatments do not exhibit reversibility. The issue of documenting reversibility in this subject population has been and continues to be a challenge in meeting the inclusion criteria of some clinical trial protocols.
Figure 6.2: Inhibition of histamine signaling in the presence of desloratadine. The active receptor favors histamine whereas the inactive receptor favors desloratadine. In the absence of desloratadine the system responds to histamine signaling leading to airway smooth muscle contraction and desloratadine would theoretically produce airway smooth muscle relaxation in the absence of histamine. When both are present the system remains in balance.
This *post hoc* observation suggests that combining an LTRA with a histamine H$_1$ receptor antagonist will significantly decrease the airway response to allergen but the inhibitory effect on the more clinically relevant response, the LAR, is dampened by multiple doses and is lost to an even greater extent with multiple high dose administration that appears to be specifically related to a loss of effect of the LTRA. One might question the clinical relevance of this given that the responses of the individual LTRA treatments and the combination treatments in the Roquet *et al.*, [1997] and Richter *et al.*, [2008] studies are still significant when compared to placebo. What is unknown at this point however is whether the apparent loss of effect amplifies with treatment regimes extending beyond 7 days. Other possible explanations of the apparent loss of efficacy on the LAR in the two earlier studies include differences in magnitude of the LAR, that is, if the overall LAR responses were greater than in our study, the effect of the LTRA may appear less, and the use of different LTRA’s and antihistamines.

*Post hoc* observations aside, the results of our LAR investigation and those of Roquet *et al.*, [1997] and Richter *et al.*, [2008] reproducibly show that the combination of an antihistamine and a leukotriene receptor antagonist clinically and significantly suppress the airflow obstruction that develops following allergen inhalation better than either agent alone. Our results add to the literature by documenting significant inhibition of the LAR after a single dose of each agent as monotherapy and taken concomitantly two hours prior to allergen exposure. Impressively, the effect on the LAR AUC and EAR AUC with combination is complete inhibition. Additionally, our first investigation showed that the combination exhibits a synergistic effect on shifting the allergen PC$_{20}$ whereas previous investigations had shown no difference between the combination of an antihistamine and an LTRA with that of an LTRA alone.

Taken together, the investigations of combined LTRA and antihistamine provide clinical evidence to suggest that there may indeed be a role for this type of treatment in individuals with atopic asthma. A single dose of a combination tablet would benefit individuals who do not require regular controller medication, yet know that exposure to a triggering agent is imminent. Importantly, this excludes individuals whose asthma is triggered by agents that cannot be avoided such as seasonal pollens and house dust mite (*i.e.*, seasonal or perennial asthma). For these individuals, we know from the Roquet *et al.*, [1997] and Richter *et al.*, [2008] data that
there is also benefit with daily use. It seems therefore that a single tablet combining an antihistamine and an LTRA would be beneficial. Interestingly, in 2007, a New Drug Application was made to the FDA by Schering-Plough and Merck Frosst for a combined montelukast and loratadine tablet for the treatment of allergic rhinitis. The application however was denied. [Schering-Plough website]. Perhaps the recent favorable evidence in suppressing allergen induced asthma will spark additional research and a future application to the FDA or the Canadian Therapeutic Products Directorate (TPD) for the treatment of atopic asthma, allergic rhinitis and the co-morbid condition of allergic rhinitis plus atopic asthma.

Insight on the mechanism of action of the combination of desloratadine and montelukast may be appreciated through pharmacological profiling or comparative pharmacology of agents that are more widely understood (Table 6.1). For example, the degree of inhibition on the EAR afforded by the combined action of H1 and cysLT1 airway smooth muscle receptor antagonism that was used in our research, has been similarly documented with β2 agonist, anti-IgE and sodium cromoglycate which is superior to that seen with anti-muscarinics, and single or stable dose inhaled glucocorticosteroids. We know that β2 agonists are functional antagonists of airway smooth muscle contraction. We know that anti-IgE binds free IgE preventing the formation of the allergen•IgE•FcεRI complex and subsequent mast cell degranulation and we know that sodium cromoglycate, at least in part, stabilizes mast cells. Collectively, these different mechanisms reinforce our current understanding of the mechanism of the EAR as being acute bronchoconstriction resulting from mast cell mediator release. Less studied, and less understood, however, is the mechanism of recovery from the EAR. Early investigations, reviewed in Chapter 3, have implicated histamine and the leukotrienes as being responsible for the initial and prolonged phases, respectively, of ex vivo bronchial contractility. Consistent with this, we do observe an earlier maximal response and a more complete recovery with less time taken to achieve complete recovery with the combination therapy (Figure 5.4). Questions that are worth asking are whether the inhibitory effect is occurring through parallel or tandem actions of two independent pathways or through the combined action of two mediators on a single pathway or through non H1 cysLT1 activation. We know that both H1 and cysLT1 receptors are coupled to the same G protein, specifically, G_{q/11} and this may play a role in the suppression of the response via a “taxing” of a common second messenger signaling pathway. Additionally, if H1 and cys
Table 6.1 Comparative Pharmacology of EAR Inhibition (Rank Order)

<table>
<thead>
<tr>
<th>Drug</th>
<th>% inhibition (max % Δ FEV₁)</th>
<th>Dose shift</th>
<th>Fold Δ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₂ agonist (salbutamol)</td>
<td>n/a</td>
<td>3.9</td>
<td>~ 15</td>
<td>Cockcroft et al, 1993</td>
</tr>
<tr>
<td>H₁ Blocker /LTRA (desloratadine/montelukast)</td>
<td>n/a</td>
<td>3.0</td>
<td>8</td>
<td>Davis et al, 2009</td>
</tr>
<tr>
<td>Anti-IgE (omalizumab)</td>
<td>n/a</td>
<td>2.7</td>
<td>6.5</td>
<td>Boulet et al, 1997</td>
</tr>
<tr>
<td>SCG (sodium cromoglycate)</td>
<td>~ 75</td>
<td>2.0</td>
<td>4</td>
<td>Pepys et al, 1974</td>
</tr>
<tr>
<td>LTRA (zafirlukast)</td>
<td>50-75</td>
<td>~ 1.5</td>
<td>3</td>
<td>Roquet et al, 1997</td>
</tr>
<tr>
<td>Regular ICS (beclomethasone dipropionate)</td>
<td>~ 50</td>
<td>1.3-1.5</td>
<td>2.3-3.0</td>
<td>Cockcroft et al, 1995 Swystun et al, 1998</td>
</tr>
<tr>
<td>H₁ Blocker (azelastine)</td>
<td>≤ 46</td>
<td>~ 0.6</td>
<td>~ 1.6</td>
<td>Richter et al, 2008</td>
</tr>
<tr>
<td>Theophylline</td>
<td>25-50</td>
<td>~ 0.7</td>
<td>~ 1.7</td>
<td>Hendeles et al, 1995</td>
</tr>
<tr>
<td>Muscarinic antagonist (ipratropium bromide)</td>
<td>~ 25</td>
<td>0.5</td>
<td>~ 1.5</td>
<td>Cockcroft et al, 1978</td>
</tr>
</tbody>
</table>

n/a = not applicable; these are EAR investigations where an actual dose shift is measured
LTRA = leukotriene receptor antagonist; IgE = immunoglobulin E; SCG = sodium cromoglycate
ICS = inhaled glucocorticosteroids
LT1 receptor antagonism leads to a localized increase in the amount of histamine and leukotrienes, these mediators and their metabolic products (e.g., N-methylhistamine) are free to activate the nearby unopposed H2 - H4 and cysLT2 receptors. This could potentially lead to, for example, a decrease in the amount of histamine and/or leukotrienes released from mast cells through a process of negative feedback regulation via H2 and/or cysLT2 receptors. Bronchodilation via H2 receptors is another possibility as is the initiation of physiological responses via H4 and cysLT2 receptor activation which, with respect to asthma, we know relatively little about.

The mechanism of action of how these agents, both as monotherapy and more importantly as combined therapy, are exerting these positive clinical actions on the LAR warrants further investigation. While it is attractive to suggest that the airway constriction is mediated through the action of histamine and the leukotrienes released from recruited eosinophils and basophils there are mounting data, most of which pertain to eosinophils, that document inhibition of leukocyte recruitment without affecting the LAR. The role of the basophil has not been studied in detail even though this cell is a major source of histamine and a temporal relationship between the LAR and basophil recruitment has been recently shown [Gauvreau et al, 2000]. Undoubtedly there are other cellular sources of histamine and the leukotrienes and cells with high levels of histidine decarboxylase (e.g., dendritic cells and T cells) have been shown to produce histamine de novo the biological role of which is not clear. The in vitro and animal data reviewed in Chapter 2 suggest both desloratadine and montelukast may be exerting anti-inflammatory actions that include decreases in IL-4, IL-5 and IL-13. [Wu et al, 2004; Maeba et al, 2005; Wu et al, 2006; Roumestan et al, 2008]. Based on our current understanding that the LAR is a Th2 driven response involving leukocyte recruitment, orchestrated primarily by IL-4, IL-5 and IL-13, these earlier mechanistic data offer a possible in vivo mechanism. However, there is evidence from investigations of agents which specifically target these cytokines or their receptors that have produced equivocal results on inhibiting the LAR in spite of decreasing inflammatory cell recruitment [Leckie et al, 2000; Wenzel et al, 2007]. Recent preclinical data support a role for histamine and the leukotrienes on dendritic cell and T cell differentiation and function. This is an attractive potential mechanism that requires further investigation. Theoretically, blocking or manipulating the immune and inflammatory
responses upstream of the plethora of mediators and cell signals that occur subsequent to T cell activation may be more effective than trying to target various individual components of a response that amplifies as it proceeds.

In closing, the prevalence of asthma is increasing, and allergies are common triggers of symptoms and exacerbations. Treatment options have undergone few changes of late and investigations of new treatments seem to be focusing on biologics that will be too costly and, for the most part, unnecessary, in the majority of patients. There are two classes of drugs, namely antihistamines, second generation H₁ antihistamines in particular, and leukotriene receptor antagonists, which are currently approved for the treatment of allergic rhinitis and asthma. Our results, together with that of Roquet et al., [1997] and Richter et al., [2008] have provided clinical evidence of superior inhibition of airway responses to allergen in mild atopic asthma when used concomitantly. Whether or not specific combinations are more efficacious than others is yet to be determined but desloratadine in combination with montelukast might be favored based not only on efficacy but on dosing convenience and superior safety profiles in both adults and children.

7.0 FUTURE RESEARCH

We will follow up our early and late response studies using sputum supernatant and peripheral blood serum samples obtained during the LAR investigation to examine changes in these tissues that may help explain our results.

We hope that other researchers in the field will also be inspired by these data to undertake mechanistic investigations that will collectively delineate how the combination provides superior efficacy and advance our knowledge surrounding the role of histamine and the leukotrienes not only in asthma pathophysiology but other inflammatory and immune disorders as well.
8.0 REFERENCES


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Effect of combined montelukast and desloratadine on the early asthmatic response to inhaled allergen

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Keywords: Antihistamine, leukotriene antagonists, allergen inhalation

The response of the airways to inhaled allergen in individuals with atopic asthma is an IgE-mediated mast-cell degranulation process leading to the early asthmatic response (EAR), which peaks about 15 to 30 minutes after inhalation and is often followed by a late asthmatic response (LAR) developing 3 to 8 hours after inhalation. The degranulation process results in the release of multiple mediators, including the leukotrienes and histamine. The EAR is thought to be due to airway smooth muscle contraction, mediated in part by histamine on histamine receptor subtype 1 (H1) receptors and leukotrienes on cysteinyl leukotriene receptor subtype 1 receptors. It follows that blocking the activity of these mediators might prevent bronchoconstriction. Investigations of the effect of H1 blockers on the EAR have produced variable and inconclusive results. Leukotriene modifiers have been shown to provide reasonable inhibition of the EAR and LAR but do not completely abolish the response. The idea that a mechanism involving multiple mediators might require multiple interventions continues to attract interest in the role of combination therapies for the treatment of atopic asthma. Desloratadine is an antihistamine that has not been clinically investigated in atopic asthma and the response to inhaled allergen. Montelukast is effective in partially attenuating the response to inhaled allergen. There are no published data reporting the effect of the combination of these 2 therapies on allergen-induced airway responses.

METHODS

Subjects

Two healthy atopic asthmatic subjects older than 18 years and with a baseline FEV1 of 65% of predicted value or greater participated in the study (Table I). Subjects had no respiratory infection or allergen exposure for 4 or more weeks before enrollment. The protocol was approved by the University of Saskatchewan Biomedical Ethics Research Board, and subjects provided written consent.

Salbutamol (n = 10) was withheld before testing for 8 or more hours. Fluticasone was used (stable dose for 3 months) by one subject. No subjects used any other asthma therapies or antihistamines.

Study design

This was a randomized, 4-way crossover, placebo-controlled trial (dexamethasone, 188 mg) investigation. Matching placebo tablets were
TABLE I. Patient demographics

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (y)</th>
<th>Height (inches)</th>
<th>Baseline FEV₁ (L)</th>
<th>% Predicted</th>
<th>Allergen</th>
<th>MCP₁₅ (mg/mL)</th>
<th>Mediations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>57</td>
<td>66</td>
<td>2.46</td>
<td>73</td>
<td>Grass*</td>
<td>0.5</td>
<td>S, prn</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>29</td>
<td>70</td>
<td>3.87</td>
<td>87</td>
<td>HDM†</td>
<td>2</td>
<td>S, prn</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>27</td>
<td>71</td>
<td>3.67</td>
<td>86</td>
<td>Cat†</td>
<td>0.67</td>
<td>S, prn</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>24</td>
<td>62</td>
<td>2.58</td>
<td>83</td>
<td>Cat†</td>
<td>0.28</td>
<td>S, prn</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>25</td>
<td>72</td>
<td>4.07</td>
<td>83</td>
<td>HDM†</td>
<td>2</td>
<td>S, prn</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>40</td>
<td>72</td>
<td>2.66</td>
<td>65</td>
<td>Grass*</td>
<td>1.8</td>
<td>S, prn</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>25</td>
<td>66</td>
<td>3.12</td>
<td>89</td>
<td>HDM†</td>
<td>2.5</td>
<td>S, prn</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>43</td>
<td>68</td>
<td>3.44</td>
<td>88</td>
<td>Grass*</td>
<td>16</td>
<td>S, prn</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>27</td>
<td>64</td>
<td>3.35</td>
<td>81</td>
<td>Cat†</td>
<td>0.86</td>
<td>S, prn</td>
</tr>
<tr>
<td>108</td>
<td>M</td>
<td>26</td>
<td>72</td>
<td>4.59</td>
<td>56</td>
<td>Cat†</td>
<td>0.75</td>
<td>S, prn</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>32</td>
<td>68</td>
<td>3.41</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S: Subcutaneous; prn, as required; F: Furosemide prophylactically, bid, twice daily.
*Grass mix 10 480,000 (IgE) human allergen metabolites.
†Standard rate mix (30,000 U/mL)
‡Bisected or pol (10 000 (IgE) metabolites).
§Not included in analysis.

Abbreviations used
- AUC: Area under the curve
- EAR: Early asthmatic response
- H₁: Histamine receptor subtype 1
- LAR: Late asthmatic response
- LTRA: Leukotriene receptor antagonist
- PC₉₀: Concentration of allergen that causes a 20% decrease in FEV₁

unavailable. Subjects were provided with 2 small brown envelopes containing either 2 placebo tablets, 1 desloratadine tablet (5 mg) and 1 placebo tablet, 1 montelukast tablet (10 mg) and 1 placebo tablet, or 1 desloratadine tablet and 1 montelukast tablet. Subjects were instructed to ingest the contents of one envelope without looking at them 26 hours before and the other 2 hours before allergen challenges, which were scheduled at intervals of 7 days or longer. No subject had previously used desloratadine, and only one subject had previously used montelukast. The investigator was blind to the treatments.

Allergen challenges
Subjects were challenged during the nonpollen season (ie, December through March) with the allergen that produced the largest response on skin prick testing. Serial 2-fold dilutions were prepared from 6.9 stock solutions diluted with sterile isotonic saline containing 0.4% glycerol. Starting concentrations for inhalation were determined with the use of an algebraic prediction of allergen PC₉₀ by using the skin test end point and airway responsiveness to methacholine. Allergen challenges began with the same concentration for each individual on each occasion. Allergens (Western Allergy Services, Victoria, British Columbia, Canada; see Table I) were aerosolized through a Wright Nebulizer (Ross-Meditech Ltd, Montreal, Quebec, Canada) calibrated to deliver 0.1 mL/min. Each concentration was inhaled during 2 minutes of tidal breathing through a mouthpiece and with nose clips in place. Ten minutes after each inhalation was completed, 2 technically acceptable FEV₁ maneuvers were performed 60 seconds apart. Exhalations were continued until

the highest postinhalation FEV₁ was at least 15% lower than the highest baseline FEV₁ (obtained from 3 reproducible flow-volume loops after a 20-minute rest period) or until the top concentration had been administered. The FEV₁ measurement was repeated at 10-minute intervals until no further decrease was observed. The allergen PC₉₀ was then calculated algebraically.12 Salbutamol (200 μg) was administered to reverse the acute bronchoconstriction. Fluticasone (500 μg) was administered to prevent the late response and associated increase in airway responsiveness.14

Data analysis
Baseline FEV₁ data and log-transformed allergen PC₉₀ data were analyzed using 2-way ANOVA, followed by pairwise comparison (least-squared difference) of means if applicable (Systat Version 7.0; Analytical Software Corp, Tallahassee, Fla). Power calculations9 showed a 90% power to detect a 1 doubling concentration difference in allergen PC₉₀ in 10 subjects (and a 97% power in 9 subjects).

RESULTS
All 10 subjects completed the study with no adverse events. A post hoc decision to exclude the data of 1 subject (no. 10) was made on the basis of the observation that the screening allergen challenge and the placebo treatment allergen challenge were not reproducible. The overall significance of the data was not affected.

Mean ± SD baseline FEV₁ measurements after placebo (3.24 ± 0.55 L), desloratadine (3.25 ± 0.54 L), montelukast (3.27 ± 0.54 L), and combination (3.31 ± 0.57 L) therapy were not significantly different from each other (P = .19, ANOVA).

Comparison of geometric mean allergen PC₉₀ differences between combination (697 U/mL), montelukast (338 U/mL), desloratadine (123 U/mL), and placebo (104 U/mL) treatments was highly significant (P < .00001, ANOVA; Fig 1). The mean ± SEM log values after combination, montelukast, desloratadine, and placebo
treatments were 2.8433 ± 0.3253, 2.5295 ± 0.2979, 2.0883 ± 0.2102, and 2.0166 ± 0.2553, respectively. Compared with placebo (least-squared difference comparison of means), combination therapy and montelukast therapy significantly increased allergen PC_{20} (P < .001 for both). Allergen PC_{20} with combination therapy was significantly greater than with montelukast alone (P = .02), and that with montelukast alone was significantly greater than that with desloratadine alone (P < .002). Desloratadine and placebo treatments produced similar results (P > .2). Analysis of individual fold increases in allergen PC_{20} (combination vs placebo, montelukast vs placebo, and desloratadine vs placebo) indicated mean fold increases of 8.9, 4.8, and 1.4-fold or 2.2, 2.3, and 0.49 doubling concentrations, respectively.

**DISCUSSION**

We have demonstrated that 5 mg of desloratadine administered 26 hours and 2 hours before allergen inhalation does not protect against the EAR. We have also demonstrated that 10 mg of montelukast administered 26 hours and 2 hours before allergen inhalation increases allergen PC_{20} by 2.3 doubling concentrations, whereas the combination increases allergen PC_{20} by 3.2 doubling concentrations.

Desloratadine is the principal metabolite of the second-generation antihistamine loratadine. Although loratadine had previously been shown to be ineffective in decreasing the EAR, desloratadine possesses superior pharmacokinetic and pharmacodynamic properties, has an excellent safety profile, and has been shown to exert anti-inflammatory effects. The lack of efficacy after desloratadine therapy reported here suggests that desloratadine alone does not protect against the EAR by blocking H_{1} receptors on airway smooth muscle.

Montelukast is a leukotriene receptor antagonist (LTRA) with proved efficacy in asthma and exercise-induced bronchoconstriction and has been shown to significantly decrease the EAR to inhaled allergen. Our data are consistent with those in the existing literature on the effect of montelukast on the EAR, although we do show a slightly greater inhibition of the EAR after montelukast when changes in the maximal decrease in FEV_{1} are equal to changes in doubling concentrations. In our study montelukast shifts the dose-response curve versus placebo more than 2 doubling concentrations. This is equivalent to a 75% decrease (vs an average of around 55% reported previously) in the maximal decrease in FEV_{1}.

It is logical to postulate that the EAR to inhaled allergen could be additive or synergistically blocked by combining cysteinyl leukotriene receptor subtype 1 and H_{1} receptor antagonist therapies. This effect was demonstrated in vitro more than 10 years ago in isolated human bronchi. Since then, only 2 studies investigating the effect of combination (antihistamine plus LTRA) therapy on the EAR could be identified. Roquet et al. investigated...
the combination of high-dose zafirlukast (80 mg twice daily) and loratadine (10 mg twice daily) administered for 8 days on the EAR and LAR and found that combination therapy was more effective at inhibiting the EAR than either drug alone; however, the difference between the inhibition after zafirlukast was not significantly different from the inhibition after combination therapy. A more recent in vitro investigation of the H1 antihistamine chlorpheniramine and the LTRA MK-571 documented a synergistic effect of the combination versus either drug alone in allergic isolated human bronchi.24 We could identify no published data describing the effect on allergen-induced airway responsiveness after montelukast in combination with an antihistamine.

We provide evidence that the combination of 5 mg of desloratadine with 10 mg of montelukast administered at 26 hours and 2 hours before allergen inhalation increases allergen PC20 by 3.2 doubling concentrations (8.9-fold) versus placebo. This is the first evidence of a clinically significant inhibition of the EAR with clinically relevant doses of the combination of an antihistamine and an LTRA that is significantly greater than the inhibition with an LTRA alone. Because no effect was observed with desloratadine alone, the significantly greater combined efficacy is difficult to interpret. The magnitude of inhibition is similar to that achieved with 10 mg of inhaled sodium cromoglycate (76%), lower than that with 200 μg of salbutamol (97%), and substantially greater than that with single-dose (200 μg) beclomethasone dipropionate (<15%) reported as maximal decreases in FEV1,25 suggesting mast-cell stabilization as a possible mechanism.

There are several methods to assess the EAR, including area under the curve (AUC40-90), maximal decrease in FEV1, and allergen PC20. These differences in methodology present difficulties in data comparison and reinforce the issue of method standardization. Efficacy reported as AUC40-90 represents changes in the airway that are occurring over 180 minutes, two thirds of which represents the spontaneous recovery portion of the EAR and one third of which represents the development of and the maximal response to the sensitizing agent. Comparison of AUC40-90 data with data reported as changes in the maximal decrease in FEV1 or allergen PC20, both of which measure changes occurring in the first 60 minutes, is therefore extremely difficult. Reporting the EAR as maximal changes in FEV1 requires the same dosing regimen and occasionally single-dose administration of allergen. Although single-dose administration protocols might raise concerns surrounding subject safety, the change in maximal decrease in FEV1 can, at least in theory, be compared with the algebraic determination of allergen PC20. Assuming a linear dose response, we estimate that a shift of 1 doubling concentration is approximately equivalent to a 50% change in the maximal decrease in FEV1, a shift of 2 doubling concentrations is approximately a 75% change, a shift of 3 doubling concentrations is approximately an 87.5% change, and so on. Therefore allergen PC20 reporting provides a more precise discrimination between treatments that generate a 50% or greater change in the maximal decrease in FEV1. The one major limitation to assessing airway response to inhaled allergen with this method is that it cannot be used to assess the LAR. Therefore the relevance of these data to the clinical features and management of asthma remains to be determined.

We present in vivo data supporting a synergistic effect of the combination of montelukast and desloratadine on the early response to inhaled allergen. The mechanism or mechanisms underlying the apparent synergism and the effect of the combination of montelukast and desloratadine on the LAR and associated events require further investigation.

REFERENCES
9.2  APPENDIX B:  Ethics Documentation for the Early Asthmatic Response Study

9.2.1  Researcher’s Summary

UNIVERSITY OF SASKATCHEWAN
RESEARCH ETHICS BOARD
(Biomedical)

http://wwwusask.ca/research/ethics.shtml

ORS USE ONLY

Date received: ___________________

File Number: ___________________

RESEARCHER’S SUMMARY

PROJECT TITLE:
Effects of combined leukotriene (montelukast), histamine (desloratadine) antagonism on the early response to inhaled allergen.

PRINCIPAL INVESTIGATOR: Dr. D.W. Cockcroft

DEPARTMENT: Medicine, Division of Respiratory Medicine

SUB-INVESTIGATOR(S): __________________________________________

DEPARTMENT: _________________________________________

RESEARCH WILL BE CONDUCTED AT: Room 346, Ellis Hall

________________________________________________________________________

1. Hypothesis (State briefly the nature and purpose of the research proposal, and the proposition the research is seeking to uphold. What potentially useful knowledge or clarification
about therapeutic options will be advanced to justify the participation of human subjects in this research project?):

Leukotriene receptor antagonism in combination with histamine receptor antagonism will provide greater protection against allergen induced bronchoconstriction than either drug administered alone.

2. Academic Validity (Provide evidence that the scientific reasoning and design of the project are sufficiently sound to meet the objectives of this project. Provide your own comments and those resulting from peer review. Indicate if any committee or other body has assessed the project’s scientific validity):

Mediators released during mast cell degranulation include the leukotrienes and histamine. Allergen exposure triggers mast cell degranulation in atopic asthmatics and is responsible for the early asthmatic response (EAR). Receptor antagonists that selectively block the binding of these endogenous autocoids should prevent or at least decrease the EAR. The leukotriene receptor antagonist (LTRA) montelukast is effective in the treatment of asthma (non-atopic) and the histamine antagonist (HA) desloratadine is effective in the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria. The current indications of these medications, speculation of possible anti-inflammatory/immunomodulatory actions and the multi-mediated mechanism of the EAR suggest that combined therapy would be beneficial in preventing the EAR.

3. Funding (indicate the source of funds supporting the research. If externally funded, state whether the grant or contract is still in application, or has already been awarded):

Supply of study medication and placebo has been requested from Merck Frosst (montelukast and matching placebo) and Schering Plough (desloratadine and matching placebo). No formal funding is expected.

4. Disclosure of Potential Conflicts of Interest (indicate any motivation or incentives for conducting this study that arise external to the objectives of the study, e.g., will the investigator
or institution be paid to conduct this research project? Note: The consent form should also include an introductory disclosure of potential conflict of interest statement indicating that this is a medical research study for which the study doctor is being paid to conduct):

N/A

5. Subjects (target population e.g., age, gender, medical condition, target enrollment at this site, proposed strategies that will be used to recruit to this study):

Twelve well controlled atopic asthmatics 18 years of age or older. Recruitment will likely be completed with individuals who have previously participated in research and have agreed to being contacted for future projects. Poster advertisement around campus as well as the university hospital may be undertaken. An ad may also be placed in the university newspaper.

6. Procedures (clearly identify treatment allocation design, and describe the medical and other procedures to be followed in obtaining research data):

The study design will be randomized, four way, placebo controlled, double blind, double dummy. Two doses of each antagonist (10mg montelukast and 5mg desloratadine) will be administered 24 hours apart. The second dose will be given two hours prior to each allergen challenge and the first dose will be given 26 hours prior to each allergen challenge. Investigations will include baseline spirometry, methacholine challenge test, and skin prick testing for eligibility. Continuing subjects will undergo a baseline allergen challenge prior to randomization. Data collection (allergen PC20) measurements will be conducted on four occasions with a minimum of seven days between challenges.

7. Time Period (indicate the dates when the research project is expected to begin and to be completed. A final status report must be filed with the Office of Research Services once data collection from the last subject is complete. ORS should be notified once the study site is closed):

8. Consent Form (include a copy of the study information / consent form that will be used, or give reasons if one is not being used):

Attached.

9. By signing below, the Principal Investigator is assuring the Biomedical Research Ethics Board that the Department Head (or corresponding senior administrator) has received a copy of this Researchers' Summary Form. (NOTE: This policy will function in lieu of the previous policy that required countersigning of this Researchers' Summary Form by the Department Head).

Dr. Donald Cockcroft 966-8346
Principal Investigator Phone

966-8694 cockcroft@sask.usask.ca
Fax E-mail

10. Contact Person and Mailing Address for Correspondence:

Beth Davis c/o Dr. Cockcroft, Division of Respiratory Medicine, 5th Floor Ellis Hall, 103 Hospital Drive, Saskatoon, SK S7N 0W8. Phone: 966-8290. E-mail: davisb@sask.usask.ca
INTRODUCTION

You are being invited to participate in a research project because you have allergies that trigger your asthma. Currently available asthma (montelukast) and allergy (desloratadine) medications and their combined effect on people who have allergies that trigger their asthma will be investigated.

VOLUNTARY PARTICIPATION

Your participation is entirely voluntary and it is up to you to decide whether or not you want to take part in this project. Before you decide, it is important for you to understand what the research involves. This document will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. If you decide to take part in this study you may still withdraw at any time and without giving any reason. If you decide not to participate, you do not have to provide any reason for your decision and you will not lose any medical care to which you are entitled or are presently receiving.
Please take time to read the following information carefully and to discuss it with your family, friends, and/or doctor before you decide.

PURPOSE

Montelukast (for asthma) and desloratadine (for allergies) are effective therapy for their current uses. Part of what happens when your allergies trigger your asthma should be prevented by either of these drugs. This project is being conducted to determine if these drugs are effective, either alone or in combination, on controlling asthma that is triggered by allergies.

PROCEDURES

The duration of the study is approximately 6 weeks and can be divided into two phases, a screening phase (testing to determine if you qualify) and a treatment phase (collection of the data to be analyzed). You will be required to attend the lab on at least 7 occasions during the course of the study. The duration of each visit will vary. Screening procedures will occur over three days. The first day will require about one hour of your time. The second and third days will require two to three hours. The treatment phase visits could last up to four hours and there are four visits. If you decide to participate in this research project, you will be required to complete the following testing:

A. Breathing Tests

Breathing tests will be conducted at all visits. You will be required to blow into a machine which you hold in your hand. The machine has a mouthpiece attached to it which you will place in your mouth and will inhale and exhale through. You will also wear noseclips. The air that goes through the machine is measured by a software program that displays the results on a computer screen.
B. Skin Prick Tests

The skin prick test will be conducted once. This will involve small droplets of common allergens (animals, pollens, etc.) being placed on your forearm. A small scratch within the droplet will be performed which will determine if you have an allergy to a particular allergen. If so, a small bump similar to a mosquito bite will appear and will likely be red and itchy.

C. Methacholine Inhalation Test

The methacholine inhalation test will be conducted once. This will involve breathing maneuvers as described above. In addition, you will be required to inhale a substance called methacholine which may cause your airway to constrict. You will be inhaling increasing concentrations of methacholine by placing a mask over your nose and mouth. The mask is attached to an aerosol generating piece of equipment which functions to provide an inhalable solution of the substance. You will inhale the substance by breathing normally for two minutes. Any constriction that may result will be monitored by the breathing maneuvers. When and if a certain value is reached (20% decrease), the test will be stopped and any constriction that has occurred will be reversed with a bronchodilator (Ventolin®) or you may choose to let the constriction reverse on its own. Either way, your breathing must return to within 10% of the value it was when the test began before you may leave the lab. You may also stop the test at any time for any reason.

D. Skin test endpoint

The skin test endpoint is identical to the skin prick test except that one allergen of various concentrations is placed on the forearm, scratched and monitored for response. This test will be done once.

E. Allergen Challenge Test

The allergen challenge test will be performed on five occasions. Each allergen challenge will be separated by at least 7 days. Again, breathing maneuvers will be performed as described above
and will be used to monitor any constriction that occurs. You will be required to inhale increasing concentrations of one of the allergens identified in the skin prick test. The inhalation is done through a mouthpiece with a noseclip on for two minutes of normal breathing. When the constriction in your airways reaches a certain value (15%) the test will be stopped. Bronchodilator (Ventolin®) and inhaled steroid (Flovent®) will be administered to reverse the immediate constriction and to prevent subsequent inflammation and constriction.

F. Administration of the drugs, montelukast and desloratadine

This is a placebo controlled study. Placebo controlled means there are tablets that look like the active drug but do not contain any active drug. There are four separate treatments and you will be required to complete all four. The order in which you complete each treatment will be random. Neither you nor the study personnel will know what treatment you are taking. This information can however be obtained if necessary.

Both montelukast and desloratadine are currently available by prescription under the trade names Singulair® and Aerius® (CAN)/Clarinex® (US) respectively. The recommended dosage is 10mg a day for Singulair® and 5mg a day for Aerius®. This will be the dose you will be required to take two times before each allergen challenge. The first dose will be taken 26 hours before the challenge and the second dose will be taken 2 hours before each allergen challenge.

RISKS AND DISCOMFORTS

A. Methacholine and allergen inhalation tests

The inhalation challenges may cause bronchoconstriction and manifest symptoms such as chest tightness, wheeze, cough and shortness of breath. Your breathing will be monitored and any discomfort that may result can and will be reversed by bronchodilator medication.
B. Study medication

The treatment medication, bronchodilator medication and anti-inflammatory medication are all available through prescription. The doses that will be administered to you in this research project do not exceed recommended daily dosages and the possible side effects from these medications are not expected. Some of the more common side effects that have been reported with the use of these medications are listed below.

Singulair® - headache (~18%), cough, dizziness, fatigue, rash, fever (~all < 5%)
Desloratadine® - headache (~6%), dry mouth (~3%), fatigue (~3%)
Ventolin HFA® - throat irritation (10%), cough (5%), viral respiratory infection (7%)
Flovent HFA® - throat irritation (4%), hoarseness (6%), oral candidiasis (4%)

There is also the potential for unforeseen or unknown risks.

The medication or treatment used in this study may pose a risk to developing fetuses or to babies who are being breastfed. If you are a sexually active woman and are of childbearing potential (sexually mature woman who has not undergone a hysterectomy or who has not been post-menopausal for 24 consecutive months), you must do one of the following while participating in this study: use a medically approved effective method of birth control or abstain from sexual intercourse that could result in pregnancy. If you plan to become pregnant during the course of this project you should not participate. If you are currently breastfeeding, you are not eligible to participate in this study.

CONFIDENTIALITY

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this
research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.

RESEARCH-RELATED INJURY

There will be no costs to you for participation in this study. You will not be charged for any research procedures. In the event that you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

BENEFITS OF STUDY PARTICIPATION

No one knows whether or not you will benefit from participation in this research study. There may or may not be direct benefits to you if you decide to participate. We hope that the information learned from this study can be used in the future to benefit other people with a similar disease.

NEW FINDINGS

If new information about either of the study medications becomes available that may influence your willingness to participate in this research project, this information will be provided to you by the study personnel.

VOLUNTARY WITHDRAWAL

Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to participate and then withdraw before completing all study procedures there will be no penalty or loss of benefits to which you are entitled. Your future medical care will not be affected. If you are a student, your academic status will not be affected.
WITHDRAWAL INITIATED BY INVESTIGATOR

The study investigator may decide to discontinue the study at any time, or withdraw you from the study at any time if it is felt to be in your best interest.

STORAGE OF DATA

The data will be stored in the lab where it is collected for a period of 5 years or more as per University regulations.

WHO TO CONTACT FOR QUESTIONS ABOUT THE STUDY

If you have any questions about this study, the procedures or treatment involved, or if you would like additional information, before or during the study, you may contact study personnel at 966-8290 or the investigator, Dr. Cockcroft, at 966-8346. Study personnel can be reached 24 hours at 229-8709.

WHO TO CONTACT ABOUT YOUR RIGHTS AS A RESEARCH SUBJECT

If you have any questions, concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, you should contact the Chair of the Biomedical Research Ethics Board, c/o the Office of Research Services, University of Saskatchewan at (306) 966-4053.

HONORARIA AND REIMBURSEMENT

You will receive an honorarium ($325.00) for participating in this research project that will cover any costs you incur (e.g., parking) as well as compensate you for the time and inconvenience of being a research subject. In the event that you begin the study and subsequently withdraw before completing the study, your honorarium will be adjusted accordingly.
CONSENT

I have read the information sheet regarding this research project.
I have had sufficient time to consider the information provided.
I have had the opportunity to ask questions about this project and to receive satisfactory answers to my questions.
I understand that the information will be kept confidential and that the results will only be used for scientific purposes.
I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
I understand that I am not waiving any of my legal rights as a result of signing this consent form.
I have read the information presented to me and I freely consent to participate in this study.
I have been told that I will receive a dated and signed copy of this form.

Participant signature __________________________ Print ___________ Date ___________

Signature of person administering Consent __________________________ Print ___________ Date ___________
9.2.3 Certificate of Approval

University of Saskatchewan
Biomedical Research Ethics Board (Bio-REB) 19-Jan-2004

Certificate of Approval

PRINCIPAL INVESTIGATOR
Donald W. Cockcroft

DEPARTMENT
Medicine (Respirology)

BMC #
03-1305

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Ellis Hall
Respiratory Medicine
103 Hospital Drive
Saskatoon SK S7N 0W8

SPONSORING AGENCIES
UNFUNDED

TITLE:
Effects of Combined Leukotriene (Montelukast), Histamine (Desloratadine) Antagonism or the Early Response to Inhaled Allergen

ORIGINAL APPROVAL DATE
19-Jan-2004

CURRENT EXPIRY DATE
01-Jan-2005

APPROVAL OF
Protocol as submitted
Information Sheet and Consent Form v.3 (19 Jan 04)

CERTIFICATION
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above-named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project, and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/research/ethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

APPROVED.

University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:
Office of Research Services, University of Saskatchewan
Room 340, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4S8
Phone: (306) 966-4053 Fax: (306) 966-2869

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9.3 Appendix C: Study flowchart for the Early Asthmatic Response Study

<table>
<thead>
<tr>
<th>PHASES</th>
<th>SCREENING</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>VISIT</td>
<td>1 2 3 4 5 6 7</td>
<td></td>
</tr>
<tr>
<td>DAY</td>
<td>-10 -9 -8 0 8 16 24</td>
<td>24</td>
</tr>
</tbody>
</table>

Consent Form ✓
Demographics ✓
Inclusion/Exclusion ✓
Concomitant Meds ✓ ✓ ✓ ✓ ✓ ✓ ✓
Spirometry ✓ ✓ ✓ ✓ ✓ ✓ ✓
SPT ✓
MCT ✓
ACT ✓ ✓ ✓ ✓ ✓ ✓ ✓
STE ✓

Study Meds 26 hours pre ACT 26 hours pre ACT 26 hours pre ACT 26 hours pre ACT

Safety Meds ✓ ✓ ✓ ✓ ✓ ✓

SPT = skin prick test
MCT = methacholine challenge test
ACT = allergen challenge test
STE = skin test endpoint
Safety meds = 125 – 250 mcg fluticasone propionate; 100-200mcg salbutamol
Montelukast and desloratadine synergistically inhibit the allergen-induced early asthmatic response. Montelukast also suppresses the allergen-induced late asthmatic response, but there are no reports on the effect of desloratadine or the combination on the allergen-induced late asthmatic response. Atopic asthmatics (n = 10) completed a multicentric randomised double-blind crossover study comparing single-dose placebo, 5 mg desloratadine, 10 mg montelukast and the combination administered 2 h prior to allergen inhalation challenge. Methacholine challenges were performed 24 h before and after allergen challenge. Exhaled nitric oxide measurements and sputum inflammatory cell counts were also carried out. All active treatments significantly decreased the late asthmatic response area under the curve. Combination therapy provided the greatest inhibition compared to desloratadine and montelukast. Montelukast was nonsignificantly better than desloratadine but not as effective as the combination. There was a trend towards a decrease in airway responsiveness following montelukast and combination. Montelukast, but not desloratadine or the combination, decreased exhaled NO levels 24 h after allergen. The allergen-induced increase in sputum eosinophil numbers was significantly suppressed at 7 h with desloratadine and combination therapy, and at 24 h with montelukast and combination therapy. Single-dose co-administration of desloratadine and montelukast 2 h prior to allergen inhalation clinically abolished the late asthmatic response and eosinophil recruitment.

PMID: 19164343 [PubMed - indexed for MEDLINE]
9.5 APPENDIX E: Ethics Documentation for the Late Asthmatic Response Study

9.5.1 Researcher’s Summary

Biomedical Research Ethics Board (Bio-REB)

RESEARCHER’S SUMMARY FORM

http://www.usask.ca/research/ethical.shtml

REB File Number: ______

PROJECT TITLE: Changes in airway responses to inhaled allergen following pharmacological inhibition of histamine and CysLT1 blockade in mild atopic asthma

PRINCIPAL INVESTIGATOR: Dr. D.W. Cockcroft

DEPARTMENT: Medicine, Division of Respiratory, Critical Care and Sleep Medicine

SUB-INVESTIGATOR(S):

DEPARTMENT:

RESEARCH WILL BE CONDUCTED AT: Room 346 Ellis Hall

1. Hypothesis (State briefly the nature and purpose of the research proposal, and the proposition the research is seeking to uphold. What potentially useful knowledge or clarification about therapeutic options will be advanced to justify the participation of human subjects in this research project?)

   Leukotriene receptor antagonism in combination with histamine receptor antagonism will provide greater protection against airway responses to inhaled allergen than either drug administered alone.
2. Academic Validity (Provide evidence that the scientific reasoning and design of the project are sufficiently sound to meet the objectives of this project. Provide your own comments and if possible those resulting from peer review. Indicate if any other committee or agency has assessed the project’s scientific validity):

Airway responses to inhaled allergen in atopic asthmatics include the early asthmatic response (EAR), the late asthmatic response (LAR) and increased airway responsiveness (AHR) to direct acting stimuli such as methacholine. The LAR occurs in about 50% of individuals with atopic asthma and is influenced by the magnitude of the EAR. IgE mediated mast cell degranulation is well documented as the mechanism triggering the EAR. Mediators released during mast cell degranulation include the leukotrienes and histamine. Recent evidence has shown superior efficacy against the early asthmatic response following combination therapy with montelukast and desloratadine. (BMC#03-1305; Davis BE, Todd DC, Cockcroft DW, J Allergy Clin Immunol. 2005 Oct;116(4):768-72). The mechanism surrounding this apparent synergism is unknown. Investigations into the effect of this particular combination of mast cell mediator inhibition on the late asthmatic response, including changes in induced sputum content, exhaled nitric oxide, leukocyte trafficking, and the subsequent airway response to methacholine are needed to extend the data surrounding the mechanism of action and potential therapeutic benefit.

If applicable, please indicate whether TPD approval has been obtained:

Yes _____  No _____  Pending _____  N/A __x__

3. Funding (indicate the source of funds supporting the research. If externally funded, state whether the grant or contract is still in application, or has already been awarded):

Internally funded.
4. Disclosure of Potential Conflicts of Interest (indicate any motivation or incentives for conducting this study that arise external to the objectives of the study, e.g., will the investigator or institution be paid to conduct this research project? Note: The consent form should also include an introductory disclosure of potential conflict of interest statement, where applicable, indicating that this is a medical research study for which the study doctor, the institution, or both are being paid):

N/A

5. Subjects

a) Target Population (e.g., age, gender, medical condition, target enrollment, significant inclusion/exclusion criteria):

Nine-twelve well controlled atopic asthmatics 18 years of age or older, male or female.

Inclusion:
Baseline FEV1 ≥ 65% predicted
Methacholine PC20 ≤ 16mg/ml
EAR ≥ 20%
LAR ≥ 15%

Exclusion:
Respiratory infection within 4 weeks of screening visit
Diagnosis of another respiratory disease other than asthma
Pregnant or lactating

b) Proposed Strategies for Recruitment (e.g., use of advertisements, brochures, physician patient records):
Recruitment will likely be completed with individuals who have previously participated in research and have agreed to being contacted for future projects. Poster advertisement around campus as well as the university hospital may be undertaken. An ad may also be placed in the university newspaper. Any advertising will be submitted to the REB for approval prior to posting.

6. Procedures (clearly identify treatment allocation design, and describe the medical and other procedures to be followed in obtaining research data, including questionnaires):

The study design will be a randomized, four way crossover, placebo controlled study. Treatments will be administered (oral) 2 hours prior to allergen challenges. Subjects will be required to undergo skin prick tests, blood tests, methacholine challenges and allergen challenges, as well as perform spirometry measurements, exhaled nitric oxide measurements and sputum induction.

See attached appendices for specific details.

7. Time Period (indicate the dates when the research project is expected to begin and to be completed. A final status report must be filed with the Ethics Office once data collection from the last subject is complete. The Ethics Office should be notified once the study site is closed.):

November 2006 - November 2007

8. Data Storage (In accordance with recommended guidelines provide a statement outlining the procedures you will use to store securely the research data. State how long and where the data will be stored and identify the person who will be assuming responsibility for data storage):

Dr. Cockcroft will store the data in Room 346 Ellis Hall for at least five years.
9. Consent Form (include a copy of the consent form and/or any study information that will be used. If not using a consent form give reasons why).

Attached

10. Signatures

______________________________ 8346 _____________________________
Principal Investigator     Phone

8294 _____________________________ cockcroft@sask.usask.ca
Fax                e-mail

______________________________
Department Head, Dean, Director, or Administrative Head

10. Contact Person and Mailing Address for Correspondence:

Beth Davis c/o Dr. Cockcroft, Division of Respiratory Medicine, 5th Floor Ellis Hall, 103 Hospital Drive, Saskatoon, SK S7N 0W8. Phone: 966-8290. E-mail: beth.davis@usask.ca
INTRODUCTION

You are being invited to participate in a research project because you have allergies that trigger your asthma. Currently available asthma (montelukast) and allergy (desloratadine) medications and their combined effect on people who have allergies that trigger their asthma will be investigated. We expect to enroll 9-12 people in this study. The University of Saskatchewan and/or Dr. Cockcroft are not being paid to conduct this study.

VOLUNTARY PARTICIPATION

Your participation is entirely voluntary and it is up to you to decide whether or not you want to take part in this project. Before you decide, it is important for you to understand what the research involves. This document will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you wish to participate, you will be asked to sign this form. If you decide to take part in this study you may still withdraw at any time and without giving any reason.
If you decide not to participate, you do not have to provide any reason for your decision and you will not lose any medical care to which you are entitled or are presently receiving. Please take time to read the following information carefully and to discuss it with your family, friends, and/or doctor before you decide.

PURPOSE

Montelukast (for asthma) and desloratadine (for allergies) are effective therapy for their current uses. Part of what happens when your allergies trigger your asthma should be prevented by either of these drugs. This project is being conducted to determine if these drugs are effective, either alone or in combination, on controlling asthma that is triggered by allergies.

PROCEDURES

The duration of the study is approximately 6 weeks and can be divided into two phases, a screening phase (testing to determine if you qualify) and a treatment phase (collection of the data to be analyzed). You will be required to attend the lab on at least 15 occasions (3 consecutive day visits on 5 different occasions) during the course of the study. The duration of each visit will vary. Screening procedures will occur over three days. The first and third days will require about two hours of your time. The second day will require about nine hours of your time. The treatment phase visits will require these same time commitments. If you decide to participate in this research project, you will be required to complete the following testing:

A. Breathing Tests

Breathing tests will be conducted at all visits. You will be required to blow into a piece of equipment that you hold in your hand. The equipment has a mouthpiece attached to it which you will place in your mouth. You will inhale and exhale through the mouthpiece with nose clips on. The air that goes through the machine is measured by a software program that displays the results on a computer screen.
B. Skin Prick Tests

The skin prick test will be conducted once. This will involve small droplets of common allergens (animals, pollens, etc.) being placed on your forearm. A small scratch within the droplet will be performed which will determine if you have an allergy to a particular allergen. If so, a small bump similar to a mosquito bite will appear and will likely be red and itchy.

C. Skin test endpoint

The skin test endpoint is identical to the skin prick test except that one allergen (chosen from the results of the skin prick test) of various concentrations is placed on the forearm, scratched and monitored for response. This test will be done once.

D. Exhaled nitric oxide

We will measure the amount of nitric oxide that you exhale through another piece of equipment that is also attached to a mouthpiece. You will need to place the mouthpiece in your mouth, inhale until your lungs are full and exhale through the mouthpiece. You do not need to wear noseclips for this test. This measurement is an indication of airway inflammation.

E. Methacholine Inhalation Test

The methacholine inhalation test will be conducted a minimum of 10 times. This will involve breathing maneuvers as described above. In addition, you will be required to inhale a substance called methacholine which may cause your airway to constrict. You will be inhaling increasing concentrations of methacholine by placing a mask over your nose and mouth. The mask is attached to an aerosol generating piece of equipment which functions to provide an inhalable solution of the substance. You will inhale the substance by breathing normally for two minutes. Any constriction that may result will be monitored by the breathing maneuvers. When and if a certain value is reached (20% decrease), the test will be stopped.
F. Sputum induction

After the methacholine challenge test, you will be given two puffs of Ventolin®. You will then be required to inhale hypertonic (salty) saline solutions which should help you produce secretions (sputum) from your airways. You do not need to wear noseclips. You will inhale the solutions through a mouthpiece. You will spit the sputum into a cup. The sputum is then processed and analyzed for changes that are occurring with the cells in your airway. This test will be done at every visit.

G. Allergen Challenge Test

The allergen challenge test will be performed on five occasions. Each allergen challenge will be separated by at least 7 days. Again, breathing maneuvers will be performed as described above and will be used to monitor any constriction that occurs. You will be required to inhale increasing concentrations of one of the allergens identified in the skin prick test and used for the skin test endpoint. The inhalation is done through a mouthpiece with a noseclip on for two minutes of normal breathing. When the constriction in your airways reaches a certain value (15%) the test will be stopped and your breathing will be monitored at various time points for the next 7 hours. Again, you may stop the test at any time for any reason.

H. Blood tests

Two tubes of blood (approximately 10mL) will be drawn from a vein in your arm at each visit.

I. Administration of the drugs, montelukast and desloratadine

This is a placebo controlled study. Placebo controlled means there are tablets that look like the active drug but do not contain any active drug. To accurately assess the effect of the treatments on blocking the response to allergen we need to have a placebo arm which serves as the baseline for calculating any protection the three active treatments might have. There are four separate treatments (montelukast alone, desloratadine alone, montelukast + desloratadine and placebo).
You will be required to complete all four, but in no particular (i.e random) order. Neither you nor the study personnel will know what treatment you are taking. This information can however be obtained if necessary and will be available at the end of the study.

Both montelukast and desloratadine are currently available by prescription under the trade names Singulair® and Aerius® (CAN)/Clarinex® (US) respectively. The recommended dosage is 10mg a day for Singulair® and 5mg a day for Aerius®. This will be the dose you will be required to take two hours before each allergen challenge.

The study table on page 7 provides an overview of what tests you will be doing on what days and approximately how long your visit will be.

ALLOWED/DISALLOWED MEDICATIONS

For safety reasons, the use of any other drugs (or herbal supplements) should be discussed with study personnel, prior to their use. The table below provides some guidance on what medications can and cannot be used.

<table>
<thead>
<tr>
<th>ALLOWED</th>
<th>DISALLOWED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventolin</td>
<td>Withhold for 6 hours before a visit</td>
</tr>
<tr>
<td>Atrovent</td>
<td>Withhold for 8 hours before a visit</td>
</tr>
<tr>
<td>Airomir</td>
<td>Withhold for 6 hours before a visit</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>No time restriction</td>
</tr>
</tbody>
</table>
RISKS AND DISCOMFORTS

A. Methacholine and allergen inhalation tests

The inhalation challenges may cause bronchoconstriction and manifest symptoms such as chest tightness, wheeze, cough and shortness of breath. Your breathing will be monitored and any discomfort that may result can be reversed by bronchodilator medication.

There is the potential for a severe allergic reaction (anaphylaxis) to occur during an allergen. Safety measures are in place to minimize the likelihood of this occurring as well as to treat such an event should it occur.

B. Study medication

The treatment medication and bronchodilator medication (Ventolin®) are available through prescription. The doses that will be administered to you in this research project do not exceed recommended daily dosages and the possible side effects from these medications are not expected. Some of the more common side effects that have been reported with the use of these medications are listed below.

Singulair® - headache (~18%), cough, dizziness, fatigue, rash, fever (~all < 5%)
Desloratadine® - headache (~6%), dry mouth (~3%), fatigue (~3%)
Ventolin HFA® - throat irritation (10%), cough (5%), viral respiratory infection (7%)

There is also the potential for unforeseen or unknown risks.

C. Reproductive risks

The medication or treatment used in this study may pose a risk to the developing fetus or to babies who are breastfeeding. If you are a sexually active woman and are of childbearing potential (sexually mature woman who has not undergone a hysterectomy or who has not been
post-menopausal for 24 consecutive months), you must do one of the following while participating in this study: use a medically approved effective method of birth control (e.g., oral or planted contraceptives, intrauterine device, diaphragm with spermicide or cervical cap) or abstain from sexual intercourse that could result in pregnancy. If you plan to become pregnant during the course of this project you should not participate. If you are currently breastfeeding, you are not eligible to participate in this study.

A urine pregnancy test will be conducted during screening for all females you are of child bearing potential.

D. Sputum induction

The inhalation of hypertonic saline may cause your airways to constrict. This is prevented by administered the 2 puffs of Ventolin® after the methacholine challenge and before the sputum induction process. Your breathing will be monitored and you can stop the test at any time for any reason.

E. Blood tests

The “needle poke” that is required to draw your blood may cause some discomfort. Bruising around the area the needle entered may also occur. The occurrence of infection following the drawing of blood is rare but has occurred.

CONFIDENTIALITY

While absolute confidentiality cannot be guaranteed, every effort will be made to ensure that the information you provide for this study is kept entirely confidential. Your name will not be attached to any information, nor mentioned in any study report, nor be made available to anyone except the research team. It is the intention of the research team to publish results of this research in scientific journals and to present the findings at related conferences and workshops, but your identity will not be revealed.
RESEARCH-RELATED INJURY

There will be no costs to you for participation in this study. You will not be charged for any research procedures. In the event that you become ill or injured as a result of participating in this study, necessary medical treatment will be made available at no additional cost to you. By signing this document you do not waive any of your legal rights.

BENEFITS OF STUDY PARTICIPATION

There is no medical or other benefit to you as an individual as a result of your being in this study. We hope that the information learned from this study can be used in the future to benefit other people with a similar disease, however, no benefit is guaranteed.

NEW FINDINGS

If new information about either of the study medications becomes available that may influence your willingness to participate in this research project, this information will be provided to you by the study personnel.

VOLUNTARY WITHDRAWAL

Your participation in this research is entirely voluntary. You may withdraw from this study at any time. If you decide to participate and then withdraw before completing all study procedures there will be no penalty or loss of benefits to which you are entitled. Your future medical care will not be affected. If you are a student, your academic status will not be affected.

WITHDRAWAL INITIATED BY INVESTIGATOR

The study investigator may decide to discontinue the study at any time, or withdraw you from the study at any time if it is felt to be in your best interest.
STORAGE OF DATA

The data will be stored in the lab where it is collected for a period of 5 years or more as per University regulations.

WHO TO CONTACT FOR QUESTIONS ABOUT THE STUDY

If you have any questions about this study, the procedures or treatment involved, or if you would like additional information, before or during the study, you may contact study personnel at 966-8290 or the investigator, Dr. Cockcroft, at 966-8346. Study personnel can be reached 24 hours at 229-8709.

WHO TO CONTACT ABOUT YOUR RIGHTS AS A RESEARCH SUBJECT

If you have any questions, concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, you should contact the Chair of the Biomedical Research Ethics Board, c/o Ethics Office, University of Saskatchewan at (306) 966-4053.

HONORARIA AND REIMBURSEMENT

You will receive an honorarium for participating in this research project that will cover any costs you incur (e.g., parking and meals) as well as compensate you for the time and inconvenience of being a research subject. In the event that you begin the study and subsequently withdraw before completing the study, your honorarium will be adjusted accordingly.
CONSENT

I have read the information sheet regarding this research project.
I have had sufficient time to consider the information provided.
I have had the opportunity to ask questions about this project and to receive satisfactory answers to my questions.
I understand that the information will be kept confidential and that the results will only be used for scientific purposes.
I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
I understand that I am not waiving any of my legal rights as a result of signing this consent form.
I have read the information presented to me and I freely consent to participate in this study.
I have been told that I will receive a dated and signed copy of this form.

Participant signature       Print       Date

Signature of person administering Consent       Print       Date
9.5.3 Certificate of Approval

UNIVERSITY OF SASKATCHEWAN

Biomedical Research Ethics Board (Bio-REB) 14-Dec-2006

Certificate of Approval

PRINCIPAL INVESTIGATOR
Donald W. Cockcroft

DEPARTMENT
Medicine (Respirology)

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Ellis Hall
Respiratory Medicine
133 Hospital Drive
Saskatoon SK S7N 0W8

SPONSORING AGENCIES
University of Saskatchewan

TITLE
Changes in Airway Responses to Inhaled Allergen Following Pharmacological Inhibition of Histamine and CysLT, Blockade in Mild Atopic Asthma

APPROVAL DATE
29 Nov 2006

STUDY APPROVAL EXPIRY
19-Nov-2006

APPROVAL OF
Protocol version 1 (October 2006)
Information Sheet and Consent Form (December 2006)

Date of Full Board Meeting: 20-Nov-2006

CERTIFICATION
The University of Saskatchewan Biomedical Research Ethics Board has reviewed the above named research project at a full-board meeting (any research classified as minimal risk is reviewed through the expedited review process). The proposal was found to be acceptable on ethical grounds. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research project and for ensuring that the authorized research is carried out according to governing law. This Approval is valid for the above time period provided there is no change in experimental protocol or in the consent process.

ONGOING REVIEW REQUIREMENTS/REB ATTESTATION
In order to receive annual renewal, a status report must be submitted to the Chair for Committee consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for further instructions: http://www.usask.ca/researchethics.shtml. In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. This approval and the views of this REB have been documented in writing.

University of Saskatchewan
Biomedical Research Ethics Board

Please send all correspondence to:
Ethics Office
University of Saskatchewan
Room 305 Kirk Hall, 117 Science Place
Saskatoon SK S7N 0C8
### APPENDIX F: Study Flowchart for the Late Asthmatic Response Study

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<th>PHASE</th>
<th>SCREENING</th>
<th>TREATMENT 1</th>
<th>TREATMENT 2</th>
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**Time Required (hours)**

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</thead>
</table>

SPT = skin prick test  
STE = skin test endpoint  
FeNO = fraction of exhaled nitric oxide  
MCT = methacholine challenge test  
ACT = allergen challenge test

Note that there is a “washout period” between the screening visits and Treatment 1 and between Treatments 1 and 2, Treatments 2 and 3 and Treatments 3 and 4. This is a minimum of 7 days. No visits are required during this time.