PROTECTIVE EFFECT OF H$_1$ AND CYSLT$_1$ ANTAGONISTS

ON ALLERGEN INDUCED AIRWAY RESPONSES IN ATOPIC ASTHMA

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy

in the Department of Pharmacology

University of Saskatchewan

Saskatoon

By

BETH DAVIS

PERMISSION TO USE

In presenting this thesis in partial fulfillment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of the University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Head of the Department of Pharmacology
University of Saskatchewan
Saskatoon, Saskatchewan, S7N 5E5

ABSTRACT

Background

The mechanism by which allergies trigger asthma occurs through the interaction of antigen, IgE and the $F_c \varepsilon R_1$ 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.

ACKNOWLEDGEMENTS

First and foremost I must thank the individuals who participated in these projects. Without their commitment and dedication this work would not have been possible.

Second, and equally as important, I am grateful to the Department of Pharmacology for providing a home for me to undertake graduate studies. There is no better discipline and no better group of people. I will remember each of you for your kindness; you have all played a valuable role in helping me achieve my goal.

Third, the AllerGen Network of Centers of Excellence and the Clinical Investigator Collaborative, in particular, Dr. Gail Gauvreau, Dr. Paul O'Byrne and Dr. Louis-Philippe Boulet, your contribution to the LAR investigation is greatly appreciated. I look forward to future collaborations.

Lastly, my supervisor, Dr. Don Cockcroft. Thank you, thank you, and thank you. Your vast knowledge, direction and friendship will always be appreciated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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

CysLT$_1$ – cysteinyl leukotriene receptor subtype 1

CysLT$_2$ – 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

$F_c\varepsilon RI$ – 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

IFNγ - 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

LTC$_4$ – leukotriene C 4

LTD$_4$ – leukotriene D 4

LTE$_4$ – leukotriene E 4

LTRA – leukotriene receptor antagonist

M$_1$ – 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

MPC$_{20}$ – concentration of methacholine that causes a 20 % decrease in FEV$_1$

NF$_\kappa$B – nuclear factor kappa B

NHLBI – United States National Heart Lung and Blood Institute

OVA – ovalbumin

PAF – platelet activating factor

PBMC – peripheral blood mononuclear cell

PC$_{20}$ – concentration of allergen that causes a 20% decrease in FEV$_1$

PD – pharmacodynamic

PDGF – platelet derived growth factor

PEFR – peak expiratory flow rate

PGD$_2$ – 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

Th_1 – T lymphocyte helper cell subtype 1

Th_2 – 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

2

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
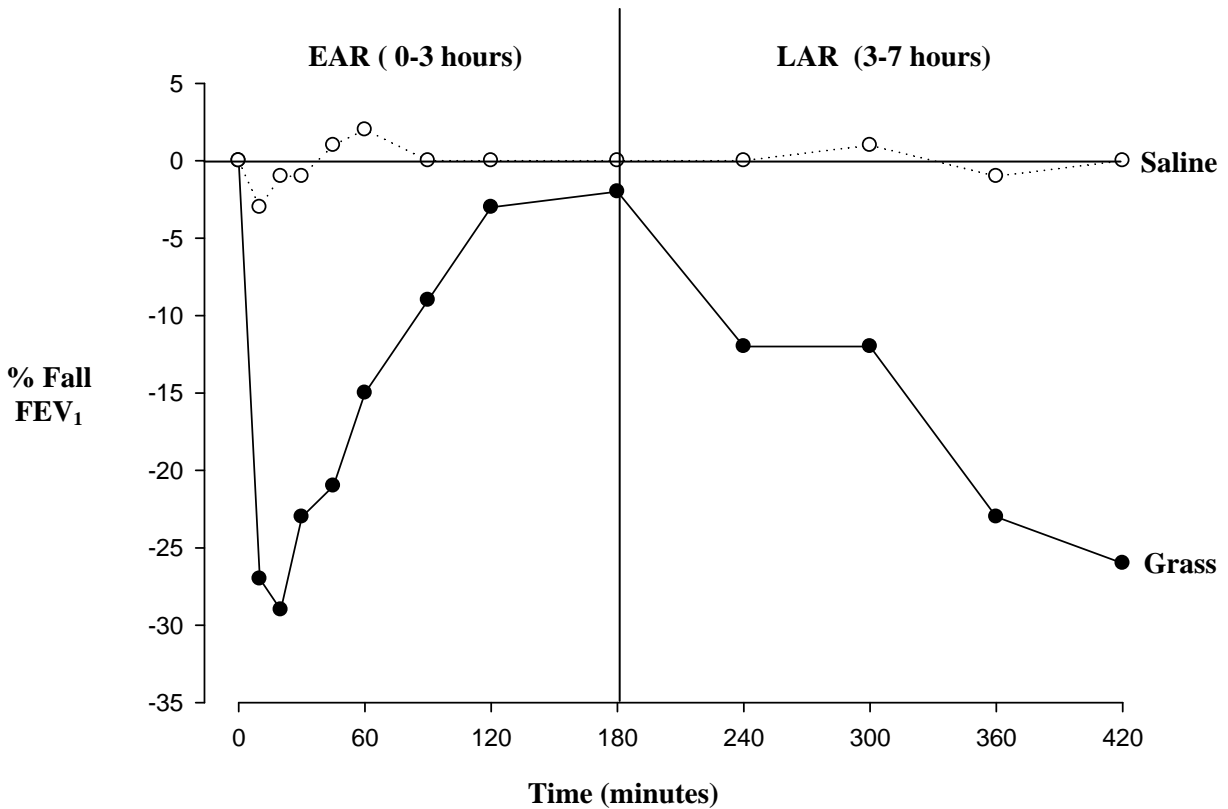
Figure 1.1:   Changes in FEV$_1$ during the early and the late asthmatic responses.

Grass inhalation results in a decrease in the FEV$_1$ 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 FEV$_1$ 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. FEV$_1$, 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 FcεRI 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-α) and some of which are produced within hours of activation (*e.g.,* numerous interleukins, monocyte chemoattractant protein - 1 (MCP-1), macrophage inhibitory protein – 1α (MIP-1α). 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.

5
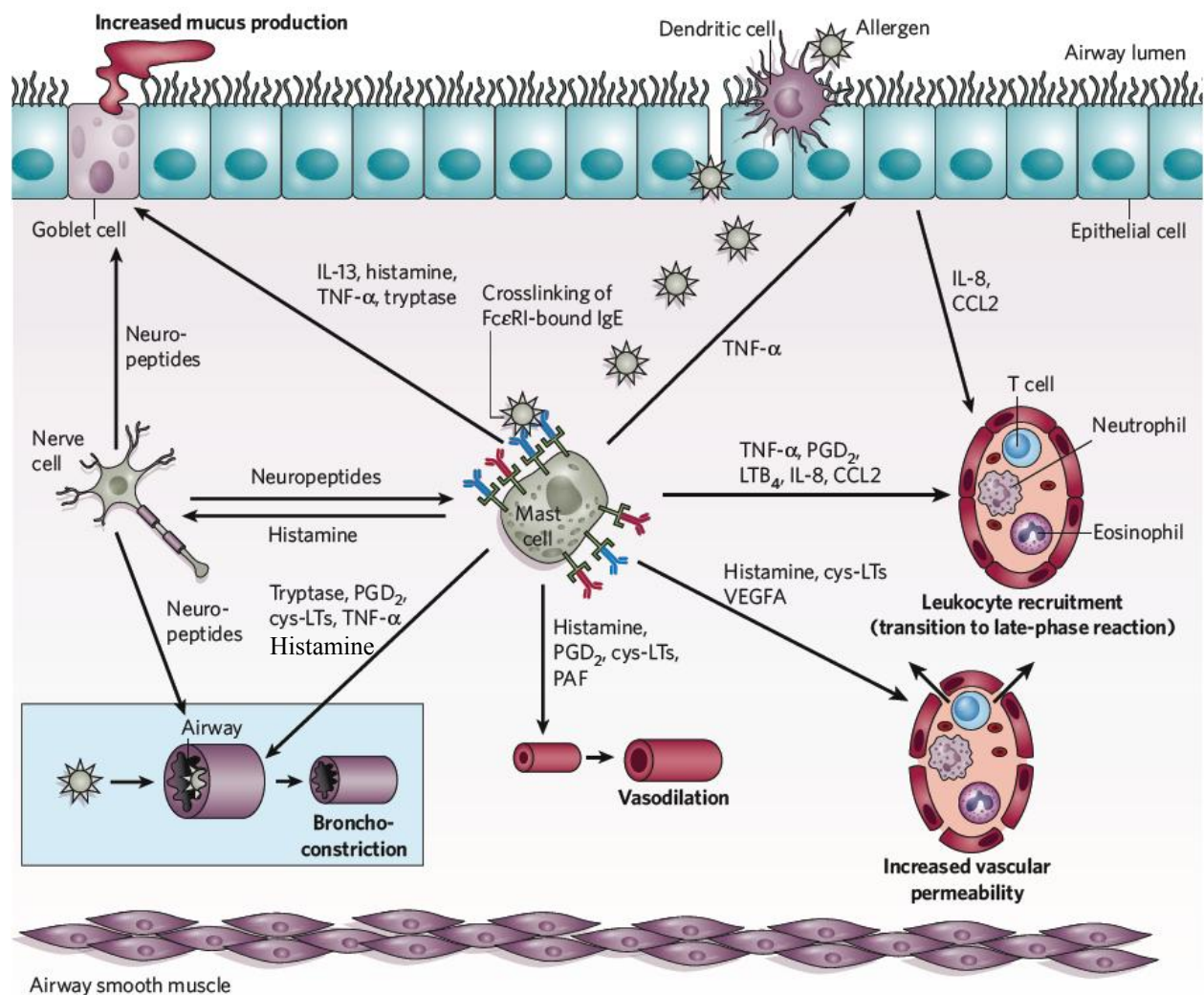
Figure 1.2: Schematic of cellular events surrounding the early allergic/asthmatic response. Inhaled allergen will stimulate the release of preformed mediators and lipid derived mediators by crosslinking allergen specific IgE molecules bound to the high affinity IgE receptor ($F_c\varepsilon RI$) on mast 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 (Th$_2$) cell inflammatory response predominantly orchestrated by the effects of Th$_2$ 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, cysteinyl leukotrienes) on the development of the LAR.
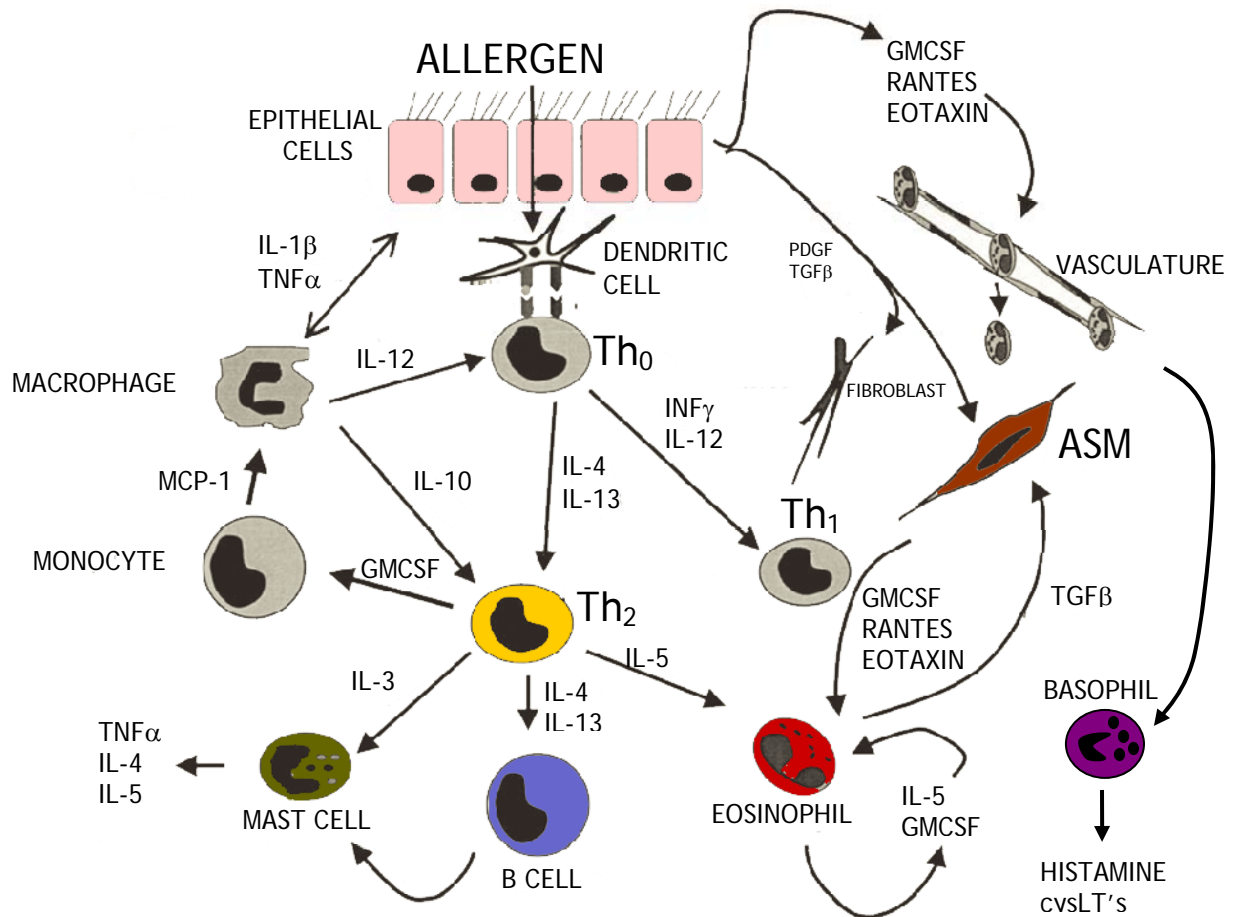
Figure 1.3: Cells and mediators involved in the late allergic/asthmatic response.

Various cells and mediators likely contribute to the LAR. Lymphocytes (notably Th$_2$ 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, cysteinyl 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. Th$_2$ 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 β_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 β_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 β_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.,* PGD_2 and LTB_4) 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, IgG_1 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 FEV_1 during the EAR (0 to 2 h post challenge) but did not report on the AUC or maximal decrease in FEV_1. 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 Beta$_2$ agonists

Beta$_2$ 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 β_2 agonist results in an increase in the amount of allergen administered to cause a 20% fall in FEV$_1$ 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 β_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 β_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 $FceR_1$ 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 $CysLT_1$ receptors and the enzyme inhibitors block the formation of LTA_4 the precursor of LTB_4 and the cysteinyl

leukotrienes LTC_4, LTD_4 and LTE_4. These agents must also be withheld prior to allergen challenge investigations.

1.4.6 Histamine H_1 blockers

Antihistamines, more specifically histamine H_1 receptor antagonists have been extensively investigated over the years. These agents produce little, if any, protection against the EAR and the LAR. First generation H_1 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 H_1 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].

17

Table 1.1 Effects of various drugs on allergen induced airway responses

DRUG TYPE	EFFECT ON EAR	EFFECT ON LAR	EFFECT ON LATE SEQUELAE
SHORT ACTING β$_2$ AGONISTS (salbutamol)	INHIBIT	NO CHANGE	NO CHANGE
LONG ACTING β$_2$ AGONISTS (salmeterol)	INHIBIT	INHIBIT	NO CHANGE
REGULAR USE OF SHORT ACTING β$_2$ AGONIST	AUGMENT	AUGMENT	AUGMENT
MUSCARINIC ANTAGONIST (ipratropium bromide)	MINOR INHIBITION	MINOR INHIBITION	NO CHANGE
GLUCOCORTICOSTEROID (beclomethasone dipropionate) SINGLE DOSE AFTER EAR	N/A	INHIBIT	MODERATE ↓AHR EOS UNKNOWN
GLUCOCORTICOSTEROID - SINGLE DOSE PRE CHALLENGE	NO CHANGE	INHIBIT	MODERATE INHIBITION
GLUCOCORTICOSTEROID - CHRONIC STABLE DOSE	MODERATE INHIBITION	INHIBIT	INHIBIT
THEOPHYLLINE	MINOR INHIBITION	MINOR INHIBITION	NO CHANGE AHR EOS UNKNOWN
CROMONES (sodium cromoglycate)	MODERATE INHIBITION	MODERATE INHIBITION	MODERATE ↓AHR EOS UNKNOWN
ANTI-IGE (omalizumab)	INHIBIT	INHIBIT	INHIBIT
LTRA (zafirlukast)	MODERATE INHIBITION	MODERATE INHIBITION	MODERATE INHIBITION (BOTH)
ANTI-HISTAMINE (loratadine)	PARTIAL INHIBITION	VARIABLE INHIBITION	UNKNOWN
SINGLE DOSE ANTI-HISTAMINE + LTRA (desloratadine + montelukast)	*INHIBIT*	*INHIBIT*	*TREND TO ↓ AHR ↓ EOS*

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 H_1 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.
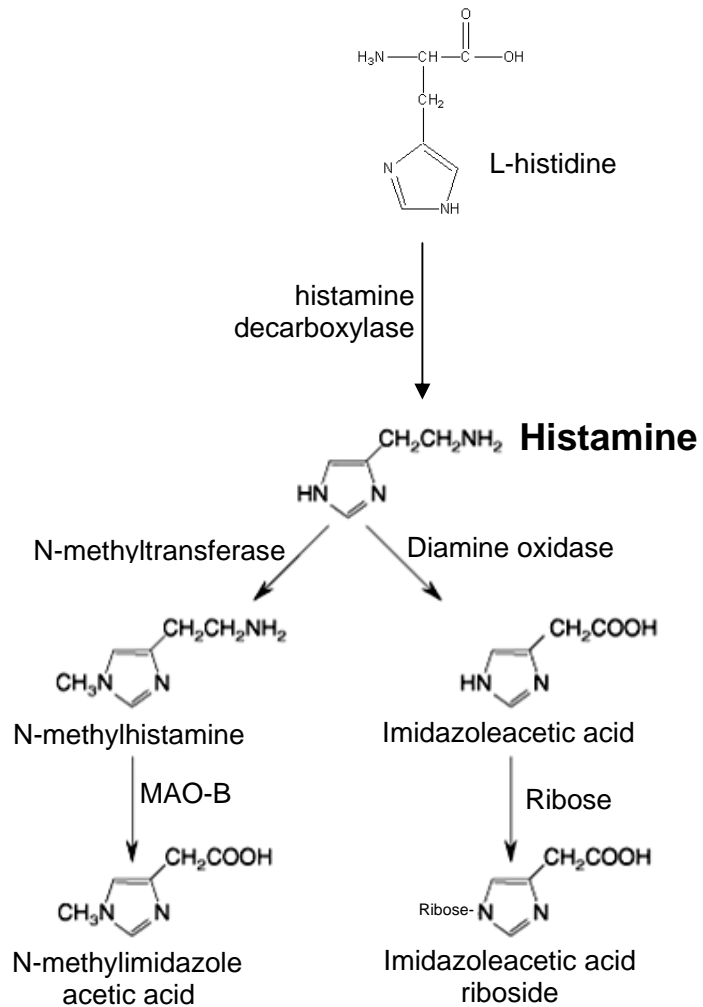
Figure 2.1 Histamine biosynthesis and metabolism. MAO, monoamine oxidase B

2.1.3 Histamine receptors

Four histamine receptors have now been identified (H_1 through H_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_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_1 receptor is coupled to $G_{q/11}$ and the binding of histamine results in the activation of phospholipase C (PLC) and the inositol trisphosphate (IP_3) diacylglycerol (DAG) intracellular signaling pathway. IP_3 mobilizes intracellular Ca^{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_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_2 receptors on airway smooth muscle) and causes bronchoconstriction. The H_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_2 receptor is coupled to the $G\alpha_s$ protein and signals through the adenylyl cyclase (AC) – cAMP – protein kinase A (PKA) second messenger system. The H_2 receptor shares similar expression patterns with H_1 and its major physiological role, to date, is the stimulation of proton release from parietal cells. H_2 antagonists are widely prescribed for the treatment of gastric ulcers (*e.g.,* ranitidine, cimetidine). There is some evidence that H_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 H_3 receptors which regulate histamine synthesis and release through an autoreceptor mechanism. This inhibitory function signals through the $G_{i/o}$ cAMP pathway. In addition to its autoregulatory function, H_3 receptor activation may also regulate the levels of other neurotransmitters such as serotonin, dopamine and GABA. There is considerable interest in the development of H_3 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 H_4 receptor which is highly expressed on leukocytes and in bone marrow. Ligand receptor interactions are coupled to the $G\alpha_{i/o}$ protein, adenylyl cyclase, cAMP pathway. Since the cloning of the gene encoding the human H_4 receptor in 2000, investigations into the role of the H_4 receptor and possible therapeutic implications have received much attention. Our current understanding suggests a major role for histamine acting via the H_4 receptor in immunoregulation and inflammation which implicates agonists or antagonists of the H_4 receptor as possible therapeutic intervention in immune and inflammatory disease. Indeed, early clinical investigations are being conducted in atopic asthma.

Table 2.1: Histamine receptor properties

RECEPTOR SUBTYPE	EXPRESSION	G PROTEIN	PREDOMINANT SIGNALING PATHWAY	PHYSIOLOGICAL EFFECTS	ANTAGONISTS	pKa
H_1	Smooth muscle Endothelial cells CNS	$G_{q/11}$	↑ PLC-IP$_3$/DAG ↑ Ca^{2++}	Bronchoconstriction Vascular leakage	Chlorpheniramine **(DESLORATADINE)**	4.2
H_2	GI tract Cardiac muscle Mast cells CNS	G_s	↑ AC-cAMP-PKA	Gastric acid secretion	Ranitidine	4.3
H_3	CNS	$G_{i/o}$	↓ AC-cAMP	Autoregulation of presynaptic neurotransmitter release	Thioperamide Clobenpropit	7.8
H_4	Hematopoietic Cells	$G_{i/o}$	↓ cAMP ↑ Ca^{2++}	Immunoregulation Anti-inflammatory	Thioperamide	8.4

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, antiserotinergic 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 H_1 receptor antagonist which binds with high affinity to the histamine H_1 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.
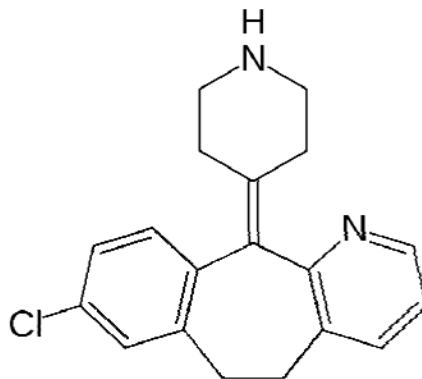
Figure 2.2: Chemical structure of desloratadine.

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 $FEV_1 \leq$ 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 H_1 antagonists, inhibited both basal and histamine induced $NF_{\kappa}B$ activity suggesting negative regulation of gene transcription of pro-inflammatory mediators as a possible mechanism [Wu *et al*., 2004]. Another *ex vivo* investigation using $Fc\varepsilon R_1$ cells from peripheral blood, skin and lung tissue (*i.e.,* mast cells and basophils) confirmed the inhibitory effect of desloratadine on histamine, tryptase, LTC_4 and PGD_2 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 Th_2 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.*, LTC_4 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 LTC_4, LTD_4 and LTE_4 – the cysteinyl leukotrienes [Holgate and Dahlen, 1997].

2.3.2   Biosynthesis (Figure 2.3)

The cysteinyl 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 A_2 releases arachidonic acid from the perinuclear membrane which serves as a substrate for 5 lipoxygenase in the formation of LTA_4 and 5-hydroperoxyeicosatetraenoic acid (5-HPETE). LTA_4 undergoes conjugation with glutathione by LTC_4 synthase to form LTC_4 which is actively transported out of the cell where further enzymatic action forms LTD_4 and subsequently LTE_4. Native LTE_4 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.
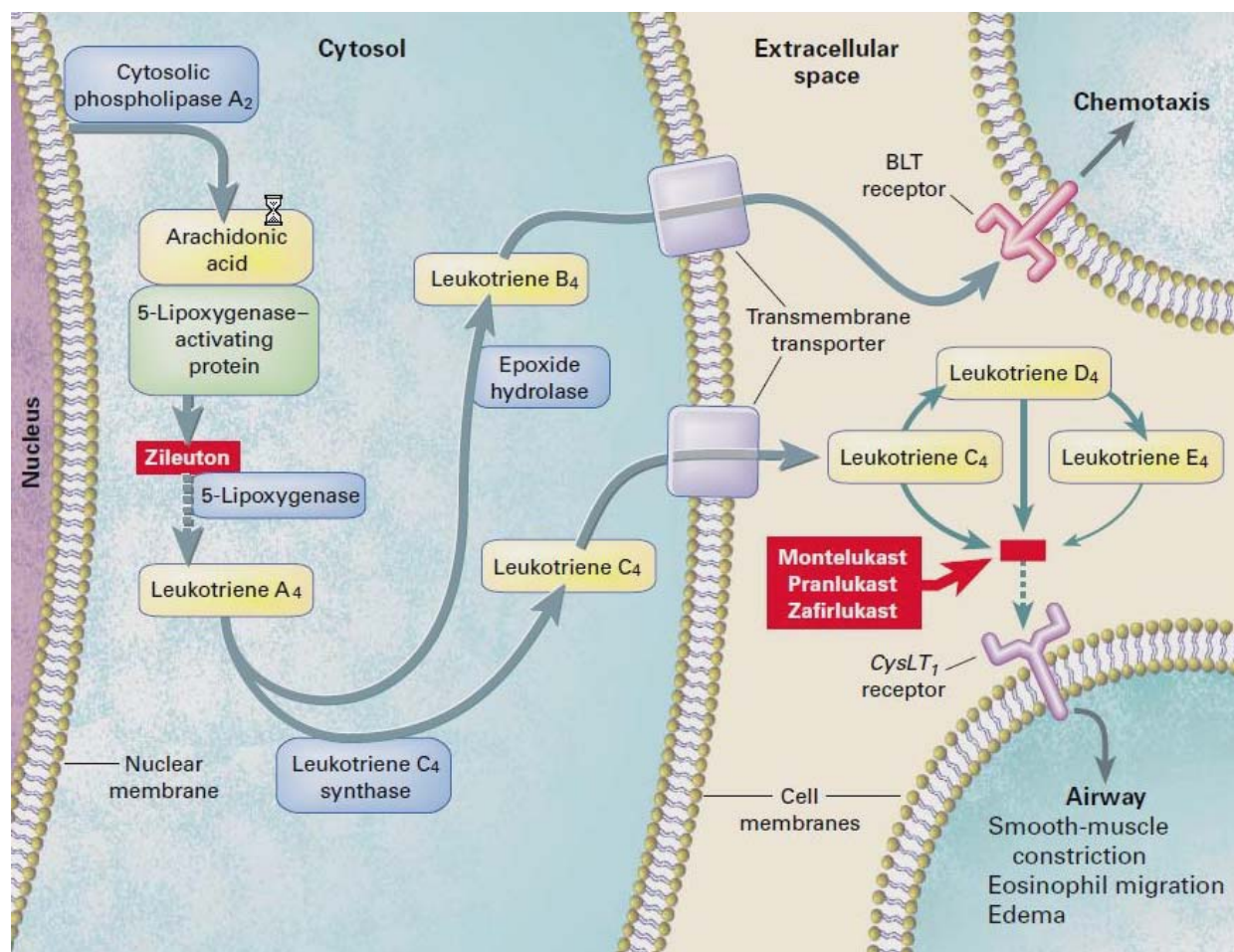
Figure 2.3: Biochemical Pathways of the Formation and Action of the Leukotrienes and Sites of Action of Leukotriene-Modifying Drugs.

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 cysteinyl 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, ($CysLT_1$, $CysLT_2$) have been
identified and evidence supporting the existence of a third is emerging. The cysteinyl
leukotrienes bind to $CysLT_1$ and $CysLT_2$ with varying affinities. $LTD_4 > LTC_4 > LTE_4$ and
$LTC_4 = LTD_4 > LTE_4$ for $CysLT_1$ and $CysLT_2$ 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_2 generated IL-4, IL-5
and IL-13.   The CysLT receptors, like the histamine receptors are coupled to G proteins.
$CysLT_1$ is coupled to Gq/11 and Gi/o and $CysLT_2$ 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_4 from arachidonic acid. In the absence of LTA_4
subsequent downstream products for which LTA_4 is a substrate are also downregulated, namely
LTB_4 and the cysteinyl leukotrienes LTC_4, LTD_4 and LTE_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 *et al*., 1991]. The negative results may
have been due to the small sample size or, as shown by Hasday *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.,* β_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 cysteinyl 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.
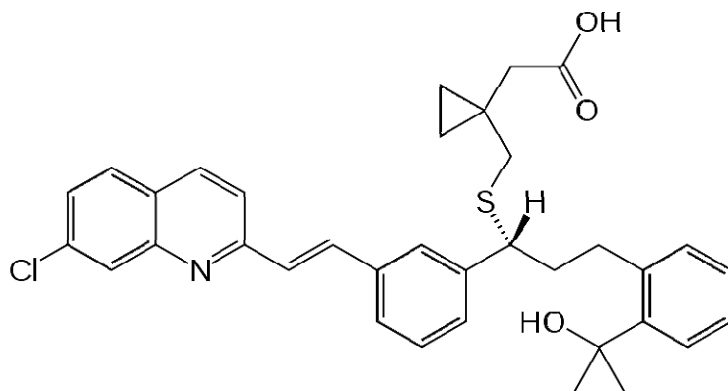
Figure 2.4 Chemical structure of montelukast.

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.,* FEV_1, 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 LTC_4 [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 $CysLT_1$ receptor expression [Zhang *et al*., 2004]. T cells selected from healthy and atopic donors have low levels of CysLT receptor expression which, for both $CysLT_1$ and $CysLT_2$ 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 $NF_{\kappa}B$ activation as has been shown in THP-1 cells [Maeba *et al*., 2005]. A recent *ex vivo* investigation documented an upregulation of $CysLT_1$ receptor expression on B lymphocytes following exposure to combined anti-CD40 and IL-4 which resulted in a subsequent increase in responsiveness to LTD_4 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 LTD_4 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 $NF_{\kappa}B$ 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 $NF_{\kappa}B$ [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 FEV_1 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 cysLT$_1$ receptor antagonism on airway smooth muscle cells and the inhibition of eosinophil recruitment and may result from the down regulation of Th$_2$ cytokines (*i.*e., IL-4, IL-5, IL-13) possibly through the inhibition of NF$_\kappa$B.

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

36

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_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 *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_1 receptor antagonist (mepyramine) and an H_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_1 in combination with H_2) but can reach 80% when pretreated with an LTRA (60% for L-648,051 and 80% for SKF 104353). Combining both histamine (H_1 and H_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.

TREATMENT	MEP + MET	L-648,051	FPL 55712	U-60,257	MK-886	Indomethacin	ICI 198,615	ICI 198,615 + MEP + MET
EFFECT	No effect	~ 50% inhibition	~ 50% inhibition	~ 50% inhibition	~ 70% inhibition	No effect	~ 65% inhibition	Complete inhibition

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 H_1 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 LTD_4 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 H_1 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 LTD_4 receptor antagonist. In normal subjects, L-649,923 shifted, to the right, the dose response curve to exogenous LTD_4 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 FEV_1 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 LTD_4, or that LTD_4 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 LTD_4 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 LTD_4 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_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_1 AUC by 32.5 % and the LAR FEV_1 AUC by 70.2 % [Rafferty *et al*, 1989]. Terfenadine has also been shown to inhibit both the mean maximal fall in FEV_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_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, desloratdine) 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_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 *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 < Desloratdine < 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 < Desloratdine < 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 H_1 receptors and leukotrienes on $CysLT_1$ receptors. It follows that blocking the activity of these mediators may prevent bronchoconstriction. Investigations of the effect of H_1 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 $\geq 65\%$ 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 PC_{20} 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 FEV_1 maneuvers were performed sixty seconds apart. Inhalations were continued until the highest post inhalation FEV_1 was at least 15 % lower than the highest baseline FEV_1 (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 PC_{20} (p < 0.001 for both). Allergen PC_{20} 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 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 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 PC_{20} 2.3 doubling concentrations while the combination increases allergen PC_{20} 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 H_1 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 FEV_1 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

Subject	Gender	Age (years)	Height (inches)	Baseline FEV$_1$ (litres)	Baseline FEV$_1$ % Predicted	Allergen used for inhalation	MPC$_{20}$ (mg/mL)	Medications
1	M	57	66	2.46	73	GRASS[a]	0.5	S; prn
2	M	29	70	3.87	87	HDM[b]	2	S; prn
3	M	27	71	3.67	86	CAT[c]	0.67	S; prn
4	F	24	62	2.66	83	CAT[c]	0.28	S; prn
5	M	25	72	4.07	85	CAT[c]	2	S; prn
6	M	40	72	2.86	65	GRASS[a]	1.8	S; prn
								S; prn
7	F	25	66	3.12	89	HDM[b]	2.5	F 125 µg bid
8	M	43	68	3.44	88	GRASS[a]	16	S; prn
9	F	27	64	3.35	101	CAT[c]	0.86	S; prn
10*	M	26	72	4.59	96	CAT[c]	0.75	S; prn
Mean		32	68	3.41	85		2.7	

* not included in analysis

S = salbutamol

MPC$_{20}$ = methacholine PC$_{20}$

F = fluticasone propionate

a: Grass Mix 10 (40,000 protein nitrogen units/mL)

b: Standard Mites Mixed (10,000 allergy units/mL)

c: Standardized Cat Pelt (10,000 bioequivalent allergy units/mL)
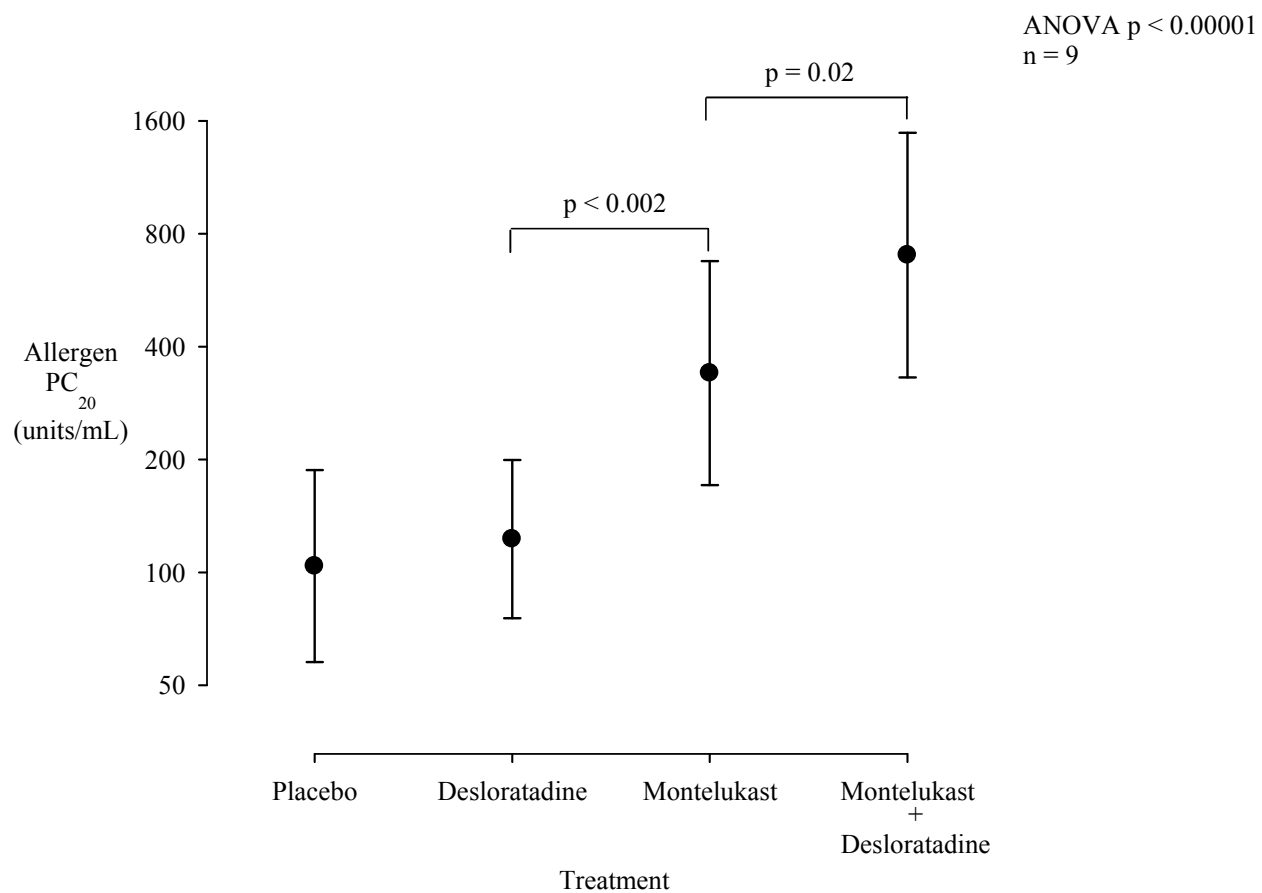
Figure 4.1: Treatment effects on the EAR allergen PC_{20}.

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 Th_2 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 ≥ 20 % fall in FEV_1 and LAR of ≥ 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 ≥ 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 ≥ 10 day intervals.

Day 1 24 h pre allergen challenge	DOSE 2 h pre allergen challenge	Day 2 ALLERGEN CHALLENGE	Day 2 7 h post allergen challenge	Day 3 24 h post allergen challenge
• eNO • MCh • SI	• Placebo • 5 mg D • 10 mg M • 5 mg D + 10 mg M	EAR (0-3h) LAR (3-7h) 4h eNO	• eNO • SI	• eNO • MCh • SI
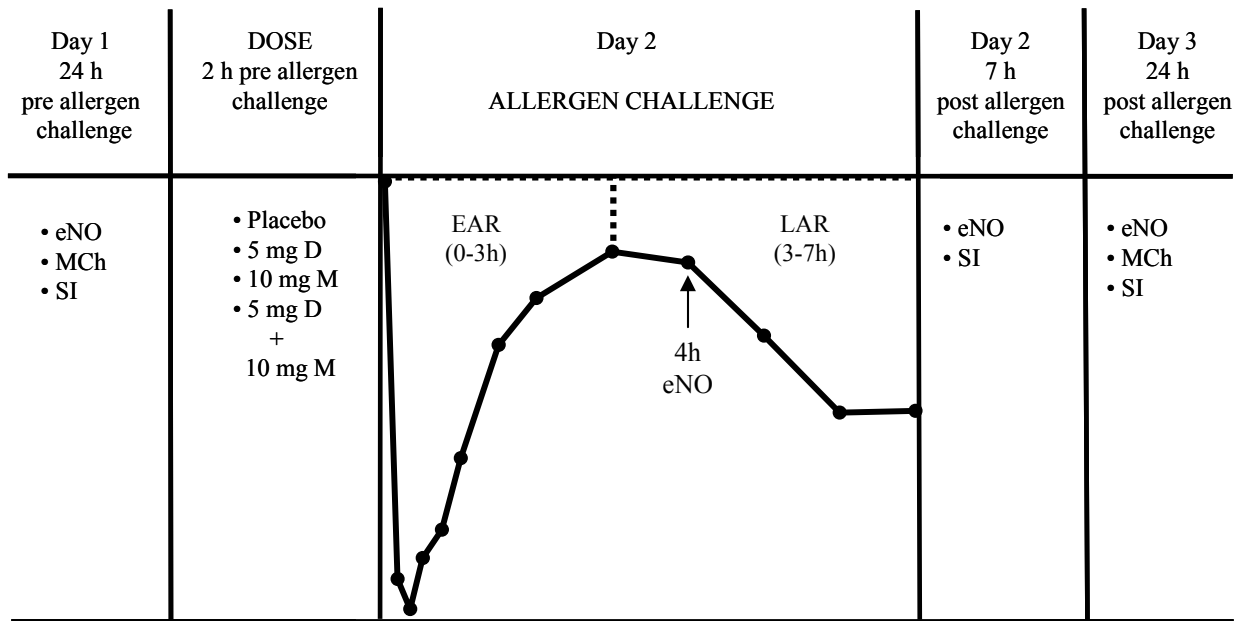
eNO, exhaled nitric oxide; MCh, methacholine challenge; SI, sputum induction; EAR, early asthmatic response; LAR, late asthmatic response

Figure 5.1:   LAR Study Design

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_{20} using the skin test endpoint and methacholine PC_{20} [Cockcroft *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 (*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_1 maneuvers were performed sixty seconds apart. Once the EAR was captured, the response remained untreated and the FEV_1 was assessed at various standardized time points, up to 7 hours post allergen inhalation, to capture the LAR [Boulet *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 *et al.*, 1999; Cockcroft *et al.*, 1977]. All methacholine challenges were performed in an identical manner (*i.e.,* same starting concentration) within a given subject. The PC_{20} was extrapolated if a concentration of 16 mg/mL or less resulted in a percent fall in FEV_1 of greater than 17 % but less than 20 % [Jokic *et al.*, 1998]; and interpolated if the percent fall was > 20% [Cockcroft *et al.*, 1983]. Salbutamol (200 µg) 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 cytospins 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 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 FEV_1 at various time points following allergen challenge is shown in Figure 5.4.   The doubling dose increase in methacholine PC_{20} was 0.66±0.19 (mean±SEM) after placebo; 0.82±0.26 after desloratadine; 0.31±0.21 after montelukast and 0.18±0.23 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 *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_1 and $CysLT_1$ 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 (*i.e.,* histamine and the cysteinyl leukotrienes) released by recruited inflammatory cells (*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_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_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_1. One possible explanation is that the relative potency of cysteinyl leukotriene induced bronchoconstriction is 1000 fold more potent than that of histamine. If $cysLT_1$ 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_1 and $cysLT_1$ receptor antagonism.

Both histamine [Thurmond *et al.*, 2008] and the leukotrienes [Busse and Kraft, 2005; Woszczek *et al.*, 2008; Woszczek *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 *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'Shaugnessy (*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, H_1 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 β_2 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

Subject	Gender	Age (years)	Height (inches)	Baseline FEV_1 (litres)	Baseline FEV_1 % Predicted	Allergen	** Allergen Dilution	*** MPC_{20} (mg/mL)	Medications
1	M	60	66	2.42	74	grass	1:256	0.56	S
2	F	24	61	3.29	106	hdm (dp)	1:256	2.5	S
3	M	30	70	3.41	77	cat	1:256	0.34	S
4	M	25	71	4.25	91	grass	1:64	1.4	S
5	M	24	73	3.49	71	grass	1:128	1.7	S B
6	F	47	66	2.47	84	cat	1:128	7.0	S
7	M	58	71	3.44	90	cat	1:128	1.3	S
8	F	26	65	3.75	110	hdm (df)	1:64	11.7	S
9	M	23	72	5.22	107	cat	1:32	5.3	S
10	F	44	68	3.72	122	cat	1:4	5.6	S F
Mean (± SD)		36.1 (14.8)	68.3 (3.8)	3.55 (0.81)	93.2 (17.3)			*2.27	

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$_1$ blockers and LTRA's on the airway response to allergen

Author	Treatment	Daily Dose	Duration	AUC EAR / max ↓ FEV$_1$ (% inhibition)	AUC LAR / max ↓ FEV$_1$ (% inhibition)
Roquet *et al*., [1997]	loratadine	20 mg	1 week	25/31	40/32
	zafirlukast	160 mg		62/62	55/36
	loratadine + zafirlukast	20 mg/160 mg		75/74	74/48
Richter *et al*., [2008]	azelastine	8.0 mg	1 week	NR/46	NR/43
	montelukast	10 mg		NR/76	NR/59
	azelastine + montelukast	8.0 mg/10 mg		NR/89	NR/78
Davis *et al*., [current study]	desloratadine	5 mg	single dose 2 hours prior to allergen challenge	32/14	43/19
	montelukast	10 mg		72/54	71/63
	desloratadine + montelukast	5 mg/10 mg		100/73	100/88

NR, not reported

n = 10
ANOVA p < 0.001
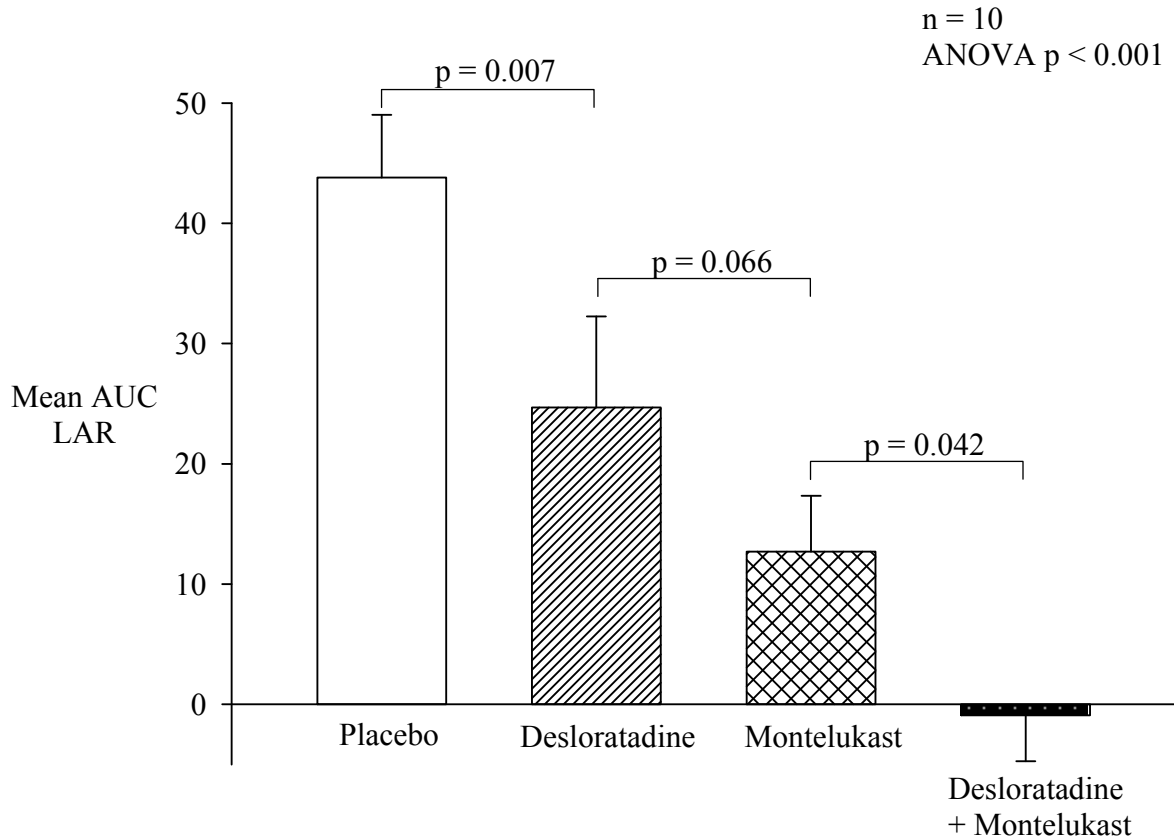
Figure 5.2   Treatment effects on the LAR AUC.

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.
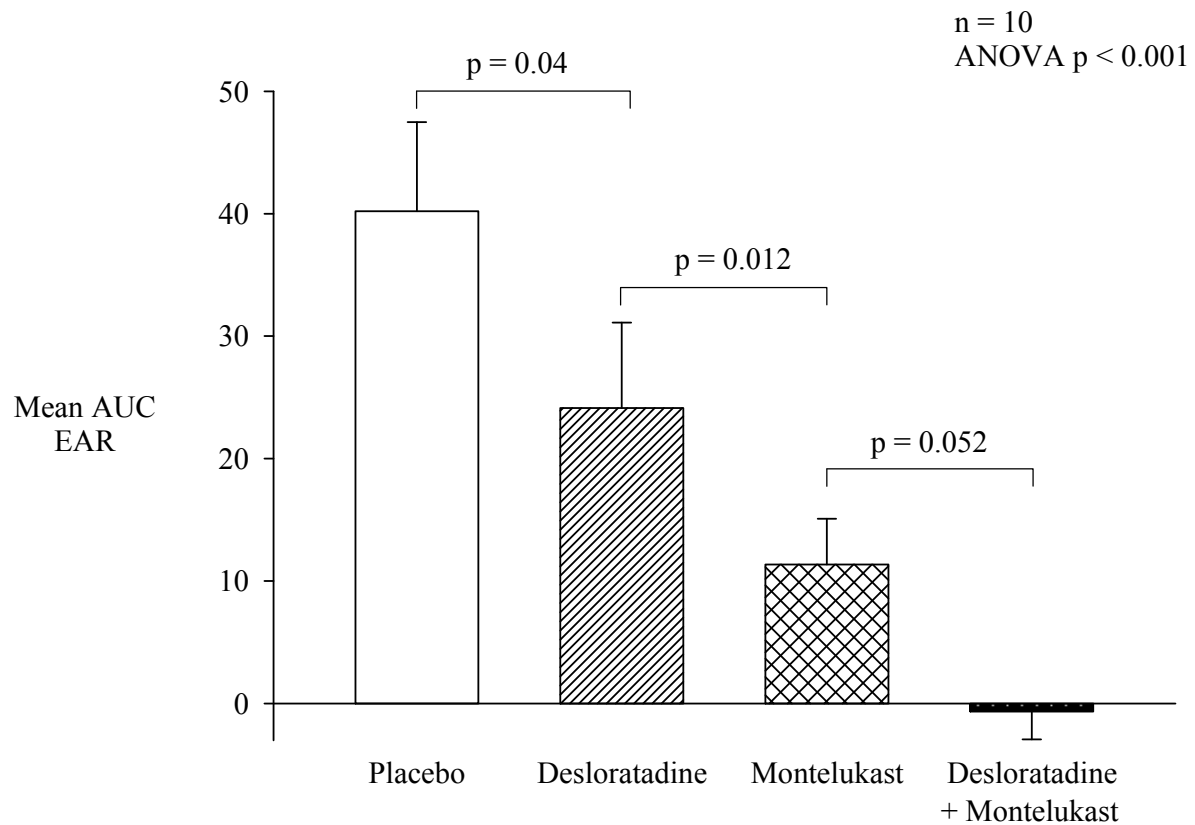
Figure 5.3   Treatment effects on the EAR AUC.

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 ($p = 0.052$).

.

n = 10
ANOVA p < 0.0001
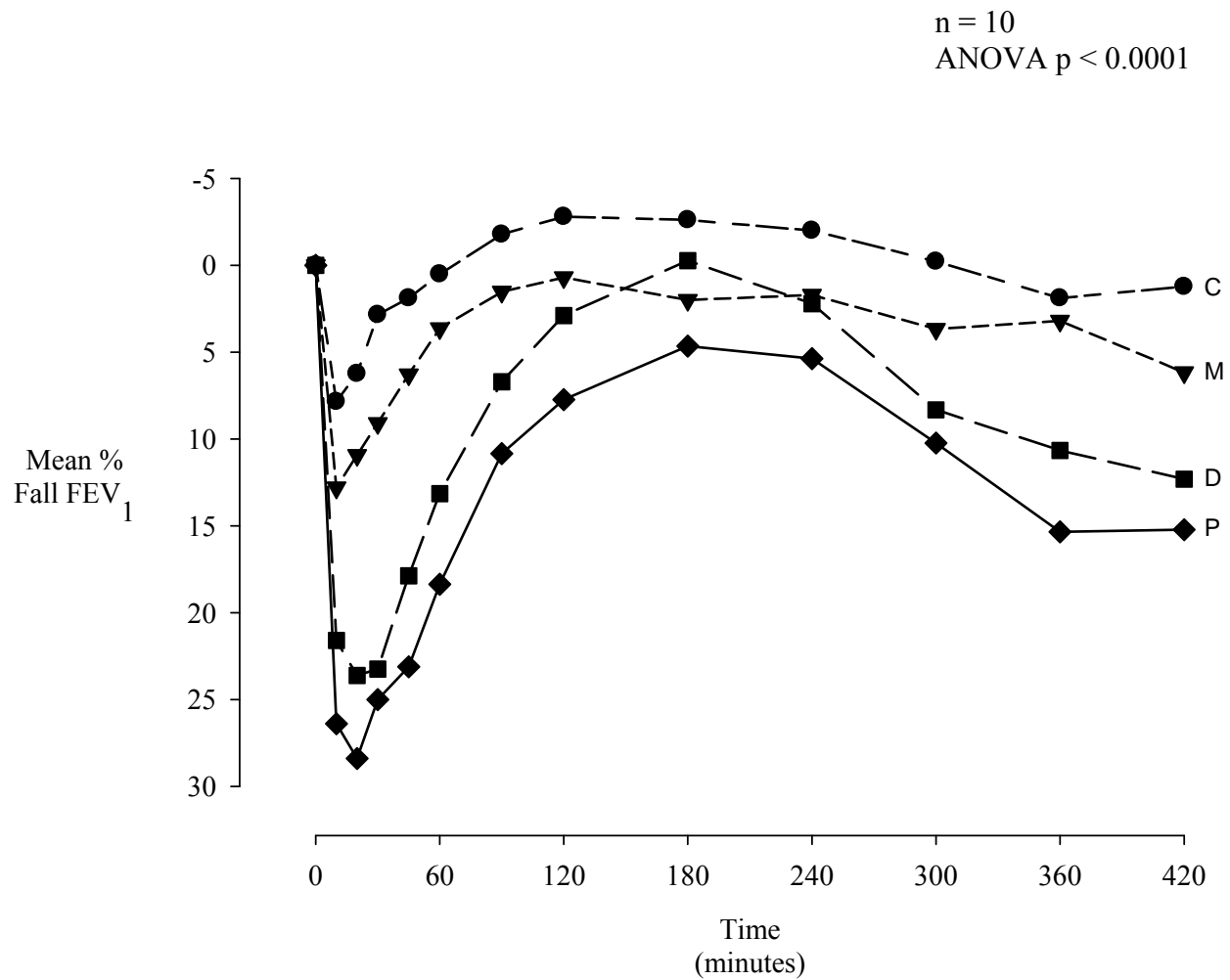
Figure 5.4   Treatment effects on the Mean % Fall in FEV_1.

Changes in FEV_1 over the 7 hours post allergen inhalation expressed as the mean percent fall in FEV_1 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.
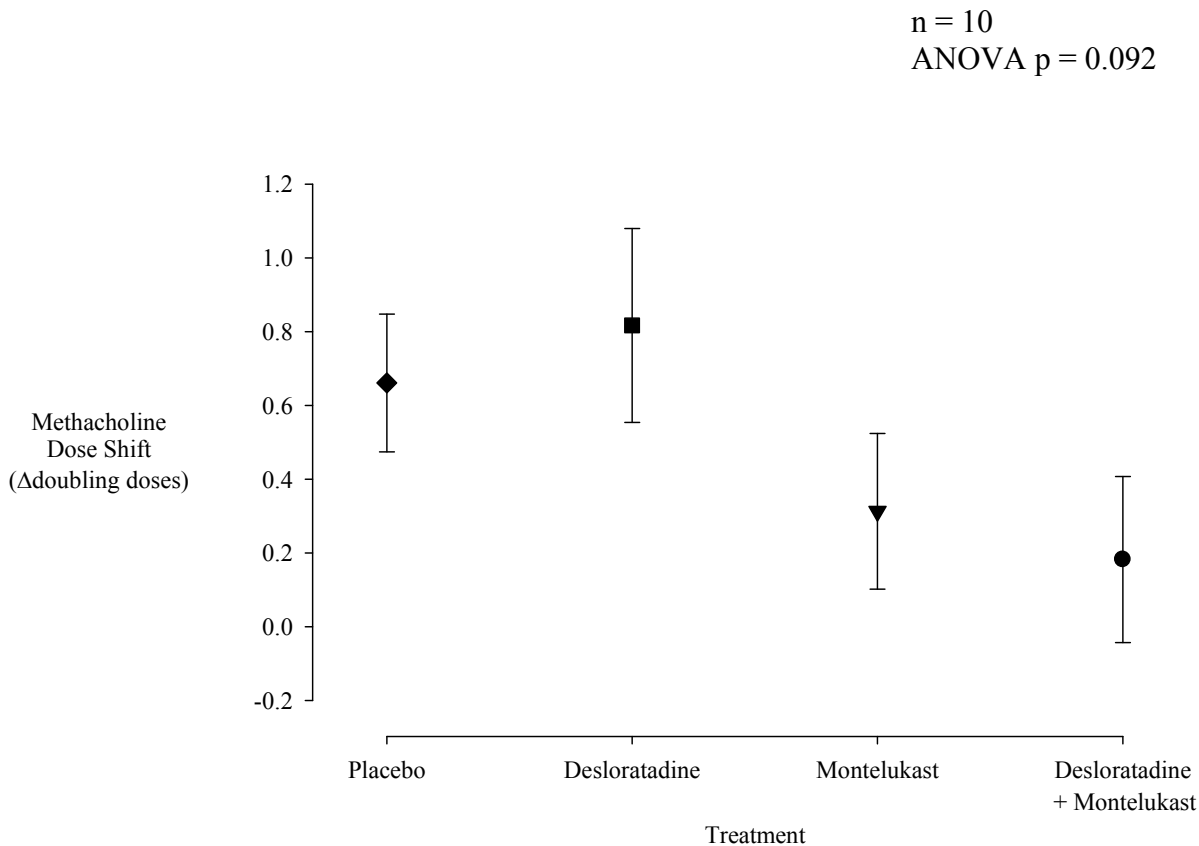
Figure 5.5   Treatment effects on the allergen induced increase in airway hyperresponsiveness to methacholine.

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.
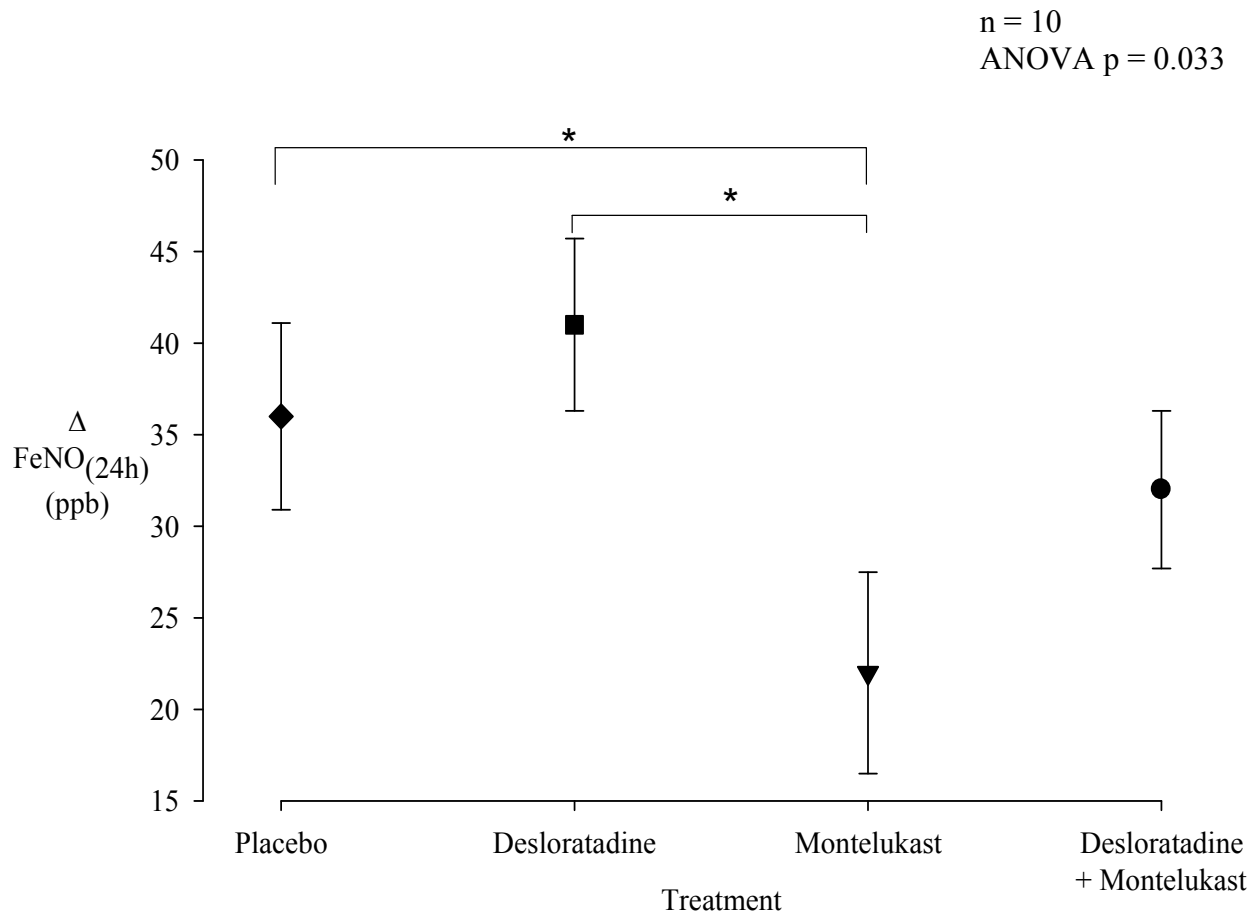
Figure 5.6   Treatment effects on the allergen induced increase in exhaled nitric oxide.

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).
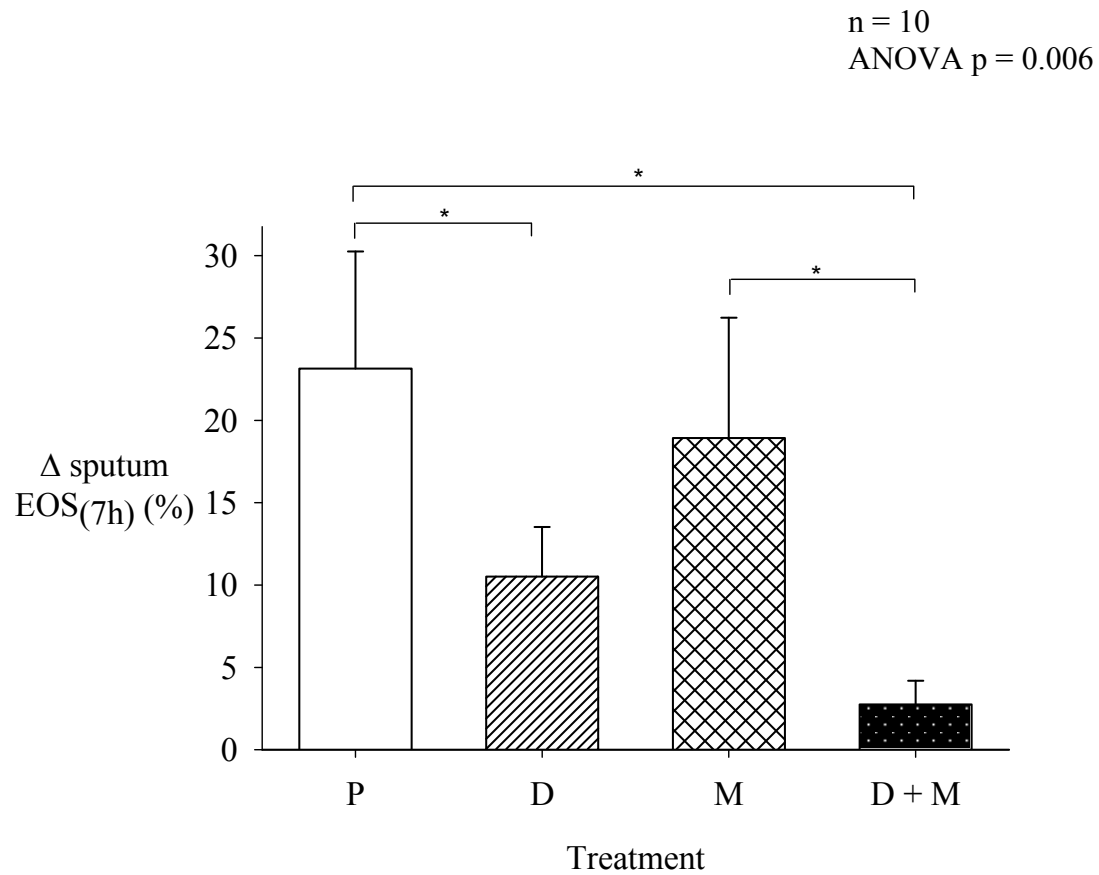
Figure 5.7   Treatment effects on allergen induced sputum eosinophilia at 7 hours.

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.
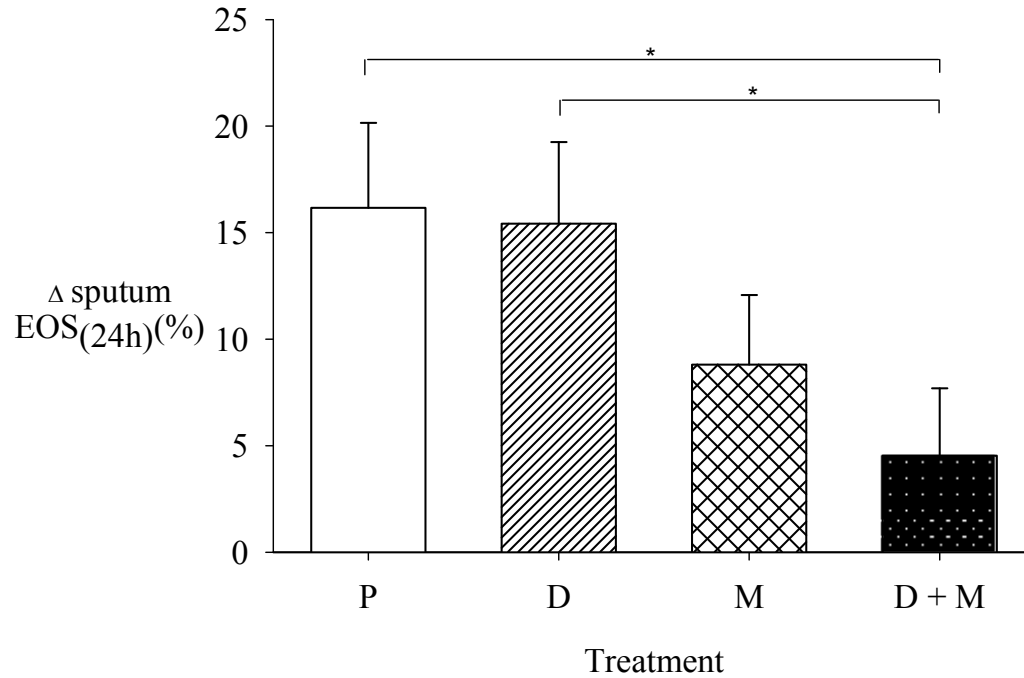
Figure 5.8   Treatment effects on allergen induced sputum eosinophilia at 24 hours.

      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 H_1 antagonist with a selective $CysLT_1$ 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 H_1 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 FEV_1 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.
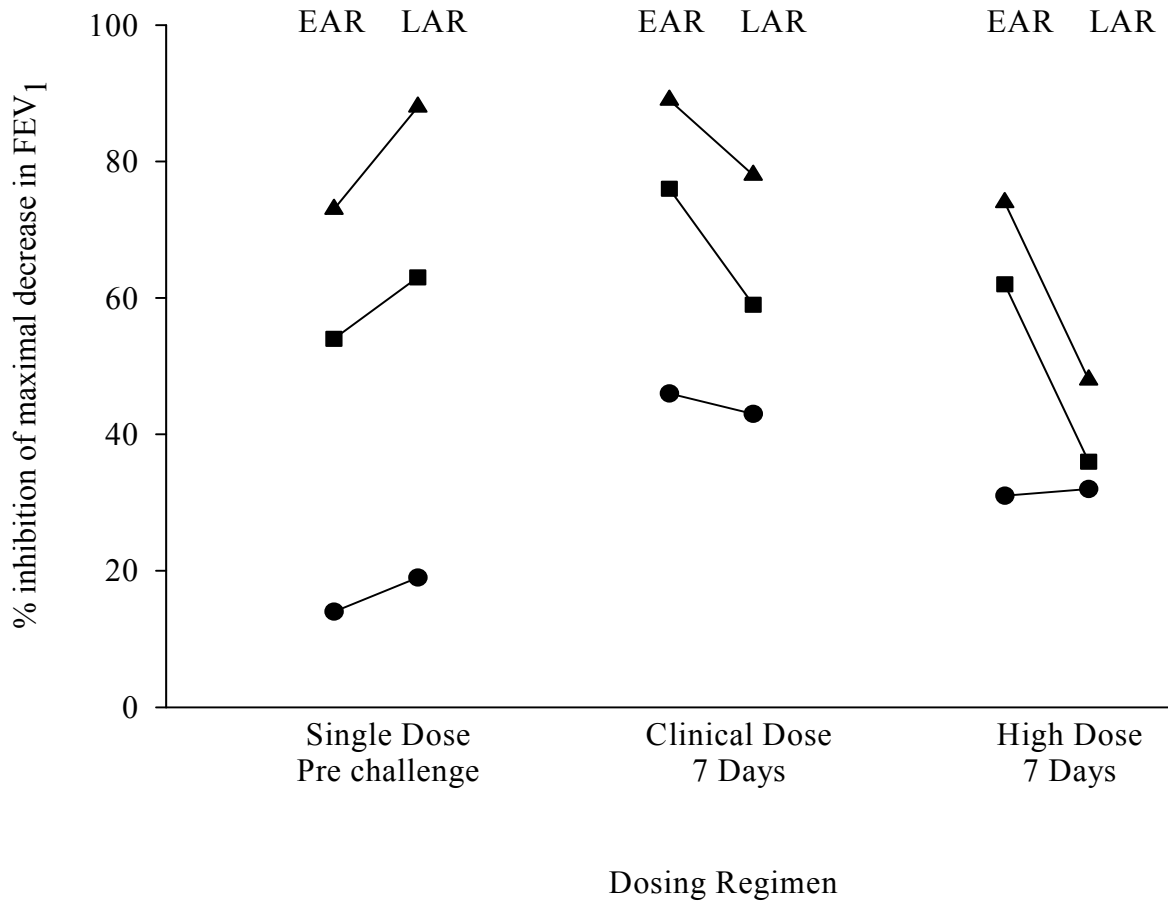
Figure 6.1:   Comparison of different doses of antihistamine and LTRA administered as monotherapy or as combination therapy on the maximal fall in FEV_1 following allergen inhalation challenge in individuals with mild atopic asthma.

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 H_1 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 H_1 receptor antagonists might be explained by the dual active/inactive conformations of the histamine H_1 receptor such that the endogenous state of being in one form or the other does not necessitate a counteractive response. All H_1 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 H_1 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 FEV_1 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.
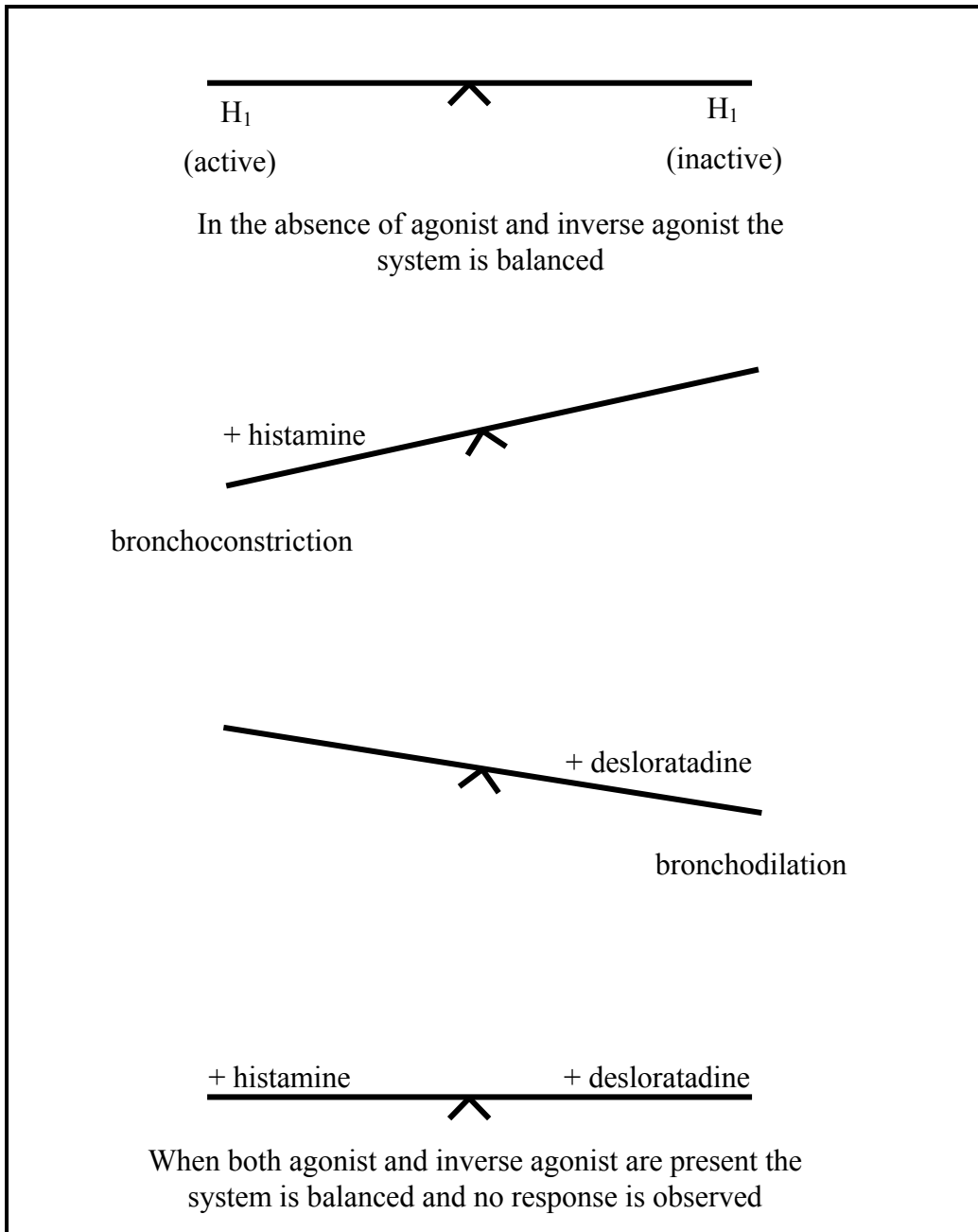
Figure 6.2: Inhibition of histamine signaling in the presence of desloratadine. The active receptor favors histamine whereas the inactive receptor favors desloratadine. In the absence of desloratadine the system responds to histamine signaling leading to airway smooth muscle contraction and desloratadine 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 H_1 and $cysLT_1$ airway smooth muscle receptor antagonism that was used in our research, has been similarly documented with β_2 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 β_2 agonists are functional antagonists of airway smooth muscle contraction. We know that anti-IgE binds free IgE preventing the formation of the allergen•IgE•$F_c\varepsilon RI$ 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 H_1 $cysLT_1$ activation. We know that both H_1 and $cysLT_1$ 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 H_1 and cys

Table 6.1   Comparative Pharmacology of EAR Inhibition (Rank Order)

Drug	% inhibition (max % Δ FEV_1)	Dose shift	Fold Δ	Reference
β_2 agonist (salbutamol)	n/a	3.9	~ 15	Cockcroft *et al*, 1993
H_1 Blocker /LTRA (desloratadine/montelukast)	n/a	3.0	8	Davis *et al*, 2009
Anti-IgE (omalizumab)	n/a	2.7	6.5	Boulet *et al*, 1997
SCG (sodium cromoglycate)	~ 75	2.0	4	Pepys *et al*, 1974
LTRA (zafirlukast)	50-75	~ 1.5	3	Roquet *et al*, 1997
Regular ICS (beclomethasone dipropionate)	~ 50	1.3-1.5	2.3-3.0	Cockcroft *et al*, 1995 Swystun *et al*, 1998
H_1 Blocker (azelastine)	≤ 46	~ 0.6	~ 1.6	Richter *et al*, 2008
Theophylline	25-50	~ 0.7	~ 1.7	Hendeles *et al*, 1995
Muscarinic antagonist (ipratropium bromide)	~ 25	0.5	~ 1.5	Cockcroft *et al*, 1978

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

LT$_1$ 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 H$_2$ - H$_4$ and cysLT$_2$ 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 H$_2$ and/or cysLT$_2$ receptors. Bronchodilation via H$_2$ receptors is another possibility as is the initiation of physiological responses via H$_4$ and cysLT$_2$ 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 Th$_2$ 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 H_1 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

Aalbers, R., Kauffman, H.F., Vrugt, B., Koeter, G.H., de Monchy, J.G. 1993. Allergen-induced recruitment of inflammatory cells in lavage 3 and 24 h after challenge in allergic asthmatic lungs. Chest. 103:1178-1184.

Adams, G.K., Lichtenstein, L. 1979. *In vitro* studies of antigen-induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. J of Immunol. 122(2):555-562.

Ahmed, T., Ungo, J., Zhou, M., Campo, C. 2000. Inhibition of allergic late airway responses by inhaled heparin-derived oligosaccharides. J Appl Physiol. 88:1721-1729.

Akdis, C.A., Blaser, K., 2003. Histamine in the immune regulation of allergic inflammation. J Allergy Clin Immunol. 112:15-22.

American Thoracic Society. 1999. Recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. Am J Respir Crit Care Med. 160:2104-2117.

Barnes, N., Piper, P.J., Costello, J. 1987. The effect of an oral leukotriene antagonist L-649,923 on histamine and leukotriene D_4 induced bronchoconstriction in normal man. J Allergy Clin Immunol. 79(5):816-821.

Beasley, R., Roche, W.R., Roberts, J.A., Holgate, S.T. Cellular events in the bronchi in mild asthma and after bronchial provocation. 1989. Am Rev Respir Dis. 139:806-817.

Beeh, K.M., Derom, E., Kanniess, F., Cameron, R., Higgins, M., van As, A. 2007. Indacaterol, a novel inhaled beta$_2$ agonist, provides sustained 24-h bronchodilation in asthma. Eur Respir J. 29(5):871-878.

Bentley, A.M., Walker, S., Hanotte, F., De Vos, C., Durham, S.R. 1996. A comparison of the effects of oral cetirizine and inhaled beclomethasone on early and late asthmatic responses to allergen and the associated increase in airways hyperresponsiveness. Clin Exp Allergy. 26:909-917.

Berry, M.A., Hargadon, B., Shelley, M., Parker, D., Shaw, D.E., Green, R.H., Bradding, P., Brightling, C.E., Wardlaw, A.J., Pavord, I.D. 2006. Evidence of a role of tumor necrosis factor alpha in refractory asthma. N Engl J Med. 354:697-708.

Bjorck, T., Dahlen, S.E. 1993. Leukotrienes and histamine mediate IgE-dependent contractions of human bronchi: pharmacological evidence obtained with tissues from asthmatic and non-asthmatic subjects. Pulm Pharmacol. 6:87-96.

Blake, K.V. 1999. Montelukast: data from clinical trials in the management of asthma. Ann Pharmacother. 33(12):1299-1314.

Blümchen, K., Gerhold, K., Thorade, I., Seib, C., Wahn, U., Hamelmann, E. 2004. Oral administration of desloratadine prior to sensitization prevents allergen-induced airway inflammation and hyper-reactivity in mice. Clin Exp Allergy. 34(7):1124-30.

Borish, L.C., Nelson, H.S., Lanz, M.J., Claussen, L., Whitmore, J.B., Agosti, J.M., Garrison, L. 1999. Interleukin-4 receptor in moderate atopic asthma. A phase I/II randomized, placebo-controlled trial. Am J Respir Crit Care Med.160:1816-1823.

Borish, L.C., Nelson, H.S., Corren, J., Bensch, G., Busse, W.W., Whitmore, J.B., Agosti, J.M.; IL-4R Asthma Study Group. 2001. Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. J Allergy Clin Immunol. 107:963-970.

Boulet, L.P., Chapman, K.R., Cote, J., Kalra, S., Bhagat, R., Swystun, V.A., Laviolette, M., Cleland, L.D., Deschesnes, F., Su, J.Q., DeVault, A., Fick, R.B., Cockcroft, D.W. 1997. Inhibitory effects of an anti-IgE antibody E25 on allergen induced early asthmatic response. Am J Respir Crit Care Med. 155(6):1835-1840.

Boulet, L.P., Turcotte, H., Laviolette, M., Côté, J., Wyile, G., Bologa, M. 1997. Inhibitory effects of BAY x 7195, a Cysteinyl leukotriene 1 receptor antagonist, on allergen-induced asthmatic responses. Ann Allergy Asthma Immunol. 79(2):155-161.

Boulet, L.P., Gauvreau, G., Boulay, M.E., O'Byrne, P., Cockcroft, D.W., Clinical Investigative Collaboration, Canadian Network of Centers of Excellence AllerGen. 2007. The allergen bronchoprovocation model : an important tool for the investigation of new asthma anti-inflammatory therapies. Allergy. 62(10):1101-1110.

Boushey, H.A., Holtzman, M.J., Sheller, J.R., Nadel, J.A. 1980. Bronchial hyperreactivity. Am Rev Respir Dis. 121:389-413.

Britton, J.R., Hanley, S.P., Tattersfield, A.E. 1987. The effect of an oral leukotriene D_4 antagonist L-649,923 on the response to inhaled antigen in asthma. J Allergy Clin Immunol. 79(5):811-816.

Brookman, L.J., Knowles, L.J., Barbier, M., Elharrar, B., Fuhr, R., Pascoe, S. 2007. Efficacy and safety of single therapeutic and supratherapeutic doses of indacaterol versus salmeterol and salbutamol in patients with asthma. Curr Med Res Opin. 23(12):3113-3122.

Bryan, S.A., O'Connor, B.J., Matti, Sl, Leckie, M.J., Kanabar, V., Khan, J., Warrington, S.J., Renzetti, L., Rames, A., Bock, J.A., Boyce, M.J., Hansel, T.T., Holgate, S.T., Barnes, P.J. 2000. Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet. 356:2149-2153.

Bryce, P.J., Geha, R., Oettgen, H.C. 2003. Desloratadine inhibits allergen-induced airway inflammation and bronchial hyperresponsiveness and alters T-cell responses in murine models of asthma. J Allergy Clin Immunol. 112(1):149-158.

Bryce, P.J., Mathias, C.B., Harrison, K.L., Watanabe, T., Geha, R.S., Oettgen, H.C. 2006. The H_1 receptor regulates allergic lung responses. J Clin Invest. 116:1624-1632.

Busse, W., Kraft, M. 2005. Cysteinyl leukotrienes in allergic inflammation. Strategic target for therapy. Chest. 127:1312-1326.

Chibana, K., Ishii, Y., Asakura, T., Fukuda, T. 2003. Up-regulation of cysteinyl leukotriene 1 receptor by IL-13 enables human lung fibroblasts to respond to leukotriene C_4 and produce eotaxin. J Immunol. 170(8):4290-4295.

Chung, K.F., Adcock, I.M. 2001. Pathophysiological mechanisms of asthma. Mol. Biotech. 18:213-232.

Cockcroft, D.W. 1983. Mechanism of perennial allergic asthma. Lancet. 2(8344):253-256.

Cockcroft, D.W., Davis, B.E. 2006. Mechanisms of airway hyperresponsiveness. Chest. 118:551-559.

Cockcroft, D.W., Davis, B.E., Boulet, L.P., Deschesnes, F., Gauvreau, G.M., O'Byrne, P.M., Watson, R.M. 2005. The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen. Allergy. 60:56-59.

Cockcroft, D.W., Keshmiri, M., Murdock, K.Y., Gore, B.C. 1992. Allergen-induced increase in airway responsiveness is not inhibited by acute treatment with ketotifen or clemastine. Ann Allergy. 68(3):245-250.

Cockcroft, D.W., Killian, D.N., Mellon, J.J.A., Hargreave, F.E. 1977. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clin Allergy. 7:235-243.

Cockcroft, D.W., McParland, C.P., Britto, S.A., Swystun. V.A., Rutherford, B.C. 1993. Regular inhaled salbutamol and airway responsiveness to allergen. Lancet. 342(8875):833-837.

Cockcroft, D.W., McParland, C.P., O'Byrne, P.M., Friend, J.L., Rutherford, B.C., Swystun, V.A. 1993. Beclomethasone given after the early asthmatic response inhibits the late response and the increased methacholine responsiveness and cromolyn does not. J Allergy Clin Immunol. 91:1163-1168.

Cockcroft, D.W., Murdock, K.Y. 1987. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. Thorax. 42:302-308.

Cockcroft, D.W., Murdock, K.Y., 1987. Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic response, late asthmatic response and increased bronchial responsiveness to histamine. J Allergy Clin Immunol. 79:734-740.

Cockcroft, D.W., Murdock, K.Y., Kirby, J., Hargreave, F.E. 1997. Prediction of responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. Am Rev Respir Dis. 135:264-267.

Cockcroft, D.W., Murdock, K.Y., Mink, J.T. 1983. Determination of histamine PC_{20}: comparison of linear and logarithmic interpolation. Chest. 84:505-506.

Cockcroft, D.W., Ruffin, R.E., Hargreave, F.E. 1978. Effect of Sch 1000 in allergen-induced asthma. Clin Allergy. 8:361-372.

Cockcroft, D.W., Swystun, V.A., Bhagat, R. 1995. Interaction of inhaled β₂ agonist and inhaled corticosteroid on airway responsiveness to allergen and methacholine. Am J Respir Crit Care Med. 152:1485-1489.

Crapo, R.O., Casaburi, R., Coates, A.L., Enright, P.L., Hankinson, J.L., Irvin, C.G., MacIntyre, N.R., McKay, R.T., Wanger, J.S., Anderson, S.D., Cockcroft, D.W., Fish, J.E., Sterk, P.J. 2000. Guidelines for methacholine and exercise challenge testing-1999. Am J Respir Crit Care Med. 161:309-329.

Currie, G.P., McLaughlin K. 2006. The expanding role of leukotriene receptor antagonists in chronic asthma. Ann Allergy Asthma Immunol. 97:731-742.

Dahl, R. 2006. Systemic side effects of inhaled corticosteroids in patients with asthma. Respir Med. 100(8):1307-1317.

Dahlen, S.E., Dahlen, B., Eliasson, E., Johansson, H., Bjorck, T., Kumlin M., Boo K, Whitney J, Binks S, King B. 1991. Inhibition of allergic bronchoconstriction in asthmatics by the leukotriene-antagonist ICI-204,219. Adv Prostaglandin Thromboxane Leukot Res. 21A:461-464.

Dahlén, B., Zetterström, O., Björck, T., Dahlén, S.E. 1994. The leukotriene-antagonist ICI-204,219 inhibits the early airway reaction to cumulative bronchial challenge with allergen in atopic asthmatics. Eur Respir J. 7(2):324-331.

Dalby, R., Spallek, M., Voshaar, T. 2004. A review of the development of Respimat Soft Mist Inhaler. International Journal of Pharmaceutics. 283:1-9.

de Bruin-Weller, M.S., Rijssenbeek-Nouwens, L.H., de Monchy, J.G. 1994. Lack of effect of cetirizine on early and late asthmatic response after allergen challenge. J Allergy Clin Immunol. 94:231-239.

Diamant, Z., Grootendorst, D.C., Veselic-Charvat, M., Timmers, M.C., De Smet, M., Leff, J.A., Seidenberg, B.C., Zwinderman, A.H., Peszek, I., Sterk, P.J. 1999. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. Clin Exp Allergy. 29:42-51.

Diamant, Z., Timmers, M.C., van der Veen, H., Friedman, B.S., De Smet, M., Depre, M., Hilliard, D., Bel, E.H., Sterk, P.J. 1995. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. J Allergy Clin Immunol. 95:42-51.

Drazen, J.M., Israel, E., O'Byrne, P.M. 1999. Treatment of asthma with drugs modifying the leukotriene pathway. NEJM. 340:197-206.

Duong, M., Cockcroft, D., Boulet, L.P., Ahmed, T., Iverson, H., Atkinson, D.C., Stahl, E.G., Watson, R., Davis, B., Milot, J., Gauvreau, G.M., O'Byrne, P.M. 2008. The effect of IVX-0142, a heparin-derived hypersulfated disaccharide, on the allergic airway responses in asthma. Allergy. 63:1195-1201.

Duong, M., Gauvreau, G., Watson, R., Obminski, G., Strinich, T., Evans, M., Howie, K., O'Byrne, P.M. 2007. The effects of inhaled budesonide and formoterol in combination and alone when given directly after allergen challenge. J Allergy Clin Immunolo. 119:322-327.

Durham, S.R., Craddock, C.F., Cookson, W.O., Benson, M.K. 1988. Increases in airway responsivness to histamine precede allergen-induced late asthmatic responses. J Allergy Clin Immunol. 82:764-770.

Eum, S.Y., Maghni, K., Hamid, Q., Campbell, H., Eidelman, D.H., Martin, J.G. 2003. Involvement of the cysteinyl-leukotrienes in allergen-induced airway eosinophilia and hyperresponsiveness in the mouse. Am J Respir Cell Mol Biol. 28:25-32.

Evans, J.M., Barnes, M.C., Zakrzewski, J.T., Sciberras, D.G., Stahl, E.G., Piper, P.J., Costello, J.F. 1989. L-648,051, a novel cysteinyl-leukotriene antagonist is active by the inhaled route in man. Brit J Clin Pharmacol. 28(2):125-135.

Fahy, J.V., Fleming, H.E., Wong, H.H., Liu, J.T., Su, J.Q., Reimann, J., Fick, R.B. Jr., Boushey, H.A. 1997. The effect of an anti-IgE monoclonal antibody on the early and late phase responses to allergen inhalation in asthmatic subjects. Am J Respir Crit Care Med. 155(6):1828-1834.

Fahy, J.V., Liu, J., Wong, H., Boushey, H.A. 1994. Analysis of cellular and viochemical constituents of induced sputum after allergen challenge: a method for studying allergic airway inflammation. J Allergy Clin Immunol. 93(6):1031-1039.

Findlay, S.R., Barden, J.M., Easley, C.B., Glass, M. 1992. Effect of the oral leukotriene antagonist, ICI-204,219, on antigen-induced bronchoconstriction in subjects with asthma. J Allergy Clin Immunol. 89(5):1040-1045.

From the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA), 2008.

Galli, S.J., Tsai, M., Piliponsky, A.M. 2008. The development of allergic inflammation. Nature. 454: 445-454.

Gauvreau,G.M., Becker, A.B., Boulet, L.P., Chakir, J., Fick, R.B., Greene, W.L., Killian, K.J., O'Byrne, P.M., Reid, J.K., Cockcroft, D.W. 2003. The effects of an anti-CD11a mAb, efalizumab, on allergen-induced airway responses and airway inflammation in subjects with atopic asthma. J Allergy Clin Immunol. 112:331-338.

Gauvreau, G.M., Boulet, L.P., Cockcroft, D.W., Baatjes, A., Cote, J., Deschesnes, F., Davis, B., Strinich, T., Howie, K., Duong, M., Watson, R.M., Renzi, P.M., O'Byrne, P.M. 2008. Antisense therapy against CCR3 and the common beta chain attenuates allergen-induced eosinophilic responses. Am J Respir Crit Care Med. 177:952-958. Epub 2008 Jan 31.

Gauvreau, G.M., Lee, J.M., Watson, R.M., Irani, A.M., Schwartz, L.B., O'Byrne, P.M. 2000. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge of atopic asthmatics. Am J Respir Crit Care Med. 161(5):1473-1478.

Gauvreau, G.M., Watson, R.M., Rerecich, T.J., Baswick, E., Inman, M.D., O'Byrne, P.M. 1999. Repeatability of allergen-induced airway inflammation. J Allergy Clin Immunol. 104:66-71.

Gelfand, E.W., Cui, Z.H., Takeda, K., Kanehiro, A., Joetham, A. 2002. Fexofenadine modulates T-cell function, preventing allergen-induced airway inflammation and hyperresponsiveness. J Allergy Clin Immunol. 110(1):85-95.

Gelfand, E.W., Cui, Z.H., Takeda K., Kanehiro, A., Joetham, A. 2003. Effects of fexofenadine on T-cell function in a murine model of allergen-induced airway inflammation and hyperresponsiveness. J Allergy Clin Immunol. 112(4 Suppl):S89-95.

Genovese, A., Patella, V., De Crescenzo, G., De Paulis, A., Spadaro, G., Marone, G. 1997. Loratadine and desethoxylcarbonyl-loratadine inhibit the immunological release of mediators from human FcεRI$^+$ cells. Clin Exp Allergy. 27(5):559-567.

Gong, H. Jr., Tashkin, D.P., Dauphinee, B., Djahed, B., Wu, T.C. 1990. Effects of oral cetirizine, a selective H_1 antagonist, on allergen- and exercise-induced bronchoconstriction in subjects with asthma. J Allergy Clin Immunol. 85:632-641.

Gordon, J.R., Swystun, V.A., Li, F., Zhang, X., Davis, B.E., Hull, P., Cockcroft, D.W. 2003. Regular salbutamol use increases CXCL8 responses in asthma: relationship to the eosinophil response. Eur Respir J. 22(1):118-126.

Hamid, M., Rafferty, P., Holgate S.T. 1990. The inhibitory effect of terfenadine and flurbiprofen on early and late-phase bronchoconstriction following allergen challenge in atopic asthma. Clin Exp Allergy. 20(3):261-267.

Hamilton, A., Faiferman, I., Stober, P., Watson, R.M., O'Byrne, P.M. 1998. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. J Allergy Clin Immunol. 102(2):177-183.

Hamilton, A.L., Watson, R.M., Wyile, G., O'Byrne, P.M. 1997. Attenuation of early and late phase allergen-induced bronchoconstriction in asthmatic subjects by a 5-lipoxygenase activating protein antagonist, BAYx 1005. Thorax. 52:348-354.

Hargreave, F.E., Nair, P. 2009. The definition and diagnosis of asthma. Clin Exp Allergy. 39:1652-1658.

Hasday, J.D., Meltzer, S.S., Moore, W.C., Wisniewski, P., Hebel, J.R., Lanni, C., Dube, L.M., Bleecker, E.R. 2000. Anti-inflammatory effects of zileuton in a subpopulation of allergic asthmatics. Am J Respir Crit Care Med. 161:1229-1236.

Hay, D.W.P., Muccitelli, R.M., Tucker, S.S., Vickery-Clark, L.M., Wilson, K.A., Gleason, J.G., Hall, R.F., Wasserman, M.A., Torphy, T.J. 1987. Pharmacologic profile of SK&F 104353: A novel, potent and selective peptidoleukotriene receptor antagonist in guinea pig and human airways. J Pharmacol Exp Ther. 243(2):474-481.

Hellings, P.W., Ceuppens, J.L. 2004. Mouse models of global airway allergy: what have we learned and what should we do next? Allergy. 59:914-919.

Hendeles, L., Harman, E., Huang, D., O'Brien, R., Blake, K., Delafuente, J. 1995. Theophylline attenuation of airway responses to allergen: Comparison with cromolyn metered-dose inhaler. J Allergy Clin Immunol. 95:505-514.

Hendeles, L., Harman, E. 1997. Use of allergen bronchoprovocation to screen drugs for anti-asthma activity. Pharmacotherapy. 17(1 Pt 2):39S-49S.

Henderson, W.R. Jr., Tang, L.O., Chu, S.J., Tsao, S.M., Chiang, G.K., Jones, F., Jonas, M., Pae, C., Wang, H., Chi, E.Y. 2002 A role for cysteinyl leukotrienes in airway remodeling in a mouse asthma model. Am J Respir Crit Care Med. 165(1):108-116.

Holgate, S.T., Finnerty, J.P. 1989. Antihistamines in asthma. J Allergy Clin Immunol;537-547.

Holgate, S.T., Dahlen, S-E. 1997. From slow reacting substance to leukotrienes: A testimony of scientific endeavour and achievement. *SRS-A to leukotrienes. The dawning of a new treatment.(Holgate, S.T., Dahlen S-E.)* Oxford. Blackwell Science

Howarth PH, Babu KS, Arshad HS, Lau L, Buckley M, McConnell W, Beckett P, Al Ali M, Chauhan A, Wilson SJ, Reynolds A, Davies DE, Holgate ST. 2005. Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. Thorax. 60:1012-1018.

Hui, K.P., Taylor, I.K., Taylor, G.W., Rubin, P., Kesterson, J., Barnes, N.C., Barnes, P.J. 1991. Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients. Thorax. 46:184-189.

Ihaku, D., Cameron, L., Suzuki, M., Molet, S., Martin, J., Hamid, Q. 1999. Montelukast, a leukotriene receptor antagonist, inhibits the late airway response to antigen, airway eosinophilia, and IL-6-expressing cells in Brown Norway rats. J Allergy Clin Immunol. 104(6):1147-1154.

Inman, M.D., Watson, R., Cockcroft, D.W., Wong, B.J., Hargreave, F.E., O'Byrne, P.M. 1995. Reproducibility of allergen-induced early and late asthmatic responses. J Allergy Clin Immunol. 95:1191-1195.

Israel, E., Cohn, J., Dube, L., Drazen, J.M. 1996. Effect of treatment with zileuton, a 5-lipoxygenase inhibitor, in patients with asthma. A randomized controlled trial. Zileuton Clinical Trial Group. JAMA. 275:931-936.

Jatakanon, A., Lim, S., Kharatinov, S.A., Chung, K.F., Barnes, P.J. 1998. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax. 53:91-95.

Jeffrey, P.K., Haahtela, T. 2006. Allergic rhinitis and asthma: inflammation in a one-airway condition.BMC Pulm Med. 30(6 Suppl) 1:S5.

Jokic, R., Davis, E.E., Cockcroft, D.W. 1998. Methacholine PC_{20} extrapolation. Chest. 114:1796-1797.

Jutel, M., Blaser, K., Akdis, C.A. 2006. The role of histamine in regulation of immune responses. Chem Immunol Allergy. 91:174-187.

Kharitonov, S.A., O'Connor, B.J., Evans, D.J., Barnes, P.J. 1995. Allergen-induced late asthmatic reactions are associated with elevation of exhaled nitric oxide. Am J Respir Crit Care Med.151:1894-1899.

Kowalski, M.L., Lewandowska, A., Wozniak, J., Makowska, J., Jankowski, A., DuBuske, L. 2005. Inhibition of nasal polyp mast cell and eosinophil activation by desloratadine. Allergy. 6(1):80-85.

Kushiya, M., Saito, K., Kikuchi, I., Kobayashi, T., Hagiwara, K., Kanazawa, M., Nagata M. 2006. Differential effects of salbutamol and montelukast on eosinophil adhesion and superoside anion generation. Int Arch Allergy Immunol. 140 (Suppl 1):17-22.

Lamoureux, J., Stankova, J., Rola-Pleszczynski, M. 2006. Leukotriene D_4 enhances immunoglobulin production in CD40-activated human B lymphocytes. J Allergy Clin Immunol. 117:924-930.

Leckie, M.J., ten Brinke, A., Khan, J., Diamant, Z., O'Connor, B.J., Walls, C.M., Mathur, A.K., Cowley, H.C., Chung, K.F., Djukanovic, R., Hansel, T.T., Holgate, S.T., Sterk, P.J., Barnes, P.J.

2000. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet. 356:2144-2148.

Lee, K.S., Kim, S.R., Park, H.S., Park, S.J., Min, K.H., Lee, K.Y., Jin, S.M., Lee, Y.C. 2007. Cysteinyl leukotriene upregulates IL-11 expression in allergic airway disease of mice. J Allergy Clin Immunol. 119(1):141-149.

Leigh, R., Vethanayagam, D., Yoshida, M., Watson, R.M., Rerecich, T., Inman, M.D., O'Byrne, P.M. 2002. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. Am J Respir Crit Care Med. 166:1212-1217.

Ma, Y., Hayglass, K.T., Becker, A.B., Halayko, A.J., Basu, S., Simons, F.E., Peng, Z. 2007. Novel cytokine peptide-based vaccines: an interleukin-4 vaccine suppresses airway allergic responses in mice. Allergy. 62:675-682.

MacFarlane, A.J., Dworski, R., Sheller, J.R., Pavord, I.D., Kay, A.B., Barnes, N.C. 2000. Sputum cysteinyl leukotrienes increase 24 hours after allergen inhalation in atopic asthmatics. Am J Respir Crit Care Med. 161:1553-1558.

Maeba, S., Ichiyama, T., Ueno, Y., Makata, H., Matsubara, T., Furukawa, S. 2005. Effect of montelukast on nuclear factor kappaB activation and proinflammatory molecules. Ann Allergy Asthma Immunol. 94(6):670-674.

Meltzer, S.S., Hasday, J.D., Cohn, J., Bleecker, E.R. 1996. Inhibition of exercise-induced bronchospasm by zileuton: a 5-lipoxygenase inhibitor. Am J Respir Crit Care Med. 153:931-935.

Minoguchi, K., Kohno, Y., Minoguchi, H., Kihara, N., Sano, Y., Yasuhara, H., Adachi, M. 2002. Reduction of eosinophilic inflammation in the airways of patients with asthma using montelukast. Chest. 121(3):732-738.

Murdoch, D., Goa, K.L., Keam, SJ. 2003. Desloratadine. An update on its efficacy in the management of allergic disorders. Drugs. 63(19):2051-2077.

O'Byrne, P. 2006. Cytokines or their antagonists for the treatment of asthma. Chest. 130:244-250.

O'Byrne, P.M., van der Linde, J., Cockcroft, D.W., Gauvreau, G.M., Brannan, J.D., Fitzgerald, J.M., Watson, R.M., Milot, J., Davis, B., O'Connor, M., Hart, L., Korducki, L., Hamilton, A., Boulet, L.P. 2009. Prolonged bronchoprotection against inhaled methacholine by inhaled BI 1744, a long-acting β_2 agonist, in mild asthmatics. J Allergy Clin Immunol. in press.

O'Connor, B.J., Fuller, R.W., Barnes, P.J. 1994. Non-bronchodilator effects of inhaled β_2 agonists. Am J Respr Crit Care Med. 150:381-387.

Palmqvist, M., Bruce, C., Sjostrand, M., Arvidsson, P., Lotvall, J. 2005. Differential effects of fluticasone and montelukast on allergen-induced asthma. Allergy. 60:65-70.

Parameswaran, K., Liang, H., Fanat, A., Watson, R., Snider, D.P., O'Byrne, P.M. 2004. Role for cysteinyl leukotrienes in allergen induced change in circulating dendritic cell number in asthma. J Allergy Clin Immunol. 114:73-79.

Parameswaran, K., Watson, R., Gauvreau, G.M., Sehmi, R., O'Byrne, P.M. 2004. The effect of parnlukast on allergen-induced bone marrow eosinophilopoiesis in subjects with asthma. Am J Respir Crit Care Med. 169:915-920.

Pawankar, R., Baena-Cagnani, C.E., Bousquet, J., Canonica, G.W., Cruz, A.A., Kaliner, M.A., Lanier, B.Q. 2008. State of World Allergy Report 2008: Allergy and Chronic Respiratory Diseases. WAO Journal. Supplement S4-S17.

Pendharkar, S., Mehta, S. 2008. The clinical significance of exhaled nitric oxide in asthma. Can Respir J. 15:99-106.

Pepys, J., Davies, R.J., Breslin, A.B., Hendrick, D.J., Hutchcroft, B.J. 1974. The effects of inhaled beclomethasone dipropionate (Becotide) and sodium cromoglycate on asthmatic reactions to provocation tests. Clin Allergy. 4(1):13-24.

Pin, I., Freitag, A.P., O'Byrne, P.M., Girgis-Gabardo, A., Watson, R.M., Dolovich, J., Denburg, J.A., Hargreave, F.E. 1992. Changes in the cellular profile of induced sputum after allergen-induced asthmatic responses. Am Rev Respir Dis. 145(6):1265-1269.

Pizzichini, E., Leff, J.A., Reiss, T.F., Hendeles, L., Boulet, L.P., Wei, L.X., Efthimiadis, A.E., Zhang, J., Hargreave, F.E. 1999. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. Eur Respir J. 14(1):12-18.

Pizzichini, E., Pizzichini, M.M., Efthimiadis, A., Evans, S., Morris, M.M., Squillace, D., Gleich, G.J., Dolovich, J., Hargreave, F.E. 1996. Indices of airway inflammation in induced sputum : reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med. 154:308-317.

Rafferty, P., Beasley, R., Holgate, S.T. 1987. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5' monophosphate in atopic asthma. Am Rev Respir Dis. 136:369-373.

Rafferty, P., Gosh, S.K., de Vos, C., Patel, K.R. 1993. Effect of oral and inhaled cetirizine in allergen induced bronchoconstriction. Clin Exp Allergy. 23(6):528-531.

Rafferty, P., Jackson, L., Smith, R., Holgate, S.T. 1990 Terfenadine, a potent histamine H_1 receptor antagonist in the treatment of grass pollen sensitive asthma. Br J Clin Pharmacol. 30:229-235.

Rafferty, P., Ng, W.H., Phillips, G., Clough, J., Church, M.K., Aurich, R., Ollier, S., Holgate, S.T. 1989. The inhibitory actions of azelastine hydrochloride on the early and late bronchoconstrictor responses to inhaled allergen in atopic asthma. J Allergy Clin Immunol. 84(5Pt 1):649-657.

Rasmussen, J.B., Eriksson, L.O., Anderson, K.E. 1991. Reversal and prevention of airway response to antigen challenge by the inhaled leukotriene D_4 antagonist (L-648,051) in patients with atopic asthma. Allergy. 46(4):266-273.

Reinartz, S.M., Overbeek, S.E., Kleinjan, A., van Drunen, C.M., Braunstahl, G.J., Hoogsteden, H.C., Fokkens, W.J. 2005. Desloratadine reduces systemic allergic inflammation following nasal provocation in allergic rhinitis and asthma patients. Allergy. 60(10):1301-1307.

Richter, K., Gronke, L., Janicki, S., Maus, J., Jorres, R.A., Magnussen, H. 2008. Effect of azelastine, montelukast, and their combination on allergen-induced bronchoconstriction in asthma. Pulm Pharmacol Ther. 21:61-66.

Roquet, A., Dahlen, B., Kumlin, M., Ihre, E., Anstren, G., Binks, S., Dahlen, S-E. 1997. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. Am J Respir Crit Care Med. 155:1856-1863.

Roumestan, C., Henriquet, C., Gougat, C., Michel, A., Portet, K., Jaffuel, D., Mathieu, M. 2008. istamine H_1-receptor antagonists inhibit nuclear factor-kappaB and activator protein-1 activities via H_1-receptor-dependent and –independent mechanisms. Clin Exp Allergy 38:947-956.

Ruck, L.M., Rizzo, C.A., Anthes, J.C., Eckel, S., Egan, R.w., Cuss, F.M., Hey, J.A. 2001. Synergistic antiallergic activity of combined histamine H_1- and cysteinyl leukotriene$_1$-receptor bloackade in human bronchus. Life Sciences. 68:2825-2834.

Schering-Plough website: www.schering-plough.com/news/news.aspx. Accessed June 10, 2009.

Schneider, E., Rolli-Derkinderen, M., Arock, M., Dy, M. 2002. Trends in histamine research: new functions during immune responses and hematopoiesis. Trends in Immunology. 23:255-263.

Schroeder, J.T., Schleimer, R.P., Lichtenstein, L.M., Kreutner, W. 2001. Inhibition of cytokine generation and mediator release by human basophils treated with desloratadine. Clin Exp Allergy. 31(9):1369-1377.

Simons, F.E. 2004. Advances in H_1-Antihistamines. N Engl J Med. 351;2203-2217.

Spinozzi, F., Russano, A.M., Piattoni, S., Agea, E., Bistoni, O., de Benedictis, D., de Benedictis, F.M. 2004. Biological effects of montelukast, a cysteinyl-leukotriene receptor-antagonist, on T lymphocytes. Clin Exp Allery. 34(12):1876-1882.

Stelmach, I., Jerzynska, J., Kuna, P. 2002. A randomized, double-blind trial of the effect of treatment with montelukast on bronchial hyperresponsiveness and serum eiosinophilic cationic protein (ECP), soluble interleukin 2 receptor (sIL-2R), IL-4, and soluble intercellular adhesion molecule 1 (sICAM-1) in children with asthma. J Allergy Clin Immunol. 109(2):210-213.

Stelmach, I., Jerzynska, J., Kuna, P. 2002. A randomized, double-blind trial of the effect of glucocorticoid, antileukotriene and beta-agonist treatment on IL-10 serum levels in children with asthma. Clin Exp Allergy. 32(2):264-269.

Stelmach, I., Majak, P., Jerzynska, J., Kuna, P. 2005. The effect of treatment with montelukast on *in vitro* interleukin-10 production of mononuclear cells of children with asthma. Clin Exp Allergy. 35(2):213-220.

Subbarao, P., Dorman, S.C., Rerecich, T., Watson, R.M., Gauvreau, G.M., O'Byrne, P.M. 2005. Protection by budesonide and fluticasone on allergen-induced airway responses after discontinuation of therapy. J Allergy Clin Immunol. 115:745-750.

Swystun, V.A., Bhagat, R., Kalra, S., Jennings, B., Cockcroft, D.W. 1998. Comparison of 3 different doses of budesonide and placebo on the early asthmatic response to inhaled allergen. J Allergy Clin Immunol. 102:363-367.

Taylor, I.K., O'Shaughnessy, K.M., Fuller, R.W., Dollery, C.T. 1991. Effect of cysteinyl-leukotriene antagonist ICI-204,219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. Lancet. 337:690-694.

Thurmond, R.L., Gelfand, E.W., Dunford, P.J. 2008. The role of histamine H_1 and H_4 receptors in allergic inflammation: the search for new antihistamines. Nat Rev Drug Discov. 7:41-53.

Town, G.I., Holgate, S.T. 1990. Comparison of the effect of loratadine on the airway and skin responses to histamine, methacholine, and allergen in subjects with asthma. J Allergy Clin Immunol. 86(6 Pt 1):886-893.

Twentyman, O.P., Ollier, S., Holgate, S.T. 1993. The effect of H1-receptor blockade on the development of early- and late-phase bronchoconstriction and increased bronchial responsiveness in allergen-induced asthma. J Allergy Clin Immunol. 91(6):1169-1178.

van der Velden, V.H.J. 1998. Glucocorticoids: mechanisms of action and anti-inflammatory potential in asthma. Mediators Inflamm. 7:229-237.

Virchow, J.C. Jr., Faehndrich, S., Nassenstein, C., Bock, S., Matthys, H., Luttmann, W. 2001. Effect of a specific cysteinyl leukotriene-receptor 1-antagonist (montelukast) on the transmigration of eosinophils across human umbilical vein endothelial cells. Clin Exp Allergy. 31(6):836-844.

Watts, A.B., McConville, J.T., Williams, R.O. 2008. Current therapies and technological advances in aqueous aerosol drug delivery. Drug Dev Ind Pharm. 34(9):913-922.

Wang, Y.H., Taché, Y., Harris, A.G., Kreutner, W., Daly, A.F., Wei, J.Y.. 2005. Desloratadine prevents compound 48/80-induced mast cell degranulation: visualization using a vital fluorescent dye technique. Allergy. 60(1):117-124.

Wenzel, S.K., Wilbraham, D., Fuller, R., Burmeister G.E., Longphre M. 2007. Effect of an interleukin-4 variant on late phase asthmatic response to allergen challenge in asthmatic patients: results of two phase 2a studies. Lancet. 370:1422-1431.

Wood-Baker, R., Holgate, S.T. 1993. The comparative action and adverse event profile of single doses of H_1 receptor antihistamines in the airways and skin of subjects with asthma. J Allergy Clin Immunology 91(5):1005-1014.

World Health Organization publication. 2003. Prevention of allergy and allergic asthma. Based on the WHO/WAO meeting on the prevention of allergy and allergic asthma. Geneva, 8-9 January 2002.

Woszczek, G., Chen, L., Nagineni, S., Kern, S., Barb, J., Munson, P.J., Logun, C., Danner, R.L., Shelhamer, J.H. 2008. Leukotriene D_4 induces gene expression in human monocytes through cysteinyl leukotriene type I receptor. J Allergy Clin Immunol. 121:215-221.

Woszczek, G., Chen, L., Nagineni, S., Shelhamer, J.H. 2008. IL-10 inhibits cysteinyl leukotriene-induced activation of human monocytes and monocyte-derived dendritic cells. J Immunol. 180:7597-7603.

Wu, A.Y., Chik, S.C., Chan, A.W., Li, Z., Tsang, K.W., Li, W. 2003. Anti-inflammatory effects of high-dose montelukast in an animal model of acute asthma. Clin Exp Allergy. 33(3):359-366.

Wu, R.L., Anthes, J.C., Kreutner, W., Harris, A.G., West, R.E. Jr. 2004. Desloratadine inhibits constitutive and histamine-stimulated nuclear factor-kappaB activity consistent with inverse agonism at the histamine H1 Receptor. Int Arch Allergy Immunol. 135(4):313-8. Epub 2004 Nov 24.

Wu, Y., Zhou, C., Tao, J., Li, S. 2006. Montelukast prevents the decrease of interleukin-10 and inhibits NF-kappaB activation in inflammatory airway of asthmatic guinea pigs. Can J Physiol Pharmacol. 84(5):531-537.

Zhang, Y.J., Ahang, L., Wnag, S.B., Shen H.H., Wei, E.Q. 2004 Montelukast modulates lung CysLT(1) receptor expression and eosinophilic inflammation in asthmatic mice. Acta Pharmacol Sin. 25(10):1341-1346.

9.0   APPENDICES

9.1   APPENDIX A: JACI Publication of the Early Asthmatic Response Study

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

Beth E. Davis, BSc, David C. Todd, MD, FRCP(C), and Donald W. Cockcroft, MD, FRCP(C)

Saskatoon, Saskatchewan, Canada

Background: The early asthmatic response (EAR) 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 might protect against the EAR better than either therapy alone.

Objective: We sought to evaluate the effect of desloratadine and montelukast, administered alone and in combination, on the EAR to inhaled allergen.

Methods: Ten adults with mild-to-moderate atopic asthma participated in a randomized, 4-way crossover study design comparing placebo, 5 mg of desloratadine, 10 mg of montelukast, and the combination administered at 26 hours and 2 hours before each allergen challenge conducted at least 7 days apart. The primary end point was the concentration of allergen that resulted in a 20% decrease in FEV_1 (PC_{20}).

Results: The geometric mean allergen PC_{20} (mean log ± SEM) for combination therapy, montelukast, desloratadine, and placebo was 697 U/mL ($2.8433 ± 0.3253$), 338 U/mL ($2.5295 ± 0.2979$), 123 U/mL ($2.0883 ± 0.2102$), and 104 U/mL ($2.0166 ± 0.2553$), respectively (n = 9; $P < .00001$, ANOVA). Montelukast increased the allergen PC_{20} 4.8-fold, and combination therapy increased the allergen PC_{20} 8.9-fold. The effect of the combination was greater than that with montelukast alone ($P < .02$). Desloratadine treatment was no different than placebo.

Conclusions: The early response to inhaled allergen was unchanged after desloratadine therapy and partially inhibited with montelukast therapy. The combination of desloratadine and montelukast provided superior efficacy to either blocker administered alone. Investigations into the possible mechanisms of the enhanced inhibition are necessary. (J Allergy Clin Immunol 2005;116:768-72.)

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 (H_1) 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 H_1 blockers on the EAR have produced variable and inconclusive results.[1-5] Leukotriene modifiers have been shown to provide reasonable inhibition of the EAR and LAR but do not completely abolish the response.[6-10] 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

Ten healthy atopic asthmatic subjects older than 18 years and with a baseline FEV_1 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 (thiamine, 100 mg) investigation. Matching placebo tablets were

768

TABLE I. Patient demographics

Subject no.	Sex	Age (y)	Height (inches)	Baseline FEV$_1$ (L)	% Predicted	Allergen	MPC$_{20}$ (mg/mL)	Medications
1	M	57	66	2.46	73	Grass*	0.5	S, prn
2	M	29	70	3.87	87	HDM†	2	S, prn
3	M	27	71	3.67	86	Cat‡	0.67	S, prn
4	F	24	62	2.66	83	Cat‡	0.28	S, prn
5	M	25	72	4.07	85	Cat‡	2	S, prn
6	M	40	72	2.86	65	Grass*	1.8	S, prn
7	F	25	66	3.12	89	HDM†	2.5	S, prn; F, 125 µg bid
8	M	43	68	3.44	88	Grass*	16	S; prn
9	F	27	64	3.35	101	Cat‡	0.86	S; prn
10§	M	26	72	4.59	96	Cat‡	0.75	S; prn
Mean		32	68	3.41	85		2.7	

S, Salbutamol; *prn*, as required; *F*, fluticasone propionate; *bid*, twice daily.
*Grass mix 10 (40,000 protein nitrogen units/mL).
†Standard mite mix (10,000 allergy units/mL).
‡Standardized cat pelt (10,000 bioequivalent allergy units/mL).
§Not included in analysis.

Abbreviations used
AUC: Area under the curve
EAR: Early asthmatic response
H$_1$: Histamine receptor subtype 1
LAR: Late asthmatic response
LTRA: Leukotriene receptor antagonist
PC$_{20}$: Concentration of allergen that causes a 20% decrease in FEV$_1$

---

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 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 PC$_{20}$ by using the skin test end point and airway responsiveness to methacholine.[11] 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 (Roxon Medi-tech Ltd, Montreal, Quebec, Canada) calibrated to deliver 0.13 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 FEV$_1$ maneuvers were performed 60 seconds apart. Inhalations were continued until

the highest postinhalation FEV$_1$ was at least 15% lower than the highest baseline FEV$_1$ (obtained from 3 reproducible flow-volume loops after a ≥20-minute rest period) or until the top concentration had been administered. The FEV$_1$ measurement was repeated at 10-minute intervals until no further decrease was observed.[12] The allergen PC$_{20}$ was then calculated algebraically.[13] 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 FEV$_1$ data and log-transformed allergen PC$_{20}$ data were analyzed by using 2-way ANOVA, followed by pairwise comparison (least-squared difference) of means if applicable (Statistix Version 7.0; Analytical Software Corp., Tallahassee, Fla). Power calculation[15] showed a 98% power to detect a 1 doubling concentration difference in allergen PC$_{20}$ 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 FEV$_1$ 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 PC$_{20}$ 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
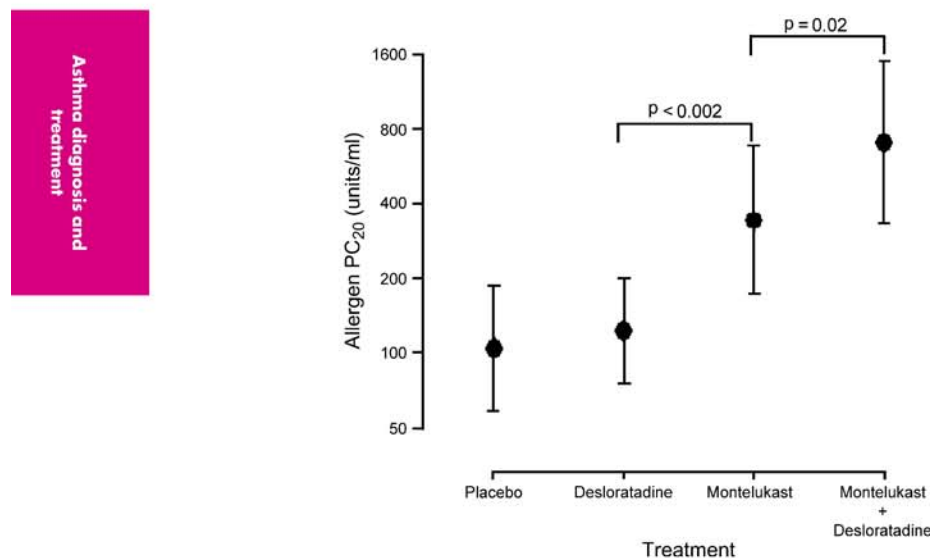
Asthma diagnosis and treatment

FIG 1. Allergen PC_{20} (geometric mean) for each treatment. Extract units are generically identified as units per milliliter. *Error bars* represent SEM.

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 3.2, 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,[16] desloratadine possesses superior pharmacokinetic and pharmacodynamic properties, has an excellent safety profile, and has been shown to exert anti-inflammatory effects.[17] 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[18] and has been shown to significantly decrease the EAR to inhaled allergen.[19-21] 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 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 (vs an average of around 55% reported previously[19-21]) in the maximal decrease in FEV_1.

It is logical to postulate that the EAR to inhaled allergen could be additively 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.[22] Since then, only 2 studies investigating the effect of combination (antihistamine plus LTRA) therapy on the EAR could be identified. Roquet et al[23] investigated

107

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 H_1 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 PC_{20} by 3.2 doubling concentrations (8.9-fold) versus placebo. This is the first evidence of 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 (<1%) reported as maximal decreases in FEV_1,[25] suggesting mast-cell stabilization as a possible mechanism.

There are several methods to assess the EAR, including area under the curve ($AUC_{[0-3h]}$), 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, 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 $AUC_{(0-3h)}$ 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 might 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 1 doubling concentration is approximately equivalent to a 50% change in the maximal decrease in FEV_1, 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 PC_{20} reporting provides a more precise discrimination between treatments that generate a 50% or greater change in the maximal decrease in FEV_1. 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

1. Rafferty P, Beasley R, Holgate ST. The contribution of histamine to immediate bronchoconstriction provoked by inhaled allergen and adenosine 5' monophosphate in atopic asthma. Am Rev Respir Dis 1987;136: 369-73.
2. Gong H Jr, Tashkin DP, Dauphinee B, Djahed B, Wu TC. Effects of oral cetirizine, a selective H_1 antagonist, on allergen- and exercise-induced bronchoconstriction in subjects with asthma. J Allergy Clin Immunol 1990;85:632-41.
3. Rafferty P, Jackson L, Smith R, Holgate ST. Terfenadine, a potent histamine H1-receptor antagonist in the treatment of grass pollen sensitive asthma. Br J Clin Pharmacol 1990;30:229-35.
4. Cockcroft DW, Keshmiri M, Murdock KY, Gore BC. Allergen induced increase in airway responsiveness is not inhibited by acute treatment with ketotifen or clemastine. Ann Allergy 1992;68:245-50.
5. Bentley AM, Walker S, Hanotte F, De Vos C, Durham SR. A comparison of the effects of oral cetirizine and inhaled beclomethasone on early and late asthmatic responses to allergen and the associated increase in airways hyperresponsiveness. Clin Exp Allergy 1996;26:909-17.
6. Dahlen S-E, Dahlen B, Eliasson E, Johansson H, Bjorck T, Kumlin M, et al. Inhibition of allergic bronchoconstriction in asthmatics by the leukotriene-antagonist ICI-204,219. Adv Prostaglandin Thromboxane Leukot Res 1991;21A:461-4.
7. Taylor IK, O'Shaughnessy KM. Effect of cysteinyl-leukotriene receptor antagonist ICI 204.219 on allergen-induced bronchoconstriction and airway hyperactivity in atopic subjects. Lancet 1991;337:690-4.
8. Diamant Z, Timmers MC, van der Veen H, Friedman BS, De Smet M, Depre M, et al. The effect of MK-0591, a novel 5-lipoxygenase activating protein inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects in vivo. J Allergy Clin Immunol 1995;95:42-51.
9. Hamilton AL, Watson RM, Wyile G, O'Byrne PM. Attenuation of early and late phase allergen-induced bronchoconstriction in asthmatic subjects by a 5-lipoxygenase activating protein antagonist, BAYx 1005. Thorax 1997;52:348-54.
10. Hamilton A, Faiferman I, Stober P, Watson RM, O'Byrne PM. Pranlukast, a cysteinyl leukotriene receptor antagonist, attenuates allergen-induced early-and late-phase bronchoconstriction and airway hyperresponsiveness in asthmatic subjects. J Allergy Clin Immunol 1998;102:177-83.
11. Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. Am Rev Respir Dis 1997;135: 264-7.
12. Cockcroft DW, Murdock KY. Changes in bronchial responsiveness to histamine at intervals after allergen challenge. Thorax 1987;42:302-8.
13. Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC_{20}: comparison of linear and logarithmic interpolation. Chest 1983;84: 505-6.
14. Cockcroft DW, McParland CP, O'Byrne PM, Friend JL, Rutherford BC, Swystun VA. Beclomethasone given after the early asthmatic response inhibits the late response and the increased methacholine responsiveness and cromolyn does not. J Allergy Clin Immunol 1993;91:1163-8.

15. Web based program for computing various sample sizes. Statistical considerations for clinical trials and scientific experiments. MGH Biostatistics Software, 2003. Available at: www.hedwig.mgh.harvard.edu/sample_sizesize.html. Accessed June 3, 2005.

16. Town GI, Holgate ST. Comparison of the effect of loratadine on the airway and skin responses to histamine, methacholine, and allergen in subjects with asthma. J Allergy Clin Immunol 1990;86:886-93.

17. Murdoch D, Goa KL, Keam SJ. Desloratadine. An update of its efficacy in the management of allergic disorders. Drugs 2003;63:2051-77.

18. Blake KV. Montelukast: data from clinical trials in the management of asthma. Ann Pharmacother 1999;33:1299-314.

19. Diamant Z, Grootendorst DC, Veselic-Charvat M, Timmers MC, De Smet M, Leff JA, et al. The effect of montelukast (MK-0476), a cysteinyl leukotriene receptor antagonist, on allergen-induced airway responses and sputum cell counts in asthma. Clin Exp Allergy 1999;29:42-51.

20. Leigh R, Vethanayagam D, Yoshida M, Watson RM, Rerecich T, Inman MD, et al. Effects of montelukast and budesonide on airway responses and airway inflammation in asthma. Am J Respir Crit Care Med 2002; 166:1212-7.

21. Palmqvist M, Bruce C, Sjostrand M, Arvidsson P, Lotvall J. Differential effects of fluticasone and montelukast on allergen-induced asthma. Allergy 2005;60:65-70.

22. Bjorck T, Dahlen S-E. Leukotrienes and histamine mediate IgE-dependent contractions of human bronchi: pharmacological evidence obtained with tissues from asthmatic and non-asthmatic subjects. Pulm Pharmacol 1993;6:87-96.

23. Roquet A, Dahlen B, Kumlin M, Ihre E, Anstren G, Binks S, et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. Am J Respir Crit Care Med 1997;155:1856-63.

24. Ruck LM, Rizzo CA, Anthes JC, Eckel S, Egan RW, Cuss FM, et al. Synergistic antiallergic activity of combined histamine H_1- and cysteinyl leukotriene1-receptor blockade in human bronchus. Life Sci 2001;68: 2825-34.

25. Cockcroft DW, Murdock KY. Comparative effects of inhaled salbutamol, sodium cromoglycate and beclomethasone dipropionate on allergen-induced early asthmatic response, late asthmatic response and increased bronchial responsiveness to histamine. J Allergy Clin Immunol 1987;79:734-40.

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.):

January 2004 – January 2005.

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

9.2.2   Information Sheet and Consent Form

<div style="border:1px solid">

INFORMATION SHEET AND
CONSENT FORM

Title: Effect of combined leukotriene (montelukast), histamine (desloratadine) antagonism on the early response to inhaled allergen

Investigator: Dr. D.W. Cockcroft

Department of Medicine, Division of Respiratory Medicine

University of Saskatchewan

5th Floor Ellis Hall, 103 Hospital Drive

Saskatoon, Saskatchewan S7N 0W8

306-966-8346

</div>

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   Print   Date
Consent

9.2.3   Certificate of Approval

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

19-Jan-2004

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	BMC #
Donald W. Cockcroft	Medicine (Respirology)	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 on the Early Response to Inhaled Allergen

ORIGINAL APPROVAL DATE	CURRENT EXPIRY DATE	APPROVAL OF
19-Jan-2004	01-Jan-2005	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 3403, 110 Gymnasium Place
Box 5000 RPO University
Saskatoon, SK S7N 4J8
Phone: (306) 966-4053   Fax: (306) 966-2369

122

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

PHASES	SCREENING			TREATMENT			
VISIT	1	2	3	4	5	6	7
DAY	-10	-9	-8	0	8	16	24
Consent Form	✓						
Demographics	✓						
Inclusion/Exclusion	✓						
Concomitant Meds	✓		✓	✓	✓	✓	✓
Spirometry	✓		✓	✓	✓	✓	✓
SPT	✓						
MCT	✓						
ACT		✓ (diluent)	✓ (control)	✓	✓	✓	✓
STE		✓					
Study Meds				26 and 2 hours pre ACT ✓	26 and 2 hours pre ACT ✓	26 and 2 hours pre ACT ✓	26 and 2 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

9.4   Appendix D: ERJ Abstract of the Late Asthmatic Response Study

Single-dose desloratadine and montelukast and allergen-induced late airway responses.

Davis BE, Illamperuma C, Gauvreau GM, Watson RM, O'Byrne PM, Deschesnes F, Boulet LP, Cockcroft DW.

Dept of Pharmacology, Division of Respiratory Medicine, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada. beth.davis@usask.ca

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.

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

9.5.2 Information Sheet and Consent Form

INFORMATION SHEET AND CONSENT FORM
Title: Changes in airway responses to inhaled allergen following pharmacological inhibition of histamine and CysLT1 blockade in mild atopic asthma.
Investigator: Dr. D.W. Cockcroft Department of Medicine, Division of Respiratory Medicine University of Saskatchewan 5th Floor Ellis Hall, 103 Hospital Drive Saskatoon, Saskatchewan S7N 0W8 306-966-8346

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.

ALLOWED		DISALLOWED	
Ventolin	Withhold for 6 hours before a visit	Singulair	Flovent
Atrovent	Withhold for 8 hours before a visit	Pulmicort	Oxeze
Airomir	Withhold for 6 hours before a visit	Salmeterol	Symbicort
Acetaminophen	No time restriction	Advair	Antihistamines

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 implanted 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   Print   Date
Consent

9.5.3 Certificate of Approval

UNIVERSITY OF SASKATCHEWAN

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

Certificate of Approval

PRINCIPAL INVESTIGATOR	DEPARTMENT	Bio #
Donald W. Cockcroft	Medicine (Respirology)	06-232

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT
Ellis Hall
Respiratory Medicine
103 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$_1$ Blockade in Mild Atopic Asthma

APPROVAL DATE	STUDY APPROVAL EXPIRY	APPROVAL OF
20-Nov-2006	19-Nov-2006	Protocol version 1 (October 2006) Information Sheet and Consent Form (December 2006)

Full Board Meeting ☒   Date of Full Board Meeting: 20-Nov-2006
Delegated Review ☐

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.

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 5C8

9.6 APPENDIX F: Study Flowchart for the Late Asthmatic Response Study

PHASE	SCREENING			TREATMENT 1			TREATMENT 2			TREATMENT 3			TREATMENT 4		
VISIT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
DAY	1	2	3	10	11	12	19	20	21	28	29	30	37	38	39
Consent Form	●														
Demographics	●														
Inclusion/Exclusion	●														
Con Meds	●														
SPT	●														
STE	●														
FeNO	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
MCT	●		●	●		●	●		●	●		●	●		●
Sputum	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Urine Pregnancy Test	●														
Blood collection	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
ACT		●			●			●			●			●	
Study Drug Admin					●			●			●			●	
Adverse Events	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Time Required (hours)	1.5	9	1.5	1.5	9	1.5	1.5	9	1.5	1.5	9	1.5	1.5	9	1.5

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.