Chapter 5

# Histamine in Asthmatic and Fibrotic Lung Disorders

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## Abstract

Histamine has long been known to mediate inflammatory and allergic responses acting predominately through $H_1$ receptors and $H_1$ receptor antagonists have been used to treat allergic rhinitis for many years. Since its discovery, histamine $H_4$ receptor has been identified as a potential drug target for the treatment of allergic and inflammatory lung disease and has been identified as a potential modulator of allergy and inflammation in animal models. The use of the $H_4$ receptor ($H_4R$) antagonist JNJ7777120 in mouse and guinea pig models of allergic bronchoconstriction highlighted the involvement of this receptor in asthma. Histamine released from tissue mast cells by the cross-linking of antigen with IgE is deaminated by amine oxidases, enzymes widely distributed among living organisms and a histaminase, purified from the pea seedlings has been shown to exert a protective effect in an experimentally animal model of asthma-like reaction in guinea pig. In an animal model of fibrosis, $H_4R$ antagonists demonstrated anti-inflammatory and anti-fibrotic properties, suggesting a possible therapeutic potential in the treatment of Th2-mediated diseases, including pulmonary fibrosis.

The combined effects of antihistamines and antileukotrienes inhibit both phases of the allergen induced bronchoconstriction in subjects with asthma. Finally, available data support $H_1$ antihistamines as part of the treatment of asthma especially in combination with other drugs, however bronchoprovocation trials or treatment studies with histamine $H_4$ antagonists are not available. In view of the interesting profile of activities of $H_4$ antagonists, it is possible to hypothesize that this class of drugs may add further clinical benefit by causing a broader inhibition of histamine effects.

## 5.1. Introduction

Since its discovery at the turn of the century, the Histamine $H_4R$ (Nakamura *et al*, 2000; Oda *et al*, 2000; Cogé *et al*, 2001; Liu *et al*, 2001 ; Morse *et al*, 2001; Nguyen *et al*, 2001; Zhu *et al*, 2001) has been a potential drug target for the treatment of inflammatory diseases.

Asthma is a heterogeneous airway inflammatory disease associated with the involvement of T helper cell, recruitment of mast cells and eosinophils into airways, production of cytokines (IL-4, IL-5 and IL-13) and other mediators leading to chronic inflammation as well as remodelling (Bousquet *et al*, 2000; Busse *et al*, 2001, Holgate, 2012). The Histamine $H_4R$ belongs to the GPCR family ($G\alpha_{i/o}$) and is expressed on many cells of the immune system, especially those associated with the pathology of asthma, such as dendritics cells, eosinophils, mast cells, monocytes, neutrophils, T-lymphocytes, NK-cells and also on fibroblasts (Zampeli & Tiligada, 2009). Many cells involved in the asthmatic inflammatory response express both $H_1$ and $H_4R$ (see review by Thurmond *et al*, 2008). Histamine is also found in increased levels in bronchoalveolar lavage from asthma patients and this is associated with worsening of lung function. In mice, genetic deletion of histamine-forming enzyme L-histidine decarboxylase or of $H_1$ receptor provides beneficial effects in experimental asthma (Ohtsu *et al.*, 2001; Miyamoto *et al.*, 2006). In animal models, the $H_4R$ has been identified as a potential modulator of the recruitment of T cell into the lungs after allergen exposure (Dunford *et al.*, 2006) and by cytokines amplifying the allergic symptoms and thus leading to chronic inflammation (Cowden *et al.*, 2010). This also leads to the activation of eosinophils and increased expression of adhesion molecules.

Evidence for the involvement of Histamine $H_4R$ in asthma was shown with the use of the $H_4R$ antagonist JNJ7777120, which inhibits T-cell infiltration to the lung and decreases Th2 cytokines in a mouse model (Cowden *et al*, 2010; Thurmond *et al*, 2004).

Although many studies in different animal models point to the role of Histamine $H_4R$ in the management of allergic disorders and asthma, there are no real human studies yet concerning asthma.

Several compounds have been produced by different pharmaceutical companies trying to address this problem. There are more than 13 pharmaceutical companies with patent applications for substances manipulating the $H_4R$ (Kiss *et al*, 2012). Among these companies, Johnson & Johnson is the most active company with 54 patent applications, and holds the patent for the most studied Histamine $H_4$ antagonist, JNJ7777120. Pfizer has also developed two other $H_4R$ antagonists, PF-3893787 and INCB38579 by IncyteCor, where both compounds have shown promising results in preclinical animal models. The first Histamine $H_4R$ antagonist to enter clinical trial Phase I was the Palau Pharma´s $H_4R$ antagonist UR-65318 and last year this compound entered the Phase IIa studies for patients suffering from chronic allergic asthma (Salcedo *et al*, 2013). Recently, another $H_4$ antagonist from Jansen Research & Development, JNJ 39758979, has been published which was well tolerated in Phase I human clinical trials and has now progressed into Phase II (Savall, 2013). The results from these clinical studies have yet to be published.

## 5.2. Histamine and Histaminergic $H_4R$ Ligands in Animal Models of Allergic Asthma and Pulmonary Fibrosis

Asthma is a heterogeneous chronic condition characterized by widespread, variable and reversible airflow obstruction which is released either spontaneously or with pharmacological treatment. The underlying patho-physiological feature of asthma is increased airway responsiveness which develops on a basis of diffuse bronchial inflammation and functional changes in the airway smooth muscle. The prevalence of asthma is increasing worldwide (Peebles & Hartert, 2002; Myers, 2000), but medications can be highly effective in reducing the burden of asthma for many people and clinical studies show that proper treatment of asthma with appropriate medications can reduce deaths, hospitalizations, and symptoms. Over the last few decades, leukotriene modifiers have emerged as one of the few pharmacological options for asthma that specifically target a pathway of pathogenesis (Drazen *et al.*, 1999; Kemp, 2003). Moreover, anti-IgE therapy has been introduced in the last ten years for the treatment of moderate to severe allergic asthma in adults and adolescent (Johansson & Buhl, 2006). Histamine has long been known to mediate inflammatory and allergic responses acting predominately through $H_1$ receptor and $H_1$ receptor antagonists have been used to treat allergic rhinitis for many years (Hill *et al.*, 1997).

Direct evidence for the role of free radicals in asthma comes from human studies that demonstrate that the levels of antioxidants in airway of asthmatics are higher than normal (Comhair *et al.,* 1999) and bronchial obstruction in asthma is associated with an increased production of oxygen free radicals

in airway lumen from inflammatory cells (Calhoun *et al.*, 1992). It has been reported that inflammatory cells produce large amount of superoxide anion and the scavenging of superoxide by superoxide dismutase (SOD) mimetics results in clear protection against ovalbumin-induced bronchospasm, lung inflammation and prostaglandin production in sensitized guinea pigs (Masini *et al.,* 2005). In another study, epigallocatechin-3-gallate (EGCG), an antioxidant molecule that enhances constitutive nitric oxide synthase (NOS) activity, reduced leukocyte infiltration as measured by myeloperoxidase (MPO) activity and eosinophilic accumulation, evaluated as eMBP-positive cells, and reduced acute bronchospasm in ovalbumin-challenged sensitized guinea pig (Bani *et al.*, 2006).

Infiltration of inflammatory cells, especially mast cells and eosinophils, is thus a prominent feature of asthmatic lungs, and acute airway response to allergens is known to depend on eosinophils and mast cells (Wardlaw *et al.,* 1988). It is improbable that only these cell types are responsible for the initiation and perpetuation of bronchoconstriction and airway inflammation, however, mast cell has long been considered to be of paramount importance in the patho-physiology of asthma (Rossi & Olivieri, 1997). In fact, the release of IgE-dependent mediators from mast cells, such as histamine, plays a central role in the pathogenesis of the disease (Galli, 1993; Bradding & Holgate, 1996; Barnes & Page, 2001). The release of histamine from mast cells infiltrating airway smooth muscle elicited by the cross-linking of antigen with IgE bound to specific receptors of cell membrane is considered of paramount importance not only in the early phase of asthmatic response, but also in the late one (Brightling *et al.,* 2002). Mast cell mediators cause bronchoconstriction and smooth muscle cell proliferation and can recruit other inflammatory cells, thereby initiating a vicious cycle that amplifies the pathological features of the disease. Studies in transgenic mast cell-deficient mice have shown that these cells act as a local amplifier of bronchoconstriction (Williams & Galli, 2000). The number of mast cells in the smooth muscle of patients with asthma is inversely correlated with the degree of airway hyperresponsiveness (Brigthling *et al.*, 2002) and endobronchial biopsies show increased mast cell degranulation in asthmatic patients compared with non asthmatic subjects (Pesci *et al.*, 1993). The levels of prostaglandin $D_2$, the major cyclooxygenase product generated by activated mast cells during allergic response (Bochenek *et al.,* 2004), are elevated in the urine of asthmatic patients after antigen exposure in both the early and late phases (O'Sullivan, 1999).

Histamine released from tissue mast cells by the cross-linking of antigen with IgE is oxidatively deaminated by amine oxidases ($AO_s$), enzymes widely distributed among living organisms (Mondovì, 1985), and is involved not only in the metabolism of histamine but of other primary amines released during anaphylactic reaction. Amine oxidases can be divided in two classes, depending whether the prosthetic group is a flavin adenine dinucleotide (FAD) or 2,4,5

trihydroxyphenylalaninequinone (TPQ), a cofactor derived from the post-translactional oxidation of a tyrosine residue (Klinmann & Mu, 1994). This second group of enzymes (E.C.1.4.3.6) contains copper ($CuAO_s$) and TPQ and within the Cu-TPQ class, plant enzymes are in general more efficient than animal ones, probably because they also function through a radical mechanism not present in the enzymes from animal sources. (Bellelli *et al.*, 2000). Recently, a copper amine oxidase (histaminase) purified from the pea seedling has been shown to exert a protective effect on cardiac anaphylactic response in guinea pig (Masini *et al.,* 2002) and a protective effect of histaminase, purified from the pea seedling, has been demonstrated in an experimentally animal model of asthma-like reaction in guinea pig (Masini *et al.*, 2004). This enzyme has a therapeutic application in various histamine-related affections, such as food histaminosis and inflammatory bowel disease and may be involved in allergic and anaphylactic responses (Mondovì *et al.*, 2013). In fact, the pre-treatment of animals with histaminases, i.p. or as aerosol solution, resulted in a marked reduction of breathing abnormalities and prevention of respiratory failure (Mondovì *et al.,* 2013).

## 5.3. Histamine $H_4$R Ligands in Animal Models of Asthma

The histaminergic system has over the last five decades proved to be a rich source of drugs. Histamine $H_1$ and $H_2$ receptor antagonists are widely used for the treatment of allergies and peptic ulcers, respectively, while $H_3$ receptor antagonists could have a therapeutic use, in a near future, in dementia, obesity and psychotic and sleep disorders (Hill *et al.,* 1997). Most importantly, growing attention is directed towards the validation of $H_4$R ligands in some inflammatory and immunological diseases, such as asthma and pulmonary fibrosis. Over the years, studies on the effect of histamine $H_4$R ligands in animal models of immune and allergic diseases have produced a large amount of information, albeit with some conflicting reports. These different responses might arise from the differential expression of histamine receptors as well as interspecies differences in the action of these molecules (Zampeli & Tiligada, 2009). Recent results obtained in animal models of airway inflammation and fibrosis are reported here.

Many animal models are available for studying human diseases from mice to primates, but we must consider that these models are not perfect replicas of human disease and may not be predictive of human outcome. Nonetheless, the growing knowledge in genomics, bioinformatics, immunology and molecular pharmacology has helped us to better understand the various animal models and apply the information to human conditions.

The widespread use of the mouse asthma model is likely due to the availability of specific molecular, immunological and genetic tools for exploring the inflammatory mechanisms contributing to the underlying pathophysiology of asthma. The $H_4R$ is present in the lung, bronchial epithelium, smooth muscle cells and microvascular endothelial cells (Gantner *et al.*, 2002) and can thus theoretically contribute to the airway disease pathobiology. The $H_4R$ mediates the synergistic sequential action of histamine and CXCL12, a chemokine constitutively expressed in skin and airway epithelium, and the migration of mast cells in the mucosal epithelium in response to the allergens (Thurmond *et al.,* 2004; Godot *et al.,* 2007). Accordingly, previous experiments with $H_4R$-deficient($H_4R^{-/-}$) mice and oral gavage administration of selective $H_4R$ antagonists in a murine model showed a reduction of lung inflammation upon allergen stimulation (Dunford *et al.*, 2006). This demonstrated the role of the $H_4R$ in modulating Th2 allergic responses, where the $H_4R$ influences $CD4^+$ T cell activation attributed to a decreased production of IL-4, IL-5 and IL-13 and chemokines from dendritic cells (Dunford *et al.*, 2006). These cytokines have individually been targeted for potential therapeutic benefit in human disease. IL-4 is the main driver of isotype switching in B cells to produce high affinity IgE and it is an important factor in Th2-cell development. A similar effect is exploited by IL-5, which is an activation factor for eosinophils, whilst IL-13 is a mediator of remodelling, airway hyperreactivity and goblet cell hyperplasia.

In the mouse model of acute lung inflammation and hyper-responsiveness, dendritic cells seem to be the target of the effects of $H_4R$ antagonists, where dendritic cells isolated from $H_4R$ knockout mice or treated with $H_4R$ antagonists were unable to properly stimulate $CD4^+$ T cells under Th2-polarizing conditions (Zhang *et al.*, 2007). In the same murine model of asthma, $H_4R$ antagonists, given only during the sensitization stage, had an inhibitory effect upon subsequent airway inflammation and T-cell cytokine production, indicating that T-cell activation was impaired at the priming stage. In another study, inhibition of airway resistance and inflammation mediated through the recruitment of T regulatory (Treg) cells was observed with 4-methylhistamine, a selective $H_4R$ agonist, given intratracheally to asthmatic mice (Morgan *et al.*, 2007). The authors attributed their different results to the local administration of the compound and to the resulting concentration gradient within the lung that would allow the migration of the Treg cells and the immune suppressive response (Morgan *et al.*, 2007).

The allergic guinea pig model, while validated many decades ago, has continued to be used to investigate the pathophysiology of asthma for many reasons. The guinea pig is a particular suitable model for studying histaminergic system and allows the reproduction of the different syndromes presented by human asthma. In fact, a model of occupational asthma has been validated using toluene-2,4-diisocyanate (Nabe *et al.*, 2005) and a model of cough variant asthma has been developed (Bani *et al.*, 1997). Guinea pigs are sensitized to

ovalbumin and bronchial responsiveness, as well as the cough reflex response, which is measured after antigen inhalation. This animal model has been widely used to evaluate the anti-asthmatic effect of different classes of compounds. For instance, a phosphodiesterase 4 inhibitor and its active metabolite reduced allergen-induced eosinophilia and airway hyper-responsiveness (AHR) in a guinea pig model (Billah *et al.,* 2002). Cyclosporine (Xie *et al.*, 2002) and FK-506 (Morishita *et al.*, 2005), administered by inhalation, inhibited AHR and cellular influx following allergen challenge, and similar effects were observed with a derivative of methotrexate. The role of neurokinins and their receptors has been extensively studied in the guinea pig model, with the evidence for a complex interplay between the three receptor subtypes (Schuiling *et al.*, 1999).

Masini and coworkers have utilized the guinea pig model for several studies. First, we studied the effect of the hormone relaxin, which was found to inhibit mast cell histamine release (Masini *et al.,* 1994) and to counteract the respiratory and histopathological abnormalities induced by inhaled antigen (Bani *et al.,* 1997). Relaxin was also found to promote dilatation of alveolar blood capillaries and to reduce the thickness of the air blood barrier. In the following years, using this model of allergic asthmatic response, we investigated several antioxidants or SOD mimetic drugs (Masini *et al.*, 2005; Bani *et al.*, 2006) as well as molecules that interfere with the production of pro-inflammatory mediators, such as ceramide (Masini *et al.,* 2008).

The recent discovery of the histamine $H_4R$, functionally expressed on many cell types associated with asthma pathology (eosinophils, basophils, mast cells, dendritic cells and $CD8^+$ T cells (Thurmond *et al.*, 2008)), prompted an investigation in the guinea pig asthma model for the ability of an $H_4R$ antagonist to modify antigen-induced oxidative stress, airway inflammation, bronchoconstriction, as well as cytokine and prostaglandin production. Ovalbumin challenge-induced cough, dyspnoea, bronchoconstriction and alveolar space dilatation were observed along with increased leukocyte infiltration and production of $PGD_2$, $LTB_4$ and TNFα in lung tissue and bronchoalveolar lavage (BAL) fluid. Compound JNJ7777120 (JNJ), a selective $H_4R$ antagonist, counteracts the functional, histopatological and biochemical changes induced by antigen challenge (Somma *et al.,* 2013). The authors tried to find a possible explanation for the protective mechanism of JNJ, evaluating the possible interaction of this molecule with lipocortin-1 (LC-1), a glucocorticoid-modulated protein, and initially characterized its ability to inhibit prostanoid release (Cirino *et al.*, 1987). LC-1 inhibits the activity of cytoplasmic phospholipase $A_2$, which plays a key role in the production of inflammatory lipid mediators, prostaglandins and leukotrienes and inhibits the extravasation of leukocytes (D'Acquisto *et al.,* 2008). Given that histamine $H_4R$ are expressed in dendritic, $CD4^+$ and $CD8^+$ T cells (Zampeli &Tiligada, 2009), it seemed logical to assume that JNJ could influence the complex cytokine interplay involved in the pathogenesis of asthma (Figure 5. 1).

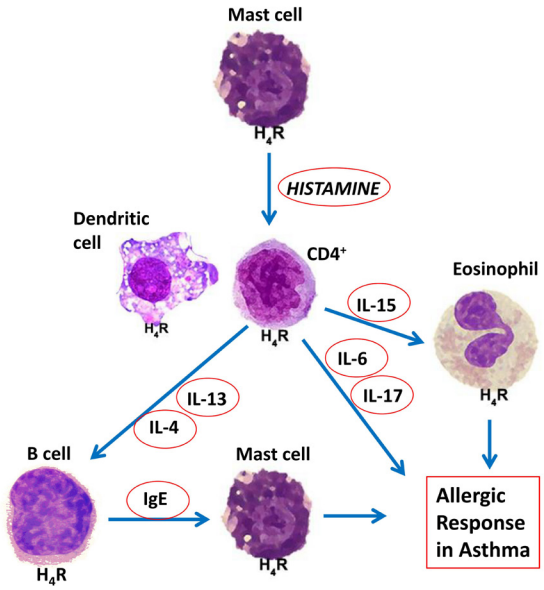


**Figure 5.1** The complex cytokine interaction in allergic response in asthma.

In fact, antigen-challenge caused leukocyte infiltration in lung tissue, especially eosinophils, which produce large amounts of free radicals and are responsible for airway remodelling (Jarjour & Calhoun, 1994). In addition, superoxide produced during the asthmatic response can promote the expression of genes encoding the pro-inflammatory cytokines IL-1, IL-6 and TNFα, which can cause endothelial cell damage (Ndengele *et al.*, 2005). Moreover, superoxide promotes mast cell degranulation and histamine release (Masini *et al.*, 2005), thus amplifying the inflammatory response. Notably, JNJ prevents lung mast cell degranulation and chemotaxis of eosinophils, which are involved in adverse airway remodelling through the release of transforming growth factor-β (TGF-β), which shifts stromal cells towards the myofibroblast phenotype, responsible for fibrosis (Kisseleva & Brenner, 2008). In particular, stimulation of $H_4R$ on eosinophils triggers cellular changes required for chemotaxis, actin polymerization, changes in cellular shape and expression of adhesion molecules (Ling *et al.*, 2004). The treatment with JNJ was effective in reducing the chemotaxis of eosinophils, confirming the hypothesis that this process is mediated by histamine $H_4R$. Considering the role of the $H_4R$ in the reduction of the asthmatic response revealed by the studies reported here as well as the literature data on the modulation of $H_4R$ antagonists on mast cell, eosinophil, T and dendritic cell functions, it is highly warranted to evaluate the future therapeutic use of $H_4R$ antagonists in asthma. Many research groups have

utilized the guinea pig model with success (reviewed by Riley et al., 2013). Smith and Broadley (2008) investigated the functional airways responses to inhaled 5'-AMP in actively sensitized, conscious guinea-pigs following administration of selective adenosine receptor antagonists and demonstrated that all four adenosine receptor subtypes play various roles in the airways response. The same group has recently stated that the sensitized guinea pig model is a suitable animal model of chronic airway inflammation, airway hyperresponsiveness and lung remodelling that can accurately predict drug effectiveness in human asthma. In OA-sensitized guinea pigs, the efficacy of the inhaled corticosteroid fluticasone propionate (FP), the phosphodiesterase 4 inhibitor roflumilast and the inducible nitric oxide synthase (iNOS) inhibitor GW274150 administered orally 24 hours and 30 min before OA exposure were compared. Fluticasone and roflumilast inhibited T cell lung influx and airway hyperresponsiveness and remodeling, while GW274150 only inhibited the inflammatory response but not remodeling (Evans *et al.*, 2012). In the clinical setting, inhaled corticosteroids and phosphodiesterase 4 inhibitors are relatively effective against most features of asthma whereas the iNOS inhibitor GW274150 was ineffective. Thus, while certain differences remain between the experimental and clinical effectiveness of antiasthma drugs, the chronic pulmonary inflammation guinea pig model does appear to be a good pre-clinical predictor of potential asthma therapeutics.

## 5.4. Histamine $H_4R$ Ligands in Animal Models of Lung Inflammation and Fibrosis

Fibrosis can be considered an excessive reparative response of tissues to chronic injury and inflammation, and features excess deposition of collagen and other extracellular matrix (ECM) components in the interstitium. ECM accumulation disrupts the normal histological architecture of an organ, eventually leading to its dysfunction (Paz & Shoenfeld, 2009; Kisseleva & Brenner, 2008). In spite of different aetiology and target organs of fibrotic disorders, one pathological hallmark is the presence of myofibroblasts, which are activated by collagen-secreting fibroblasts originating from stromal precursor cells that are induced to proliferate and differentiate by pro-fibrotic factors released in the local inflammatory micro-environment, such as TGF-β and angiotensin II (Kisseleva & Brenner, 2008; Wynn 2007). In particular, fibrosis takes place when the synthesis of new collagen by myofibroblasts exceeds its degradation rate, leading to accumulation of collagen over time (Wynn, 2008).

Pulmonary fibrosis is the end stage of a wide range of chronic lung inflammatory diseases leading to progressive parenchymal destruction. Histopathologically it is characterized by alveolar and capillary loss due to pneumocyte and endothelial

apoptosis, accumulation of myofibroblasts and an excess of interstitial collagen and abnormal remodelling of lung parenchyma (Hardie *et al.*, 2009). This process results in progressive airway stiffening and thickening of the air-blood membrane, which makes breathing difficult and eventually leads to respiratory failure. Idiopathic pulmonary fibrosis (IPF), the most common fibrotic disease of the lungs, has a particularly poor prognosis and represents a therapeutic challenge for pneumologists. In fact, the classical anti-inflammatory drugs are nearly ineffective in improving its clinical course, being unable to prevent or delay the onset of respiratory failure. Therefore, the current therapeutic approaches to IPF are oriented towards novel substances which could override the limitations of the existing anti-inflammatory drugs, such as molecules targeting TGF-β signalling, the upstream activation pathway of myofibroblasts (Gharaee-Kermani *et al.*, 2009). In this context, cyclooxygenase (COX)-inhibiting nitric oxide (NO) donors (CINODs), designed to inhibit COX-1 and COX-2 while releasing NO, have shown a significantly higher efficacy than classic anti-inflammatory drugs in reducing lung inflammation and preventing collagen accumulation in bleomycin-induced lung fibrosis in male C57BL/6 mice (Pini *et al.*, 2012), agreeing with previous studies on the cirrhotic liver (Casini *et al.,* 1997; Failli *et al.,* 2000). In the latter situation, NO supplementation by NO donors decreased liver cirrhosis by inhibiting the profibrotic activation of hepatic stellate cells induced by reactive oxygen species (ROS) or platelet-derived growth factor (Casini *et al.,* 1997; Failli *et al.,* 2000, Svegliati-Baroni *et al.*, 2001). Moreover, experimental data in cultured rat mesangial cells suggests that NO could have direct antifibrotic effects in kidney cells by down-regulating the expression of fibrosis-related genes (Wani *et al.,* 2007). In this context, a novel antifibrotic peptide with relaxin-like activity, which exerts an inhibitory effect on TGF-β1-induced collagen deposition in human dermal fibroblasts and enhanced matrix metalloproteinase (MMP)-2 expression, caused a significant reduction in lung inflammation and injury and ameliorated adverse airway remodelling and peribronchial fibrosis (Pini *et al.*, 2010).

Based on the above evidence and on the results of Kohyama and coworkers (2010), which demonstrated that the profibrotic effect of histamine on human foetal lung fibroblasts is a consequence of $H_4$R activation and fibronectin-induced lung fibroblast migration is blocked by JNJ, we investigated whether $H_4$R ligands could have a therapeutic effect in the same mouse model of bleomycin-induced lung fibrosis (Kaminski *et al.*, 2000; Moeller *et al.*, 2008).

The results indicate that $H_4$R antagonists have significant anti-inflammatory and anti-fibrotic properties, where $H_4$R antagonists consistently decreased inflammatory and oxidative stress parameters and reduced the relative number of goblet cells, the thickness of smooth layer, the level of TGF-β and collagen deposition. All of these parameters were also accompanied by a decrease in airway resistance to inflation (PAO) (Figure 5. 2) (Lucarini *et al.,* 2013).

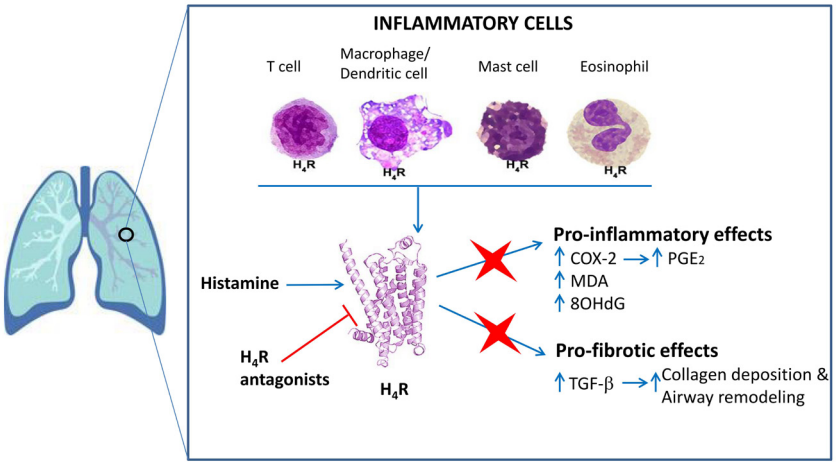


**Figure 5.2** A schematic description of the protective action of $H_4$ receptor ($H_4R$) antagonists on histamine-induced pro-inflammatory and pro-fibrotic effects. COX-2: cyclooxygenase-2; MDA: malonyldialdehyde; 8OHdG: 8-hydroxy-2'-deoxyguanosine; TGF-β: transforming growth factor β.

As previously reported, histamine $H_4R$ are functionally expressed in cells of the innate immune system and histamine is a robust chemotactic factor for these cells (Damaj *et al.*, 2007). Furthermore, $H_4R$ antagonists inhibit the migration of immune cells, decreasing the production of the pro-fibrotic cytokine TGF-β, which has been recently identified as a target for the development of novel antifibrotic agents.

In conclusion, the dual effect of $H_4R$ antagonists on inflammatory and fibrotic mediator production points to their therapeutic potential for the treatment of Th2-mediated diseases, including pulmonary fibrosis.

## 5.5. Conventional Antihistamines as Effective Treatment for Asthma in New Combination with Other Mast Cell Inhibitors

Current clinical guidelines do not support treatment of asthma with antihistamines. However, this conclusion has not been reassessed recently. The recommendation rests on early clinical data with the first generation of $H_1$ antagonists. This report advocates the opinion that it is now timely to revisit the use of antihistamines for the treatment of asthma. The strategy should not be through the use antihistamines as single medications, however, but as adjuvant

therapy in combination with drugs that block one or two of the other major mast cell mediators. This has great potential to become new, effective treatments of asthma.

Accordingly, as reviewed in other parts of this volume, there is strong evidence from different experimental models to support this specific combination therapy. Whereas singular inhibition of one mediator class such as histamine or cysteinyl-leukotrienes (CysLT) provides small or sometimes insignificant protective effects against mast cell-dependent bronchoconstriction, a combination of inhibitors against two or more classes of mediators generally produces significant effects (e.g. Hay *et al.*, 1987; Wikström-Jonsson, 1994; Sundström *et al.* 2003). This is illustrated in Figure 5.3 where the antagonism of histamine, CysLTs and prostaglandins was studied in a step-wise fashion in allergic bronchoconstriction evoked in the peripheral airways of the guinea pig (Ressmeyer *et al.*, 2006).

These results demonstrate that antagonism of one mediator class has only a small effect on the response, antagonism of two classes has mostly greater but incomplete effects, but intervention with all three pathways confers complete abolishment of the reaction. The absolute degree of this effect with the different interventions in different systems are variable, but the overall concept that triple mediator antagonism essentially eliminates the responsiveness to antigen-challenge has been confirmed in other guinea pig models and on isolated human bronchi (Dahlén *et al.*, 1983; Hay *et al.*, 1987; Björck & Dahlén 1993; Wikström-Jonsson, 1994; Sundström *et al.* 2003;). This is not surprising, as

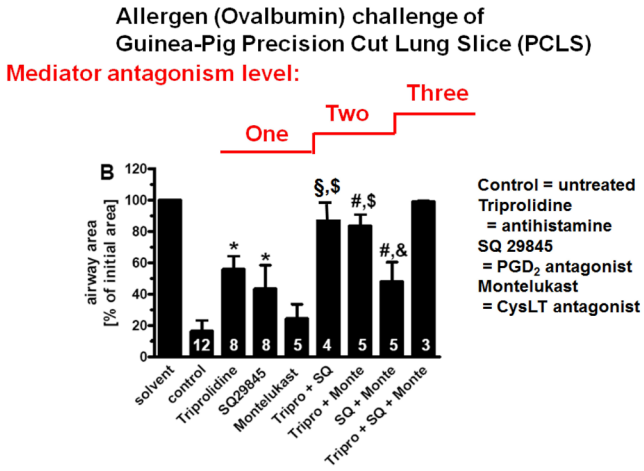


**Figure 5.3** Video-microscopy of antigen challenge of guinea pig peripheral airways, 100% represents completely open airways (solvent and triple antagonism), control is antigen without antagonist. Modified from Ressmeyer et al, 2006.

the antigen-induced activation of mast cells results in the release of histamine, prostaglandin (PG) $D_2$ and the CysLTs (Levi-Schaffer *et al.*, 1987), which are all effective bronchoconstrictors.

Furthermore, following the original work by Adams and Lichtenstein (1979), several studies in isolated human bronchi have shown that the combination of a CysLT antagonist and an antihistamine have profound inhibitory effects on the Schultz-Dale contraction induced by challenge with a specific antigen or anti-IgE. In a series of publications during the 1980-90's, this has been extensively documented both in human bronchi from non-asthmatic subjects and occasionally in airway preparations from subjects with asthma (e.g. Dahlén *et al.*, 1983; Hay *et al.*, 1987; Björck & Dahlén, 1993). Together, these studies in human tissues confirm the value of the basic strategy of combined antagonism of mast cell mediators outlined in the predictive guinea-pig models.

However, the critical standard for establishing proof-of-concept and predicting clinical efficacy in asthma is the allergen bronchoprovocation setting. In fact, there are no currently used clinical treatments of asthma that do not display effectiveness in this human *in vivo* model (Boulet *et al.*, 2007). In the first comprehensive study of the combined effects of antihistamines and antileukotrienes (Roquet *et al.*, 1997), it was reported that the antihistamine loratadine by itself caused significant inhibition of both early and late phase allergen-induced bronchoconstriction (Figure 5.4). Although the effect of the

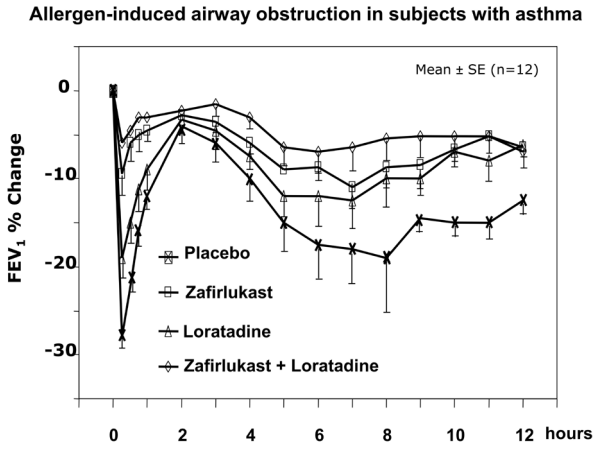


**Figure 5.4** Allergen bronchoprovocation of subjects with allergic asthma in a cross-over trial where each subject was provoked four times after one week double-blind pre-treatment with placebo, the $H_1R$ antagonist loratadine, the $CysLT_1$ antagonist zafirlukast or loratadine plus zafirlukast. At least four weeks of washout between the sessions. Airway response monitored as drop in FEV1 from prechallenge baseline. Modified from Roquet et al, 1997.

antihistamine was smaller than that of the antileukotriene zafirlukast on the early phase reaction, the effect on the late phase reaction was significant and closely similar to that of the antileukotriene. Moreover, when both drugs were combined, there was a substantial ~75% inhibition in both phases of allergen-induced bronchoconstriction (Figure 5.4).

More recently, these effects have been replicated using another leukotriene receptor antagonist (montelukast) in combination either with the metabolite of loratadine, desloratadine (Davis *et al.*, 2009) or azelastine (Richter *et al.*, 2008). In addition, Davis and coworkers found that this combination significantly blunted the allergen-elicited sputum eosinophila (Davis *et al.*, 2009).

In a 10 week placebo-controlled clinical trial with cross-over design, performed in 117 subjects with asthma, it has been further established that the combination of loratadine and montelukast had greater clinical effect than montelukast alone (Reicin *et al.*, 2000). A shorter 6 week cross-over study in 406 patients with asthma mostly agreed with this observation and also showed that the effect of the combination therapy was comparable to treatment with an ordinary dose of inhaled beclomethasone (Lu *et al.*, 2009). Considering both the clinical trials well as the extensive experimental data collected in the allergen bronchoprovocation studies, it is in our opinion support for the use of a combination of an $H_1$ anti-histamine drug with an antileukotriene for the treatment of mast cell-dependent asthma.

Furthermore, this implies that a combination treatment should not be restricted to allergen-induced reactions but also to asthma triggered by other stimuli such as exercise, inhalation of cold air or mannitol, as well as aspirin in aspirin/NSAID-intolerant asthma (AIA), as mast cell activation is the final pathway for inducing the bronchoconstriction in all of these responses (Sladek & Szczeklik 1993; O'Sullivan *et al.*, 1996; O'Sullivan *et al.*. 1998a; Brannan *et al.*, 2003). While the protective effects of leukotriene receptor antagonists have been extensively documented in bronchoconstriction induced by exercise (Manning *et al.*, 1990; Leff *et al.*, 1998; Dahlén *et al.*, 2002) or aspirin in AIA (Christie *et al.*, 1991; Dahlén *et al.*, 1993), the response to cumulative challenge with mannitol was found to be inhibited only with respect to the recovery phase, not the sensitivity to this cumulative challenge (Brannan *et al.*, 2001). For bronchoconstriction induced by exercise, however, the protective effect of a single intervention with antihistamines appears to have minimal effects both in children and adults (Dahlén *et al.*, 2002; Peroni *et al.*, 2002). There is also substantial release of $PGD_2$ after exercise (O'Sullivan *et al.*, 1998b) and mannitol challenges (Brannan *et al.*, 2003) and that TP receptor antagonists, which block bronchoconstriction to $PGD_2$, demonstrate protective effects on the airway response to exercise challenge (Magnussen *et al.*, 1992), which highlights the importance of this major mast cell mediator in the asthmatic responses. In line with the findings in the animal models, complete attenuation of mast cell-

dependent bronchoconstriction requires antagonism of all three major classes of bronchoconstrictors. As indicated, the relative importance of histamine, CysLTs and $PGD_2$ varies depending upon the characteristics of the challenge and possibly also between individuals, but we postulate that when all three mediators are blocked, the airway response will be almost completely abolished (Figure 5.5).

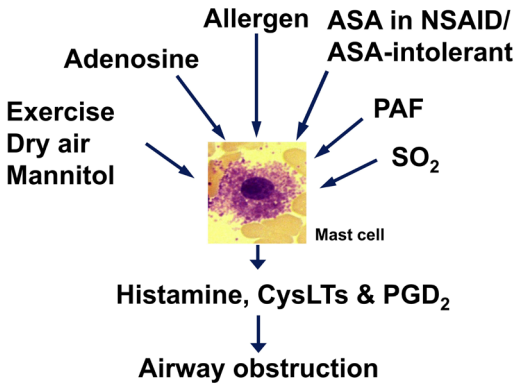


**Figure 5.5** Mast cell activation as the final common path for a number of trigger-factors in asthma.

This hypothesis again rests on the large databank of results from various experimental models, including isolated human bronchi (Dahlén *et al.*, 1983: Hay *et al.*, 1987; Björck *et al.*, 1993).

Finally, whereas the currently available data discussed above supports $H_1$ antihistamines as part of the treatment of asthma, there is as of yet no bronchoprovocation trials or treatment studies with $H_4$ antagonist that provide definitive data on the use of that particular class of antagonists in asthma. As discussed in other parts of this volume, there is experimental data supporting future trials addressing $H_4R$ as another target in combination therapy. In light of the interesting profile of activities of $H_4R$ antagonists in experimental models, it is hypothesized that this class of drugs may add further clinical benefit by causing a broader inhibition of histamine. Likewise, in order to establish the introduction of the triple mast cell mediator concept that this communication advocates, it is necessary to perform well designed, long-term treatment trials to support changes to the current clinical guidelines. The concept of combined mediator antagonists has received comparatively little attention because there are no patents protecting the development of such therapy, and thus provides minor commercial incentive for its development. Meanwhile, many patients, especially those with occasional or seasonal troubles, are looking for simple and easy to use oral treatments for their asthma.

In conclusion, it is clear that histamine contributes to the pathophysiology of asthma. Although current clinical guidelines do not support treatment of asthma with antihistamines. This affirmation is however based on early clinical data with first generation of $H_1$ antagonists and it is current opinion to revisit the use of this class of drugs for the treatment of asthma. The strategy should, however, not be to use antihistamines as single medications, but rather as adjuvant therapy in combination with other drugs acting on other classes of mast cell mediators. The interesting profile of preclinical activities of histamine $H_4$ receptor antagonists raises the potential that a broader inhibition of histamine might add clinical benefit and introduce a new effective treatment of asthma.

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