Handbook of Experimental Pharmacology 237

Clive P. Page
Peter J. Barnes *Editors*

Pharmacology and Therapeutics of Asthma and COPD

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We have dedicated this volume to Dr Dom Spina who sadly passed away on December 5, 2016. Dr Spina had made major contributions to the field of Pulmonary Pharmacology throughout his career, not least in the area of xanthines and phosphodiesterase inhibitors, the subject of his contribution to this volume. He will be sorely missed by all who knew and had the privilege of working with him.

Preface

In 2004 we edited Volume 161 on the *Pharmacology and Therapeutics of Asthma and COPD* as part of this prestigious series. Over the last decade there have been substantial increases in our understanding of the mechanisms underlying asthma and COPD, as well as in the treatment of these important diseases. We have brought together internationally recognized authorities to review the most important new information on the advances in our understanding of the pathogenesis and treatment of these diseases, including the substantial advances in the topical delivery of inhaled medicines. It is hoped that this book will be invaluable for research scientists and clinicians involved in research into asthma and COPD, and that this volume will be a major reference resource for chest physicians and those involved in the development of novel pharmaceutical entities for these diseases.

Each chapter is extensively referenced, generously illustrated with clear diagrams and photographs, and represents a state-of-the-art review of this important area of respiratory medicine.

London, UK
December 2016

C.P. Page
P.J. Barnes

Contents

Pathogenesis of COPD and Asthma

Clive Page, Blaze O'Shaughnessy, and Peter Barnes

Contents

Abstract

Asthma and COPD remain two diseases of the respiratory tract with unmet medical needs. This review considers the current state of play with respect to what is known about the underlying pathogenesis of these two chronic inflammatory diseases of the lung. The review highlights why they are different conditions requiring different approaches to treatment and provides a backdrop for the subsequent chapters in this volume discussing recent advances in the pharmacology and treatment of asthma and COPD.

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Keywords
Asthma • Bronchial hyperresponsiveness • COPD • Pathogenesis

1 Pathology of COPD

The major pathological features of COPD are obstructive bronchiolitis, emphysema and, in many cases, mucus hypersecretion (chronic bronchitis) (Fig. 1), but the relative contributions of each of these pathologies to COPD vary between patients (Hogg and Timens 2009). Even in early or mild COPD, there is evidence of airflow obstruction and a significant loss (disappearance) of small airways (McDonough et al. 2011). A novel CT imaging technique for quantifying small airway disease shows that this small airways loss is an early feature of disease and might account for the initial progression of airway obstruction in COPD (Galban et al. 2012). Structural changes in small pulmonary arterioles are also common in patients with COPD, with increased intimal thickening and vascular smooth muscle proliferation, perhaps resulting from inflammation in these vessels, as well as hypoxic

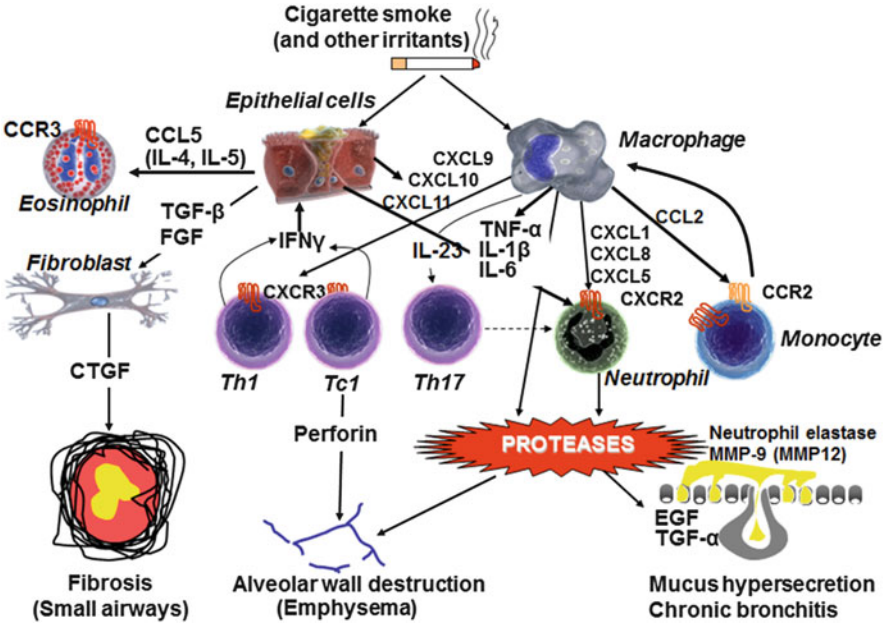

Fig. 1 Pathogenesis of COPD. Cigarette smoke (and other irritants) activate macrophages in the respiratory tract that release chemotactic factors that attract inflammatory cells from the circulation and fibrogenic factors such as transforming growth factor-β (TGF-β) and connective tissue growth factor (CTGF) which stimulate fibrosis in peripheral airways. Various cells release proteases in the airways, including matrix metalloproteinases (MMPs) that break down connective tissue in the lung parenchyma, resulting in emphysema, and stimulate mucus hypersecretion (chronic bronchitis). T cells play an important role in the persistence of inflammation

vasoconstriction (Peinado et al. 2008). However, pulmonary hypertension is usually not marked in COPD, except for a small group of patients with disproportionate pulmonary hypertension who can develop right heart failure (Seeger et al. 2013).

2 Chronic Inflammation

COPD is associated with chronic inflammation that predominantly affects peripheral airways and lung parenchyma, although large airways also show inflammatory changes (Barnes 2014). The degree of inflammation increases – with increased numbers of neutrophils, macrophages and lymphocytes in the lungs – as the disease progresses (Hogg and Timens 2009). Chronic inhalation of irritants, including cigarette smoke, biomass fuel smoke and air pollutants, activates pattern recognition receptors, such as Toll-like receptors (TLRs), resulting in an innate immune response, which leads to increased numbers of neutrophils and macrophages in the lungs as well as activation of airway epithelial cells and mucus secretion (Brusselle et al. 2011). Activation of adaptive immunity occurs later in the course of the disease and leads to increased numbers of T lymphocytes and B lymphocytes in the lungs. These cells might be organized into lymphoid follicles, which involves an increase in the number and activation of dendritic cells. During this adaptive immune response there is also an increase in the number of $CD8^+$ cytotoxic T (Tc1) and $CD4^+$ T helper (Th)1 cells in lung tissue (Barnes 2008a). The number of $CD4^+$ Th17 cells is also increased in the lungs and might further amplify neutrophilic inflammation (McAleer and Kolls 2014). Some patients with COPD have increased eosinophils in their airways and sputum and share some features of asthma, such as reversibility of the airway obstruction and a greater response to corticosteroids compared with patients with typical COPD (Barrecheguren et al. 2015). This has led to the description "Overlap Syndrome" to describe such patients, which has recently been reviewed elsewhere (Postma and Rabe 2015).

The levels of many different inflammatory mediators are increased in the lungs of patients with COPD, including lipid and peptide mediators, as well as a network of cytokines and chemokines that maintain inflammation and recruit circulating cells into the lungs (Barnes 2008b). Many of these proinflammatory mediators are regulated through the activation of the pro inflammatory transcription factor, nuclear factor-kB (NF-kB), and mitogen-activated protein kinases (MAPK), particularly p38 MAPK (Di Stefano et al. 2002; Renda et al. 2008). In addition, several proteases that degrade elastin fibres are secreted from airway resident neutrophils, macrophages and epithelial cells in patients with COPD. In larger airways, elastase from neutrophils might be an important stimulator of mucus hypersecretion, whereas matrix metalloproteinases (MMP9 and MMP12) in the lung parenchyma might be more important in the elastolysis that is observed in those patients having emphysema.

Even cigarette smokers with normal lung function have increased airway inflammation, suggesting this might be the normal response of the respiratory mucosa to inhaled irritants. However, this inflammation seems to be amplified in COPD

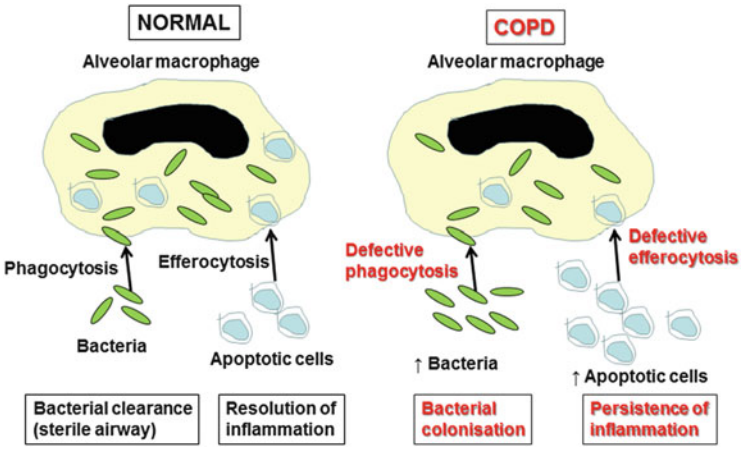

Fig. 2 Defective phagocytosis in COPD. Normally, macrophages phagocytose bacteria in the lung periphery and respiratory tract to maintain lung sterility. These macrophages also phagocytose apoptotic cells (efferocytosis) resulting in resolution of inflammation. In chronic obstructive pulmonary disease (COPD), macrophages are defective at phagocytosing bacteria, which results in chronic bacterial colonization of the lower airways. In addition, these macrophages have an impaired ability to carry out uptake (efferocytosis) of apoptotic cells, which results in failure to resolve inflammation

patients, particularly during acute exacerbations. The amplified inflammatory response in COPD might be explained by reduced expression of the nuclear enzyme histone deacetylase 2 (HDAC2, encoded by *HDAC2*) in macrophages and epithelial cells found in the lungs of those with COPD, resulting in activation of multiple inflammatory genes (Ito et al. 2005). The lung inflammation in COPD patients persists even after smoking cessation, suggesting that it is maintained by some autonomous mechanism that is not yet understood.

The lower respiratory tract of patients with COPD is often colonized with bacteria, such as *Haemophilus influenzae* and *Streptococcus pneumoniae*. This chronic bacterial colonization has been linked to a defect in the uptake (phagocytosis) of bacteria by macrophages (Taylor et al. 2010; Donnelly and Barnes 2012), and, particularly with *H. influenzae*, might be a factor driving chronic airway and systemic inflammatory responses in these patients (Fig. 2) (Singh et al. 2014). This defect in phagocytosis might also apply to defective uptake of apoptotic inflammatory cells (efferocytosis) and so might contribute to the impairment in resolution of lung inflammation observed in patients with COPD (Donnelly and Barnes 2012; Mukaro and Hodge 2011). Autoimmune mechanisms might also have a role in the persistence of bacterial infections in the lungs of such patients as there is evidence for the presence of autoantibodies, such as endothelial cell antibodies and antibodies against carbonyl modified proteins, in the lungs of those with COPD, at least in patients with severe disease (Kirkham et al. 2011). Finally, the peripheral lung inflammation might also 'spill over' into the systemic circulation and contribute to the systemic inflammation in COPD that is associated with various

comorbidities, such as cardiovascular disease and metabolic diseases (Barnes 2010). However, not all patients with COPD have evidence of systemic inflammation (Agusti et al. 2012) and comorbidities might be part of multimorbidity with similar mechanisms, such as accelerated ageing, affecting several organs at the same time.

3 Accelerated Ageing

COPD is largely a disease of the elderly and there is increasing evidence that emphysema is caused by accelerated ageing of the lung parenchyma due to defective endogenous anti-ageing mechanisms, such as those that involve sirtuins (Ito and Barnes 2009), with the activation of pathways leading to telomere shortening and cellular senescence (Fig. 3) (Mercado et al. 2015; Mitani et al. 2015). Cellular senescence and decreased sirtuin-1 have also been found in circulating endothelial progenitor cells of COPD patients, which are less effective at vascular repair than cells from age-matched normal individuals, which predisposes these individuals to cardiovascular disease and other comorbidities (Paschalaki et al. 2013). Indeed, stem cell senescence might be a common mechanism in COPD and its comorbidities, with consequent failure to repair tissue damage (Barnes 2015). Autophagy is a process whereby cells keep their cytoplasm clean by removing damaged organelles and proteins which is impaired with ageing (Madeo et al. 2015). There is increasing evidence that autophagy is defective in COPD, so that the accumulation of damaged proteins and organelles, such as mitochondria, result in accelerated cellular senescence and death (Mizumura et al. 2012).

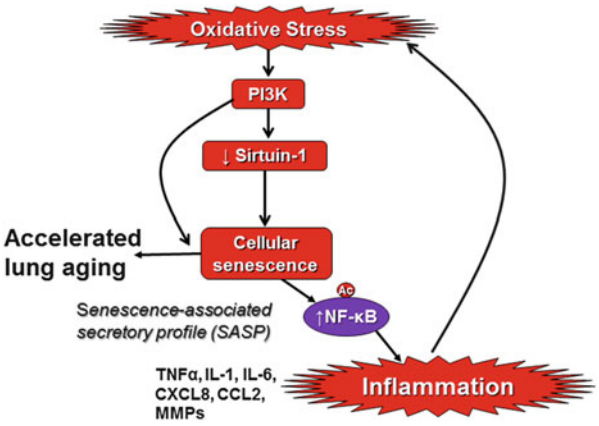

Fig. 3 Accelerated ageing and inflammation in COPD. Oxidative stress drives accelerated ageing though activation of phosphoinositide-3-kinase (PI3K) and reduction in sirtuin-1 which leads to cellular senescence and the release of inflammatory proteins (SASP), which further increase oxidative stress

4 Oxidative Stress

Increased oxidative stress is a key driving mechanism in the pathophysiology of COPD and accounts for many of the features of the disease (Kirkham and Barnes 2013). Oxidative stress is increased in patients with COPD from cigarette smoke exposure, but also endogenously from the activation of inflammatory cells, particularly neutrophils and macrophages. Reactive oxygen species (ROS) contribute to the pathophysiology of COPD in several ways (Fig. 4). For instance, ROS activate NF-kB and p38 MAPK, resulting in increased expression of inflammatory genes and proteases. ROS also inhibit endogenous antiproteases, such as α1-antitrypsin, resulting in increased elastolysis. Oxidative stress also leads to DNA damage, which is normally repaired by the efficient DNA repair machinery, but there might be a failure to repair double-stranded DNA breaks in COPD patients, which might also lead to increased risk of developing lung cancer (Caramori et al. 2011). ROS induce carbonylation of proteins, which, particularly in severe COPD, might lead to the generation of circulating autoantibodies that might perpetuate inflammation and lung injury (Kirkham et al. 2011). ROS also activate transforming growth factor β (TGF-β), leading to fibrosis. In addition, oxidative stress reduces corticosteroid responsiveness through a reduction in HD2 activity

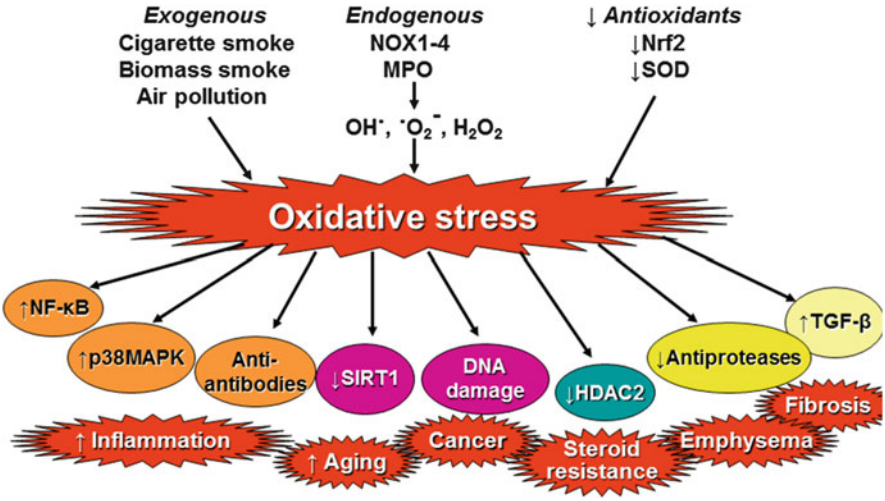

Fig. 4 Increased oxidative stress in COPD. Oxidative stress might be increased in chronic obstructive pulmonary disease (COPD) by a reduction in the expression of transcription factor NRF2, NADPH oxidases (NOX), myeloperoxidase (MPO) and superoxide dismutase (SOD) and other antioxidants, which might be triggered by inflammatory stimuli. Oxidative stress is a key mechanism that drives the development and progression of COPD through activation of the proinflammatory transcription factor nuclear factor-kB (NF-kB), p38 mitogen-activated protein kinase (MAPK), generation of autoantibodies to carbonylated proteins, reduced expression of sirtuin-1 (SIRT1), DNA damage, reduced histone deacetylase 2 (HDAC2) expression, reduced activity of antiproteases and increased release of transforming growth factor (TGF)-β

and expression (Barnes 2013). ROS also reduce the expression and activity of SIRT1, which is markedly reduced in lungs of patients with COPD and has a role in maintaining genomic stability, regulating autophagy and protecting against cellular senescence and ageing (Nakamaru et al. 2009). There is also evidence for defective endogenous anti-oxidant defences in patients with COPD. The transcription factor nuclear factor erythroid 2-related factor 2 (NRF2) plays a key part in the regulation of multiple antioxidant and cytoprotective genes in response to oxidative stress. NRF2 function is impaired in patients with COPD (Malhotra et al. 2008) and is not appropriately activated by oxidative stress due its increased acetylation as a result of reduced HDAC2 activity (Mercado et al. 2011).

Evidence is emerging that mitochondria are an important source of ROS in COPD and that there is a disruption of mitochondrial function in patients which leads to impaired oxidative phosphorylation and reduced intracellular ATP. Mitochondria are fragmented in epithelial cells of COPD patients and these changes are mimicked by cigarette smoke exposure in vitro, which leads to mitochondrial ROS production and cellular senescence (Hara et al. 2013). Cigarette smoke induces the autophagic uptake of mitochondria (mitophagy) in airway epithelial cells resulting in mitochondrial deficiency and cell death (necroptosis) that is mediated by the mitophagy regulator serine/threonine-protein kinase PINK1, mitochondrial [also called PTEN-induced putative kinase protein 1 (PINK1), encoded by *PINK1*] (Mizumura et al. 2014). It has been demonstrated that there is an increased expression of PINK1 in epithelial cells of patients with COPD and *Pink1* knock-out in mice do not develop emphysema or mucus secretion induced by chronic cigarette smoke exposure.

5 Pathophysiology

The airway obstruction in COPD is predominantly in the small airways of the lung periphery and results in a reduction in FEV_1 and the FEV_1/FVC ratio, which progresses over time. An acceleration of the normal FEV_1 decline with age can be observed in most patients, although poor lung function might result from the normal decline in lung function of developmentally impaired lungs. The fixed narrowing of small airways and the loss of alveolar attachments due to emphysema result in premature closure of small airways upon expiration, resulting in air trapping (Fig. 5). This causes lung hyperinflation (increased total lung capacity) and an increase in resting lung volume (functional residual capacity). Air trapping worsens in response to exercise (dynamic hyperinflation), resulting in exertional dyspnoea and reduced exercise tolerance (Guenette et al. 2012). Although the obstruction of small airways due to fibrosis is irreversible, superimposed cholinergic tone markedly increases airway resistance. This cholinergic tone is reversible by muscarinic antagonists and β_2-adrenergic receptor agonists (β_2-agonists), which results in reduced air trapping and thus reduced symptoms. Emphysema results in reduced alveolar surface area, resulting in impaired gas transfer and eventually to hypoxia.

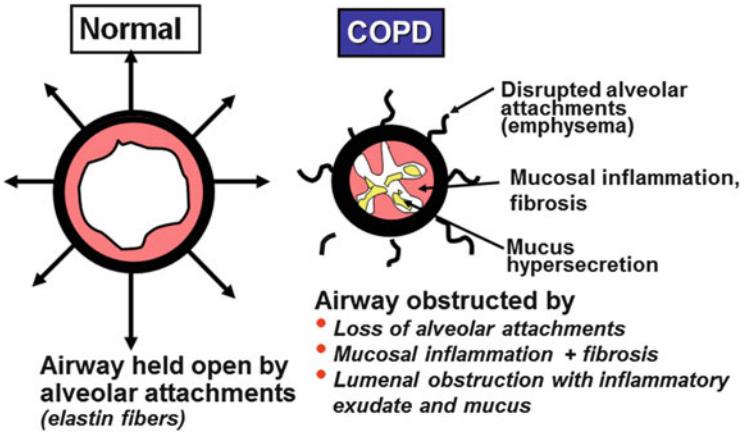

Fig. 5 Airway obstruction in COPD. In healthy lungs, the small airways (bronchioles) are held open by alveolar wall attachments that contain elastin fibres. In chronic obstructive pulmonary disease (COPD), small airways are narrowed through thickening of the bronchiolar wall through inflammation and fixed narrowing as a result of fibrosis, disruption of alveolar attachments as a result of emphysema and luminal occlusion by mucus and inflammatory exudate

6 Causes and Pathogenesis of Exacerbations

COPD exacerbations are episodes of symptom worsening that are usually associated with increased airway inflammation and systemic inflammatory effects (Fig. 6) (Wedzicha and Seemungal 2007). Most COPD exacerbations are triggered by respiratory viral infections, especially rhinovirus, which is the cause of the common cold and thus exacerbations are more common in winter. Respiratory viruses can be identified in the airway by PCR in up to 60% of exacerbations (Seemungal et al. 2001). Exacerbations associated with viruses tend to have more airway and systemic inflammation than those without any evidence of viral infection, are more common in the winter months and are associated with more chance of hospital admission (George et al. 2014). Pollutants that reach the airways might also be associated with precipitating exacerbations, especially by interacting with respiratory viruses, although significant effects of pollution are only seen in regions of high urban pollution (Peacock et al. 2011). Airway bacteria are also involved in causing exacerbations, though their precise role in triggering exacerbations is controversial. Although airway bacterial load increases during exacerbations, it is now considered that bacteria are often not the primary infective cause of the exacerbation, but are secondary invaders after an initial viral trigger (Wedzicha and Seemungal 2007).

Some COPD patients are susceptible to exacerbations irrespective of disease severity. These patients have been called ‘frequent exacerbators’ and over time their exacerbation frequency is relatively stable (Seemungal et al. 1998; Hurst et al. 2010; Wedzicha et al. 2013). The main risk of developing frequent

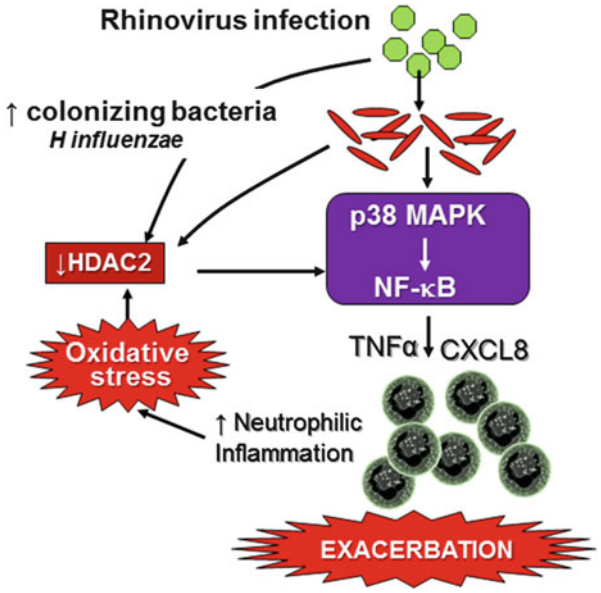

Fig. 6 Mechanisms of COPD exacerbations. Exacerbations are commonly triggered by rhinovirus infection, which may increase growth of colonising bacteria, such as *Haemophilus influenzae*. This activates nuclear factor-kB (NF-kB) and p38 mitogen-activated protein kinase (MAPK), which together activate inflammatory genes leading to increased cytokines, and chemokines that attract neutrophils into the airways. Activated neutrophils generate oxidative stress, which reduces histone deacetylase-2 (HDAC2) that is also reduced by virus and bacterial infection, resulting in further amplification of the increased inflammation, leading to an exacerbation

exacerbations is a history of exacerbations in the previous year. Frequent exacerbators have a worse prognosis with more hospital admissions, faster disease progression and worse health status than those who experience infrequent exacerbations.

7 Pathology of Asthma

In contrast to COPD, asthma is a disease that usually occurs in the first 5 years of life and is often associated with allergy (Barnes 2011). Asthma is a chronic inflammatory disorder of the airways, characterized by variable airflow obstruction resulting from underlying airways inflammation and bronchial hyperresponsiveness (Pepe et al. 2005), which are characteristic of this condition. A number of asthma guidelines define asthma as 'reversible airway obstruction and airway hyperresponsiveness in response to a variety of external stimuli' (Brusasco et al. 1998). However, it is now recognized that asthma is an umbrella term that has a number of phenotypes and these have been recently reviewed in some detail elsewhere (Corren 2013).

Asthma is exacerbated by acute exposure to respiratory allergens or other irritants including cold air, inhalation of cigarette smoke and exercise (Fireman 2003). These are some triggers that may provoke airway obstruction, resulting in wheezing, coughing, shortness of breath and tightening of the chest (Cookson 1999). In addition to bronchoconstriction and inflammatory cell recruitment, airway remodelling is suggested to be an underlying mechanism leading to the development of severe asthma (Pitchford 2007).

A wide spectrum of disease severity is observed in patients with asthma (Corren 2013; Cookson 1999), with recurrent attacks varying in frequency and gravity from person to person. Typically, airway obstruction is reversible in patients displaying a milder form of the disease, however with disease progression airway obstruction is often irreversible (Benayoun et al. 2003).

According to the World Health Organization, asthma represents one of the most serious of the allergic diseases (Web.archive.org 2014), with the prevalence associated with this disease at 235 million people and the average cost of treating asthma at £1 billion per annum in the United Kingdom (Cookson 1999).

8 Airways Inflammation

Asthma is a complex and chronic inflammatory disease of the airways, with eosinophils, mast cells, platelets (Pitchford 2007; Page and Pitchford 2014) and T lymphocytes playing a requisite role in the pathophysiology of the disease (Janeway 2001; Bousquet et al. 2000). Although predominantly mediated by the T helper 2 (Th2) response, the inflammation associated with asthma is now considered highly heterogeneous (Fig. 7) (Murdoch et al. 2010). Both acute (the infiltration of immune and inflammatory cells) and chronic inflammatory events (airway remodelling) contribute towards the pathogenesis of asthma (Pitchford 2007).

The response to inhaled allergen exposure in asthma typically consists of the acute inflammatory response, which comprises of an early phase response, followed by a late phase response. The early phase response is characterized by bronchoconstriction, inflammatory cell recruitment and vasodilation (Barnes 2011; Janeway 2001), and usually occurs within two hours following allergen challenge. This first phase of the allergic response is initiated by interactions between IgE and its high affinity receptor FcεRI, which is expressed on mast cells, basophils and epithelial cells (Barnes 2011; Amin 2012; Nakanishi 2010). The interaction between IgE and its receptor results in the activation of these inflammatory cell types and the release of pro inflammatory mediators including histamine, reactive oxygen species, leukotrienes and prostaglandins (Barnes 2011; Amin 2012). These inflammatory mediators induce bronchoconstriction, vasodilation, mucus secretion (Pepe et al. 2005), and microvascular leakage, resulting in oedema and further narrowing of the airway lumen and airway obstruction.

The late phase response typically occurs 6–9 h following allergen provocation and is characterized by early inflammatory cell recruitment, tissue remodelling and mucus secretion (Janeway 2001). The late phase response is associated with the

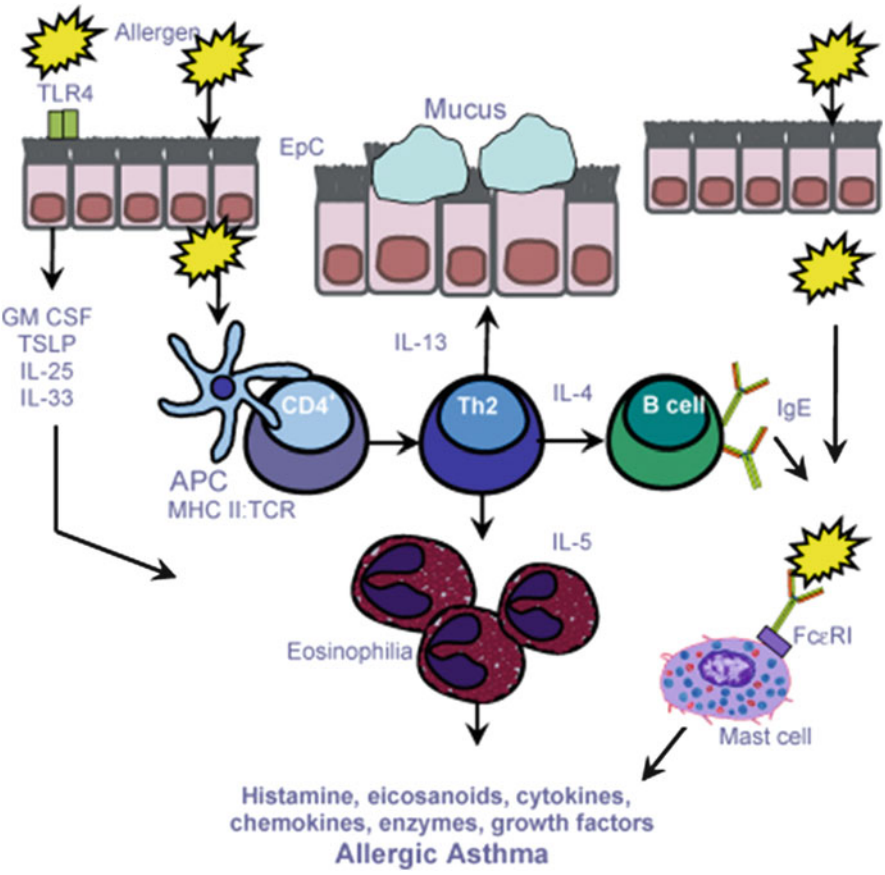

Fig. 7 The complex inflammatory processes involved in the pathogenesis of asthma. Modified from Murdoch et al. (2010)

recruitment and activation of neutrophils, basophils, eosinophils, macrophages and $CD4^+$ T cells (Bousquet et al. 2000; Wenzel et al. 1997). This inflammatory cell recruitment leads to further release of pro inflammatory mediators, such as major basic protein, peroxidases, reactive oxygen species and degradative enzymes (Wardlaw et al. 2000). In addition, an upregulation of adhesion molecules, such as CD11b, CD18, CD11A, ICAM-1, VLA-4 and V-CAM occurs (Bousquet et al. 2000), which is a requisite step for the recruitment of inflammatory cells.

The release of inflammatory mediators in the early and late acute inflammatory responses contributes towards bronchospasm and may lead towards the development of non-specific airway hyper-responsiveness, a state of excessive bronchial narrowing and heightened sensitivity to various inhaled stimuli (Fig. 8) (Holgate 2012; Grootendorst and Rabe 2004; Deo et al. 2010; Rt 2014).

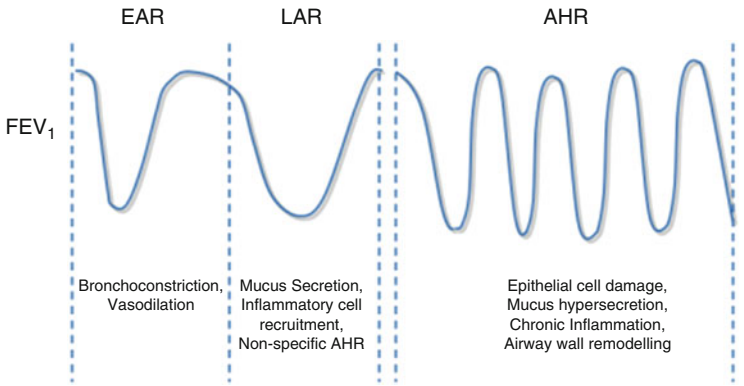

Fig. 8 The acute inflammatory response in the pathogenesis of asthma. Modified from Rt (2014)

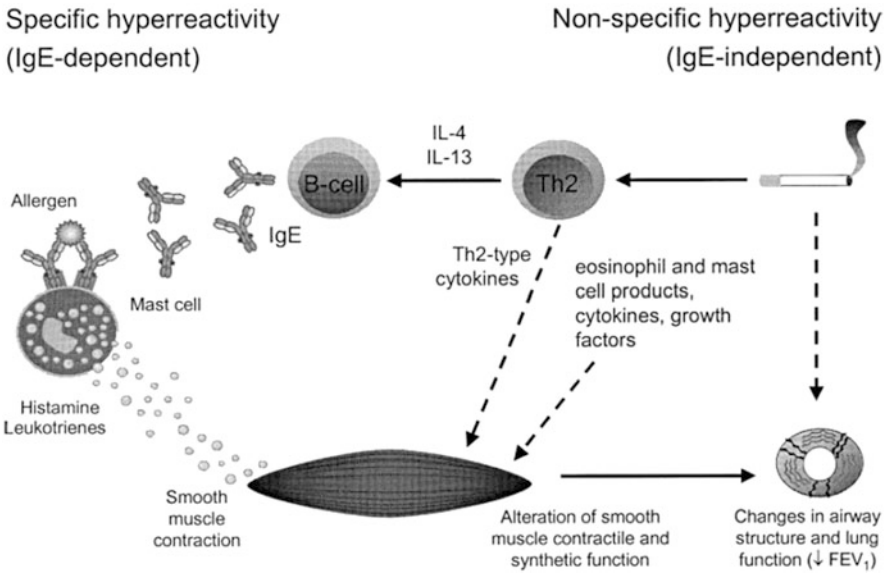

Fig. 9 The mechanisms leading towards bronchial hypersensitivity in asthma. Modified from Grootendorst and Rabe (2004)

Chronic inflammatory events are a feature of asthma and have been suggested to contribute to airway remodelling (Pitchford 2007), and to airway hyperresponsiveness. Th2 cells are thought to orchestrate allergic airway inflammation in the chronic inflammatory phase (Fig. 9) (Holgate 2012; Grootendorst and Rabe 2004). Th2 cells differentiate from the $CD4^+$ subset of T cells via MHCII and antigen presenting cells, such as dendritic cells and mast cells. Once activated, the Th2 subset generate and secrete type 2 cytokines, including interleukin-4, 5, 6, 9, 13, RANTES and eotaxin, which mediate the allergic inflammatory response

(Barnes 2011; Deo et al. 2010). Type 2 cytokines (IL-4 and 5) are particularly important in pulmonary eosinophil recruitment (Hershey et al. 1997). Eosinophilia in bronchoalveolar lavage fluid is a key characteristic feature in allergen induced asthma (De Monchy et al. 1985), with eosinophils playing an important role in airway remodelling, via the release of growth factors and a range of cationic proteins (De Monchy et al. 1985).

Additionally, the secretion of type 2 cytokines from Th2 cells causes the proliferation of B cells (Janeway 2001; Holgate 2012; Rt 2014) and differentiation into the plasma subset of b cells, which are antibody secreting. IL-4 and IL-13 induce isotype switching from IgM to IgE (Wedzicha et al. 2013; Janeway 2001), the antibody predominantly involved in asthma and allergic inflammation. The production of IgE results in further cross linking to the FcεRI, receptor, enabling the hypersensitivity reaction (Wardlaw et al. 2000). The release of histamine, leukotrienes (LTB4) and reactive oxygen species leads are also major causes of the bronchoconstriction in subjects with asthma (Deo et al. 2010).

Although asthma has been considered to be classically a Th2 mediated chronic inflammatory disease, it is now recognized that this diseases is far more complex (Murdoch et al. 2010). It has recently been suggested that there are in fact many different phenotypes of the disease, including atopic asthma, neutrophilic asthma, non-atopic asthma and late onset asthma (Fireman 2003). Consequently, therapeutically targeting eosinophilic airways inflammation or a single inflammatory mediator may not be optimal treatment for all forms of asthma.

Both IL-4 and IL-5 have been implicated in allergen induced eosinophilia (Hershey et al. 1997), suggesting anti-cytokine therapy would alleviate symptoms of asthma. However, although IL-5 monoclonal antibodies demonstrate inhibition of eosinophilia, no effect on bronchial hyper-responsiveness (BHR) or asthma symptoms was noted in patients with mild asthma undergoing allergen provocation (Leckie et al. 2000). Nonetheless the anti-IL5 monoclonal antibody mepolizumab has recently been approved for treating severe asthma as a result of its ability to reduce exacerbations of asthma (Ortega et al. 2014). Furthermore, Rh-IL-12 inhibited allergen induced eosinophilia but not BHR in patients with mild asthma undergoing allergen provocation (Matsuse et al. 2003). This suggests that the Th2 response is not solely responsible for the pathophysiology of asthma.

9 Bronchial Hyper-Responsiveness

Non-specific BHR may be defined as a state of increased narrowing of the airway wall and increased sensitivity in response to a variety of stimuli (Brusasco et al. 1998). BHR is recognized as a hallmark of chronic asthma and there are many underlying factors that contribute towards BHR (Murdoch et al. 2010; Grootendorst and Rabe 2004), including airway inflammation, airway remodelling, damage to the airway epithelium and neural humoral pathways (Woisin et al. 2001).

The level of bronchial hyper-responsiveness has been suggested to be related to the number of eosinophils, mast cells and T cells in bronchoalveolar lavage and sputum (Sont et al. 1996), and therefore airways inflammation contributes to the

pathogenesis of BHR. However, as observed above, the inability of anti-IL5 and Rh-IL12 to reduce BHR, despite significantly reducing eosinophils questions this relationship and indeed there are now many reports suggesting that airways inflammation and BHR may be dissociated (Woisin et al. 2001). For example, treatment of patients with asthma with the inhaled glucocorticosteroid budesonide for 10 years inhibited all signs of airways inflammation, yet patients still had BHR (and asthma, albeit asymptomatic) (Lundgren et al. 1988). Such observations question the direct relationship between airways inflammation and BHR and suggest other mechanisms must contribute to this important feature of the diseases.

Damage to the airway epithelium is another key feature of asthma (Wang et al. 2008). The airway epithelium acts as a protective diffusion barrier (Wang et al. 2008; Lambrecht et al. 2012) and consequently when damaged, this contributes towards BHR and airway inflammation via increased permeability. The airway epithelium secretes various inflammatory mediators that regulate vascular smooth muscle, consequently modulating airway smooth muscle tone (Farmer et al. 1990). Destruction of the airway epithelium reduces the synthesis and release of endogenous bronchodilator agents, such as PGE_2 (Leikauf et al. 1985; Manning et al. 1989) and nitric oxide, thus perpetuating airway irritability and bronchial contractility. The airway epithelium acts as a site for the metabolism of peptides by production of neutral endopeptidase (Dusser et al. 1988). A loss of a site for the metabolism of peptides such as bradykinin and substance p will enhance sensory nerve function via excitatory neural pathways (Widdicombe 2003) (activation of TRPA1/V1 and ASIC channels) and further contribute to BHR (Manning et al. 1989), Sensory nerve endings become exposed as a result of the loosening of tight junctions (Corren 2013), which may also contribute towards BHR in asthma. The epithelium acts as a source of an array of cytokines (IL-3, 4, 5, 9, 33, TSLP) (Wang et al. 2008), chemokines and growth factors (GM-CSF, EGF2), which may promote airway remodelling and BHR.

BHR in healthy and asthmatic subjects can be characterized by measuring the percentage fall in FEV_1 in response to an inhaled provoking stimulus such as histamine or methacholine (Fig. 10) (Grootendorst and Rabe 2004; O'Byrne

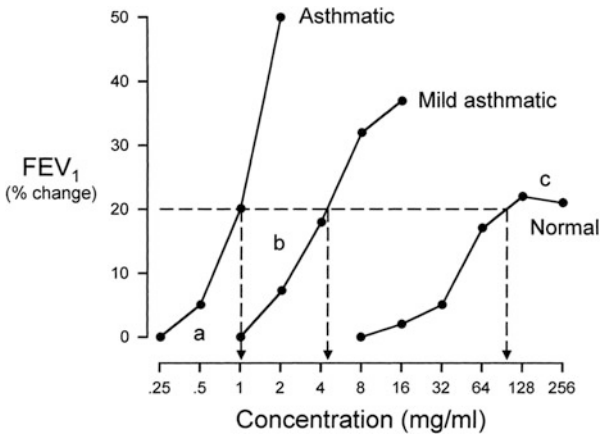

Fig. 10 A dose–response curve of inhaled provoking stimuli to measure BHR. Modified from O'Byrne et al. (2003)

et al. 2003). In patients with severe asthma the position of the dose response is steeper and shifted to the left in comparison with healthy individuals, suggesting increased sensitivity to the provocation. In healthy individuals the fall in FEV_1 will reach a plateau after inhalation of increasing doses of the stimuli. The concentration of a direct acting spasmogen such as histamine or methacholine that produces a 20% fall in FEV_1 is commonly used to indicate BHR (Grootendorst and Rabe 2004). More recently however there is a recognition that certain "indirect" agents such as mannitol, bradykinin and adenosine may be more discriminatory for the investigation of new drugs as healthy populations fail to respond to these agents (Indirect agents such as mannitol, bradykinin and adenosine may be more discriminatory for the investigation of new drugs as healthy populations fail to respond to these agents (van Schoor et al. 2005)).

10 Airway Remodelling in Asthma

Airway remodelling refers to persistent inflammation of the airways and permanent structural changes to lung architecture (Cookson 1999). Airway remodelling is suggested to be an underlying mechanism leading towards the development of severe asthma (Pitchford 2007) with studies indicating the severity of disease is dependent upon the extent of airway remodelling (Pepe et al. 2005). Airway remodelling encompasses a range of structural changes (Pepe et al. 2005), comprising of loss of epithelial integrity, airway smooth muscle (ASM) cell hyperplasia and hypertrophy, mucus hypersecretion, increased airway vascularity and thickening of the basement membrane (Fig. 11) (Cookson 1999; Elias et al. 1999; Fahy 2015). These structural changes to the airways lead to airway obstruction, BHR and airway edema. Airway remodelling is associated with poor clinical outcomes and consequently early diagnosis and prevention are critical to prevent development to severe asthma.

ASM is requisite in modulating airway tone. It has been demonstrated that the airway wall of asthmatic patients is thicker versus that of a healthy individual (Murdoch et al. 2010; Elias et al. 1999). An array of parameters contribute towards the thickening of the airway, including inflammatory cell infiltration, connective tissue deposition and an increased ASM mass represented by ASM hypertrophy and hyperplasia (Elias et al. 1999). There is now considerable literature showing that airway wall thickening contributes substantially to airway obstruction, AHR and bronchospasm, and may cause airway collapse (Murdoch et al. 2010; Elias et al. 1999).

It has been suggested that ASM cells may alter their phenotypic characteristics in allergic inflammation, from a contractile cell to a secretory and/or proliferative cell (Frossard 2000), releasing a range of pro inflammatory cytokines, extracellular matrix proteins and chemokines. This suggests ASM plays a direct role in the remodelling and inflammatory processes in patients with asthma (Bergeron et al. 2010).

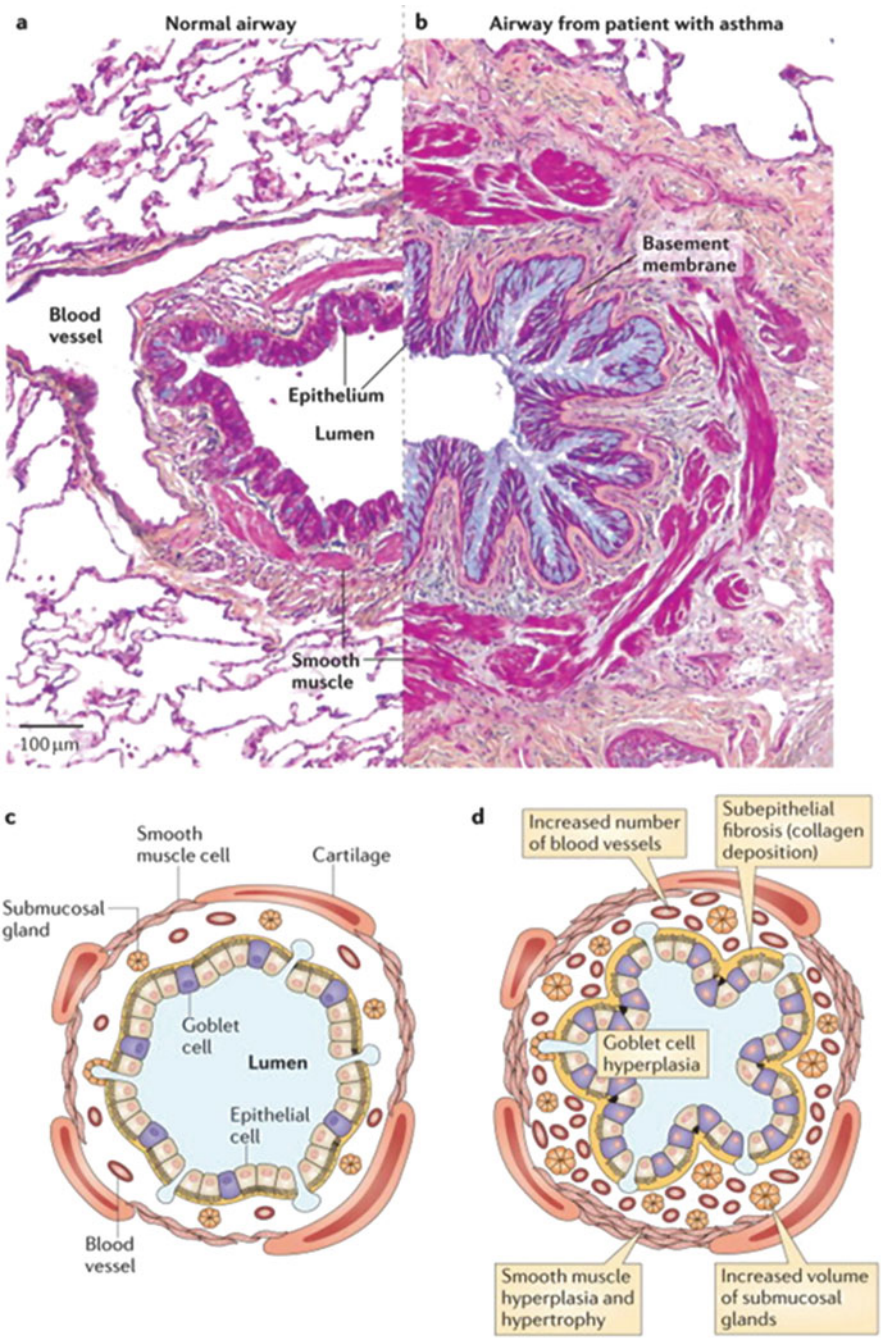

Fig. 11 Airway remodelling observed in asthma. Modified from Fahy (2015)

Goblet cell hyperplasia has been observed in asthmatic airways (Elias et al. 1999), resulting in the narrowing of the airway lumen and airway obstruction. Excessive sputum production results in mucus plugging in fatal asthma, whereby the surface tension at the air liquid interface is increased (Elias et al. 1999; Bergeron et al. 2010).

An additional feature of airway remodelling in asthma is sub-epithelial basement membrane thickening and fibrosis. Fibrosis is characterized by an imbalance in extra cellular matrix (ECM) protein production and degradation (Bergeron et al. 2010). Increased matrix metalloproteinase (MMP-2, 3, 8 and 9) and collagen deposition (types I, III and V) has been reported (Elias et al. 1999) in asthma. Studies show a correlation between sub-epithelial basement membrane thickness and asthma exacerbations (Elias et al. 1999).

11 Severe Asthma and Frequent Exacerbations

Asthma exacerbations are triggered by an array of factors, although the frequency and severity of exacerbations is highly variable amongst patients. The extent to which the airways are obstructed in asthma is variable, with reversibility observed in a milder form of the disease, and irreversible airway obstruction demonstrated with severe asthma (Benayoun et al. 2003). Several risk factors can predict those individuals prone to frequent exacerbations, for example inflammatory biomarkers, co-morbidities and a low pre-bronchodilator FEV_1 (<60%) (Thomson et al. 2008). Individuals with severe asthma and frequent exacerbations require high dose corticosteroids or hospitalization as a result of asthma (Thomson et al. 2008).

Studies suggest individuals with severe asthma and recurrent exacerbations are at risk of excessive airway narrowing and total closure of the airways (Veen et al. 2000). Persistent chronic inflammation and airway remodelling are associated with disease progression (Pitchford 2007), with a mucus plug or marked increase in goblet cells a key characteristic feature of patients with severe asthma attack (Fig. 12) (Aikawa et al. 1992; Jeffery et al. 2006).

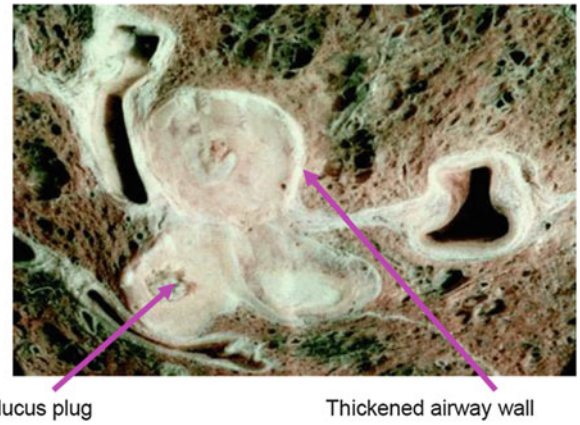

Fig. 12 A mucus plug leading to total airway obstruction in severe asthma. Modified from Jeffery et al. (2006)

There remains an unmet medical need for new treatments for respiratory diseases, particularly for severe asthma and as an alternative to steroids in patients with COPD, given recent concerns about their poor efficacy in the majority of patients and the increased risk of pneumonia (Barnes 2012; O'Byrne et al. 2011; Suissa et al. 2013). Furthermore, despite the continuous use of β2 agonists and inhaled corticosteroids, some patients continue to display chronic symptoms (Benayoun et al. 2003). The remainder of this volume reviews the current state of play in the development of new therapies and improved delivery of existing drugs for the treatment of these important conditions.

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β_2 Agonists

Charlotte K. Billington, Raymond B. Penn, and Ian P. Hall

Contents

Abstract

History suggests β agonists, the cognate ligand of the β_2 adrenoceptor, have been used as bronchodilators for around 5,000 years, and β agonists remain today the frontline treatment for asthma and chronic obstructive pulmonary disease (COPD). The β agonists used clinically today are the products of significant expenditure and over 100 year's intensive research aimed at minimizing side effects and enhancing therapeutic usefulness. The respiratory physician now has a therapeutic toolbox of long acting β agonists to prophylactically manage bronchoconstriction, and short acting β agonists to relieve acute exacerbations. Despite constituting the cornerstone of asthma and COPD therapy, these drugs

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are not perfect; significant safety issues have led to a black box warning advising that long acting β agonists should not be used alone in patients with asthma. In addition there are a significant proportion of patients whose asthma remains uncontrolled. In this chapter we discuss the evolution of β agonist use and how the understanding of β agonist actions on their principal target tissue, airway smooth muscle, has led to greater understanding of how these drugs can be further modified and improved in the future. Research into the genetics of the β_2 adrenoceptor will also be discussed, as will the implications of individual DNA profiles on the clinical outcomes of β agonist use (pharmacogenetics). Finally we comment on what the future may hold for the use of β agonists in respiratory disease.

Keywords
Airway smooth muscle • Asthma • β adrenoceptor • β agonists • Cyclic AMP • Isoprenaline

1 Introduction

β agonists constitute the frontline treatment for both asthma and COPD. They exert their bronchodilatory effects via β_2 adrenoceptors (β_2ARs) located on airway smooth muscle (ASM) cells. Activation of these receptors results in ASM, and thus airway, relaxation via the molecular processes outlined later in the "Mechanisms of Action" section of this chapter and also shown in Fig. 1. In addition to the receptors expressed on ASM cells, β_2ARs are also found on a number of other cell types within the lungs including epithelial cells, submucosal glands, vascular endothelium, vascular smooth muscle, and inflammatory cells including mast cells, macrophages, and eosinophils (Barnes 2004).

The β_2AR is a member of the G-protein coupled receptor (GPCR) family and was in fact the first GPCR to be cloned (Dixon et al. 1986). In common with all GPCRs, it is composed of seven transmembrane spanning domains and has an intracellular C-terminus and an extracellular N-terminus. GPCRs have long been overrepresented as targets for drug therapy with an estimated 30–50% of medicines acting via GPCRs either directly or indirectly (Garland 2013). Both an historical and ongoing challenge in all drug development is of course ensuring selectivity of beneficial over unwanted effects. The β agonist story is no different and many of the major side effects related to these drugs are due to cross-activation of β_1ARs, with activation of these leading to anxiety, tachycardia, tremor, and sweating. As we will describe later, there is increasing awareness of not only the selectivity for the β_2AR but within β_2AR-mediated signalling pathways the need to select for "good" vs "bad" effects. There is a third βAR subtype, the β_3AR, however these are located predominantly in adipose tissue and do not contribute to the adverse effects profile.

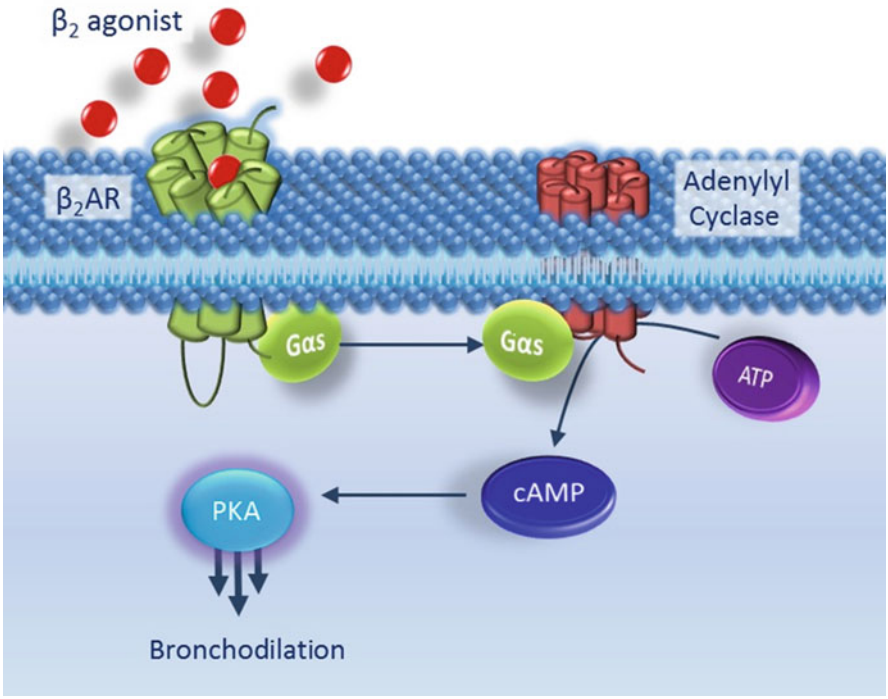

Fig. 1 The classic β_2AR signalling pathway. Binding of β_2 agonist to β_2AR induces a conformational change allowing the α-subunit of the G-protein to dissociate and bind to adenylyl cyclase. Adenylyl cyclase is thus activated and catalyzes the formation of cyclic AMP (cAMP) from ATP. cAMP molecules bind to PKA which induces the dissociation of the catalytic and regulatory subunits from each other. Once released, the PKA catalytic subunits phosphorylate and hence activate myriad cellular targets which results in airway smooth muscle relaxation and hence bronchodilation

The history of β agonist use in respiratory disease is a fascinating one and the reader is directed to a number of excellent reviews and books for further reading (Barnes 2006; Chu and Drazen 2005; Jackson 2009). It is thought that β agonist-mediated airway relaxation was first used around 5,000 years ago in Chinese medicine when the ephedrine-containing plant ma huang was used to alleviate respiratory conditions. Ephedrine activates the β_2AR pathway indirectly via heightening activity of noradrenaline at βARs. However, in western medicine it was not until the early twentieth century that ephedrine-mediated bronchodilatory effects were described (Melland 1910 and as reviewed by Chu and Drazen 2005). Throughout the twentieth century further research and enlightenment led to increased use of β agonists in respiratory disease particularly following the introduction of the first pure (but nonselective between β_1AR and β_2ARs) β agonist, isoprenaline (isoproterenol in the USA), in the 1940s. Isoprenaline became the most commonly used inhaled treatment for asthma in the next 20 years. Indeed, in just the 10 years following its availability as a metered dose inhaler in 1956, usage increased

fourfold (Jackson 2009). However in the 1960s an epidemic of deaths across six countries, likely due to usage of a higher dose form of isoprenaline, led to the realization that more refined therapies were required. The first β_2AR-selective agonist, salbutamol, was synthesized in 1968 by a team at Glaxo and, in addition to reducing the side effects associated with the non-selective β agonist, isoprenaline, it was also superior in terms of duration of effect (Brittain et al. 1968; Cullum et al. 1969). However, it still remains relatively short acting, and hence is considered a short acting β agonist or SABA. The same team at Glaxo proceeded to further modify salbutamol producing salmeterol, capable of exerting its bronchodilatory effect for up to 12 h: this is a member of a class of drugs called long acting β agonists, or LABAs for short. The subsequently developed LABA formoterol was also shown to produce effects for 12 h and more recently the discovery of even longer acting β_2AR agonists such as indacaterol, has allowed for once daily dosing: these agents have therefore been called ultra-LABAs. Other drugs in these classes are discussed further below.

Whilst these longer acting β_2 agonists constitute the cornerstone of treatment for people with asthma and COPD, the manner in which their use is recommended differs dramatically. In 2011 the US Food and Drug Administration (FDA) published a warning that, when treating asthma, LABAs must only be used in combination with an Inhaled CorticoSteroid (ICS). However for COPD, in at least some countries, LABA monotherapy remains an option for frontline treatment. In addition the ultra-long-acting β agonist indacaterol is only indicated for COPD and not asthma at present. In the next section we will explore the different classes of β agonists available and consider their current clinical use.

2 Clinical Classification of β Agonists

As discussed above, β agonists are grouped into three classes, namely Short-Acting β Agonists (SABAs), Long-Acting β Agonists (LABAs), and Ultra-Long Acting β Agonists (ultra-LABAs). Table 1 lists the β agonists used clinically. As suggested by the names SABAs have short half-lives and are used as rapid relievers, whereas LABAs and ultra-LABAs provide sustained symptomatic relief due to their longer duration of action. As noted above, LABA monotherapy for asthma is contraindicated due to safety concerns. The prolonged duration of action of the LABAs currently used in clinical practice is not thought to be due to a difference in receptor kinetics but rather retention within the cell membrane and hence a continued presence of the drug near to the receptor.

2.1 SABAs

SABAs (e.g., salbutamol) delivered via metered dose or dry powder inhalers provide almost instant symptomatic relief and are the frontline therapy in asthma to combat bronchoconstriction and acute exacerbations. Their bronchoprotective

Table 1 Clinically used β_2 agonists with their respective times of onset of action, duration of effects, dosing regimen, and specificity at the β_2 AR (β_2/β_1)

	Onset of action (min)	Duration of effect (h)	Therapeutic use	Specificity at β_2 AR (β_2/β_1)
SABAs				
Salbutamol	<5	3–6	100–200 μg As required (up to 4 times per day)	27
Terbutaline	<5	4–6	500 μg As required (up to 4 times per day)	63
LABAs				
Salmeterol	~15	12	50–100 μg Twice daily	3,000
Formoterol	~7	12	12–24 μg Twice daily	150
Olodaterol	~5	12	5 μg Once daily	65
Vilanterol	~5	12	55 μg Once daily	2,400
Ultra-LABA				
Indacaterol	~5	24	150–300 μg Once daily	16

Doses given are for inhalation. Adapted from Tamm et al. (2012), Baker (2010), and BNF

effect is evident in minutes and remains for 4–6 h. These drugs are also available for oral administration in some countries; however, this method is of less therapeutic value with the patient being more prone to systemic side effects and it is thus rarely employed. SABAs are recommended to be used only on an "as required" basis rather than a regular basis in asthma and an escalation of use should prompt clinicians to review patient management. British Thoracic Society (BTS) guidelines currently recommend the use of inhaled SABAs "as required" for mild intermittent asthma in adults.

Similar to asthma, for COPD SABAs are recommended as the initial treatment for the relief of breathlessness and exercise limitation (NICE clinical guidelines). As would be predicted in a disease where reversibility is by definition limited, the efficacy in COPD of these agents is less than in asthma.

2.2 LABAs

BTS guidelines for adults with asthma recommend an inhaled LABA as the initial add-on therapy in patients already taking a regular inhaled steroid but with inadequate control of disease. Monotherapy with a LABA in asthma is contraindicated. This is partly due to LABA monotherapy proving less clinically effective than treatment with ICS but mainly due to the safety issues highlighted in the "Adverse

Effects" section of this chapter. If asthma is still persistently poorly controlled even following increased doses of steroids, further add-on drugs are advised to be trialled including leukotriene receptor antagonists, slow release theophylline, or antimuscarinic agents including the new long acting muscarinic antagonists (LAMAs) such as tiotropium.

LABAs are a frontline treatment for COPD. They are recommended to be offered as maintenance therapy either alone (if $FEV_1 \geq 50\%$ predicted) or certainly in the UK, more commonly in combination with an ICS (if $FEV_1 < 50\%$ predicted) (NICE guidelines). In people with stable COPD and an $FEV_1 \geq 50\%$ who remain breathless or have exacerbations despite maintenance therapy with a LABA a combination inhaler comprising a LABA and ICS is also recommended. Long acting muscarinic antagonists (LAMAs) can be used interchangeably with LABAs depending on the patients' symptomatic response and preference in addition to the drug's potential to reduce exacerbations, its side effects and cost (NICE guidelines).

2.3 Ultra-LABAs

The ultra-LABA indacaterol was given approval by the European Medicines Agency (EMA) in 2009 and by the FDA in 2011 for the maintenance treatment of patients with COPD. Indacaterol is delivered by inhalation as a dry powder and has a fast onset of action due to its rapid absorption. In December 2014 a combination ultra-LABA/LAMA (indacaterol/glycopyrronium bromide) was launched in the UK, also indicated for maintenance bronchodilator treatments for patients with COPD. Also available in other European countries, authorization of this product in the USA is ongoing. No specific NICE guidance currently exists surrounding the use of indacaterol as mono- or combination therapy in COPD. Indacaterol has not yet been approved for use in the treatment of asthma; however, clinical trials to ascertain its suitability for asthma therapy are ongoing.

3 Mechanisms of Action

The β_2AR is the most exhaustively studied GPCR, with respect to both its signalling and its regulation, and is therefore frequently referred to as the "prototypical GPCR." Although for a time there appeared to be a consensus as to what constituted "canonical" β_2AR signalling, recent studies identify a complexity of β_2AR signalling that portends a new era of research in β_2AR biology and pharmacology.

Results from studies involving numerous cell and cell–free systems have contributed to the description of canonical β_2AR (Fig. 1), which also serves as an example of prototypical heterotrimeric G protein signalling. Early studies by Gilman, Lefkowitz, Birnbaum, Bourne, Perkins, and others (reviewed in Penn and Benovic 1998) characterize transmembrane signalling involving GPCR (β_2AR), heterotrimeric G protein (Gs for the β_2AR) and an effector (adenylyl

cyclase downstream of β_2AR-Gs). Adenylyl cyclase mediates the hydrolysis of ATP into cAMP, which in turn activates the cAMP-dependent protein kinase [aka PKA (Protein kinase A)]. PKA is the first discovered cAMP effector, and has been shown to phosphorylate numerous intracellular substrates to effect various functions in a cell type-dependent manner. This classical signalling paradigm resulting in PKA activation was presumed to be the predominant pathway stimulated by β agonists in all cell types; the functional consequences of activation of this pathway depended on the specific PKA substrates expressed in a cell and whatever downstream signalling/targets these substrates regulate. Thus, functional diversity of β agonist signalling was thought to be determined by the stoichiometry of signalling elements, and signalling targets, in a given cell. For example, in ASM important PKA substrates include various Gq-coupled receptors, Gq, phospholipase C, myosin light chain kinase (MLCK), IP3 receptors, K Ca channels, heat shock protein 20, and phosphorylation of each is believed to antagonize pro-contractile Gq-coupled receptor signalling or directly inhibit mechanisms important to ASM contraction. In addition, phosphorylation of the MAP kinase kinase kinase Raf-1 as well as the transcription factor CREB inhibits mitogenic signalling and pro-mitogenic gene induction in many mesenchymal cell types to regulate cell growth (for an extensive discussion of β_2AR signalling and regulation in ASM cells, the reader is referred to Billington and Penn 2003; Penn 2008; Walker et al. 2011).

Although some instances of cAMP-independent β_2AR signalling had been identified (Kume et al. 1994), cAMP-dependent PKA actions were for years presumed to mediate most of the functional consequences of β_2AR activation. However, in 1998 the Bos laboratory discovered the cAMP effector Epac, which was able to activate the small GTPase Rap1 in the presence of PKA inhibition (Kawasaki et al. 1998). Epac1 and Epac2 were determined to be GTPase-activating proteins of Rap1, and subsequent studies identified various cAMP-dependent/PKA-independent functions, attributable to Epac, in various cells (reviewed in Roscioni et al. 2008).

The discovery of Epac has led to questioning of the widely held assumption that β_2AR actions in a given cell type are entirely PKA-dependent. In many instances, the functional consequences of β agonists or other agonists of Gs-coupled receptors in a given cell have been dogmatically ascribed to PKA signalling, despite the lack of any direct evidence in such cells. This lack of direct evidence stems from difficulties in selectively inhibiting PKA in intact cells or tissue; all existing small molecular inhibitors lack specificity, and genetic ablation of catalytic PKA is lethal (reviewed in Morgan et al. 2014; Penn et al. 1999). Consequently, a role for PKA was often asserted when agents classically known to induce intracellular cAMP [e.g., Gs-coupled receptor agonists, or forskolin (which activates adenylyl cyclase downstream of GPCRs)] to activate PKA could generate a similar functional effect to the agent/receptor in question.

This logic, and not direct evidence, was for years employed to assert a role for PKA in mediating bronchorelaxation and growth inhibition of ASM. In 2011 Zieba et al. (2011) demonstrated that Epac-selective cAMP analogues could relax

contracted smooth muscle, including ASM, presumably via inhibition of RhoA activity. Moreover, in rat aortic smooth muscle cells, β agonist induced Rap1 activity that could be inhibited by Epac1 knockdown. This study raised the intriguing possibly that Epac is a novel therapeutic target for obstructive lung diseases, and questioned long held beliefs regarding the mechanisms of bronchodilatory actions of β agonists. However, a recent study by Morgan et al. (2014), employing molecular means of selective PKA inhibition demonstrated a clear, dominant role of PKA in mediating the relaxant effect of β agonists in both cell- and tissue-based models of ASM contraction. This study, in addition to a prior study ascribing the anti-mitogenic effect of Gs-coupled receptors in ASM to PKA (Yan et al. 2011), suggests that indeed PKA is the main effector through which β agonist promotes its therapeutic actions on ASM, yet leaves open the possibility that Epac targeting has therapeutic utility.

To further complicate the story of β_2AR signalling, an increasing body of evidence accumulated over the last 15+ years demonstrates the ability of the β_2AR to signal *independent of G protein activation*. Most of these studies (recently reviewed in Kenakin 2011; Reiter et al. 2012; Walker et al. 2011) have focused on the ability of arrestin proteins to function as a scaffold capable of coordinating signalling complexes and to initiate signalling events often distinct, and sometimes antithetical, to those mediated by G proteins (see Fig. 2). Arrestins were originally discovered as GPCR-interacting proteins that function to both uncouple GPCRs from G proteins and to mediate GPCR internalization (for recycling or lysosomal degradation) (Kang et al. 2014; Shenoy and Lefkowitz 2011). Prompted by the discovery of arrestin-dependent signalling, investigation into *qualitative signalling* or *biased agonism* has exploded based on the underlying assumption that G protein-dependent and -independent signalling events can be linked with distinct functional outcomes, thus allowing a great range of function diversity (and possibly greater therapeutic utility) among GPCR ligands.

Although the relevance of qualitative β_2AR signalling in airway biology and disease is unknown, studies of arrestin and PKA function in the airway have led to speculation that G protein/PKA-dependent signalling is therapeutic to inflammatory lung diseases such as asthma, whereas arrestin-dependent signalling may be pathological (Penn et al. 2014; Walker et al. 2011). As noted above, β agonist-stimulated Gs/PKA signalling in ASM mediates bronchorelaxation through actions on ASM. Early studies by Walker et al. demonstrated that β-arrestin2 gene ablation inhibited the development of allergic inflammation in a mouse model (Walker et al. 2003). More recent studies by Bond and colleagues note that "antagonism" of the β_2AR, in the form of either certain β-blockers (Callaerts-Vegh et al. 2004), β_2AR gene ablation (Nguyen et al. 2009), or depletion of systemic epinephrine (Thanawala et al. 2013), also thwarted the development of the asthma phenotype (airway inflammation, mucous production, and airway hyperresponsiveness) in mice sensitized and challenged with allergen. Interestingly, the unbiased β-blocker nadolol (which functions as an inverse agonist for both G protein- and arrestin-dependent signalling (Stallaert et al. 2012; Wisler et al. 2007)) was more effective than either (arrestin-activating but G protein-inhibiting) carvedilol or

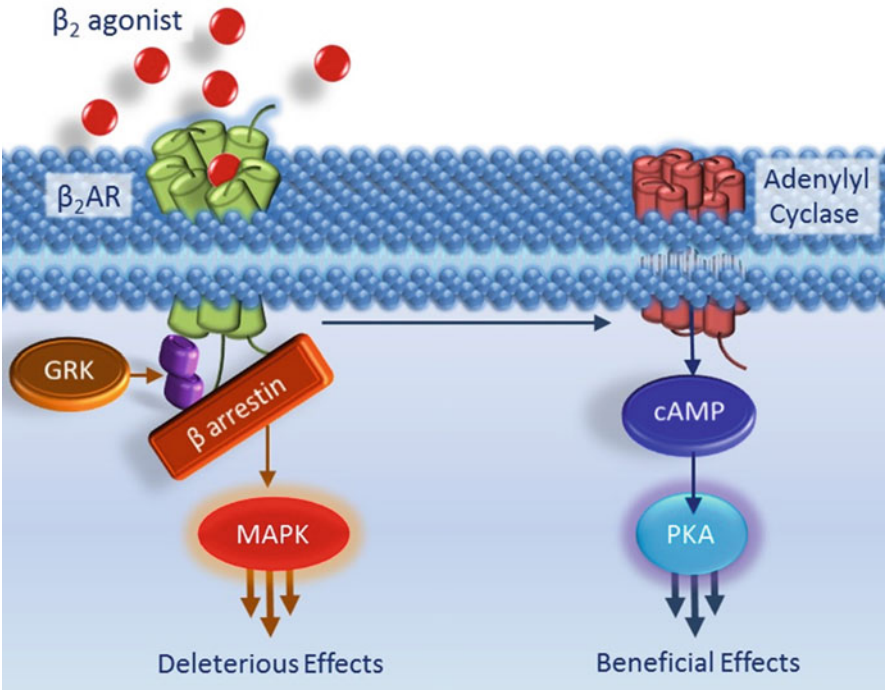

Fig. 2 The pros and cons of β_2AR activation. As shown in Fig. 1, activation of the β_2AR induces bronchorelaxation (i.e., a beneficial effect) via its activation of the adenylyl cyclase-cAMP-PKA pathway. In this figure, parallel deleterious effects are highlighted whereby the activation of β_2ARs is controlled by βarrestins. As shown in the *left-hand side* pathway in this figure, following exposure to β_2 agonists, β_2ARs are phosphorylated by G Protein Coupled Receptor Kinases (GRKs) and rapidly desensitized meaning that regardless of continued β_2 agonist presence, cAMP production is diminished. GRK regulates β_2AR activity in part by uncoupling the β_2AR from the Gα subunit of the G protein but also by promoting the binding of βarrestin molecules to the β_2AR. βarrestin physically blocks further β_2AR and Gα subunit interaction and hence further prevents the beneficial, pro-relaxant signalling pathway shown in Fig. 1 and also, in shortened form, on the *right-hand side* of this diagram. However, the GRK- and βarrestin-mediated effects on cAMP signalling are not the only deleterious effects. βarrestin acts as a scaffold protein bringing together other molecules and initiating signalling via pathways not involving G-proteins, for example the MAPK pathway

alprenolol, suggesting that all "β-blockers" are not equal, and that their functional effects may be linked to their signalling bias. Interestingly, restoration of systemic β agonist (in epinephrine-depleted mice) by infusion of formoterol (capable of both G protein and arrestin signalling) fully restored the asthma phenotype in allergen challenged mice (Thanawala et al. 2013). Finally, it is interesting to note that in a pilot study, human asthmatics treated with nadolol exhibited a decrease in airway hyperreactivity in the medium term (Hanania et al. 2008), although in a separate study asthmatics treated with propranolol (a β-blocker capable of promoting arrestin signalling) showed no clinical improvement (Short et al. 2013).

4 Adverse Effects

Despite being used extensively for the treatment of asthma for over half a century, β agonists have an almost equally long history of adverse effects. Although the reasons for such adverse effects are multiple, with some still unknown, the majority of adverse effects can be attributed to either: (1) a lack of selectivity for the β_2AR, resulting in "off-target" effects mediated by either α or β1 ARs; or (2) ill-defined β_2AR-mediated effects that appear to involve either β_2AR desensitization or exacerbation of airway inflammation and its consequences. Although a thorough discussion of adverse effects associated with β agonist use is beyond the scope of this review, we will summarize below the current consensus beliefs.

β_1AR Agonism Promotes Several Adverse Effects of Therapeutic Relevance Whereas numerous side effects including tachycardia, arrhythmia, tremor, and headache occurred with the early therapeutic use of nonselective β agonists such as adrenaline (activating both α and β ARs) and isoprenaline (activating both β_1 and β_2ARs), receptor subtype discrimination enabled by the landmark studies of Ahlquist (1948) and Lands et al. (1967) ultimately resulted in the development of the β_2AR-selective salbutamol and terbutaline. Waldeck (2002) provides an elegant history of the discovery and clinical application of bronchodilatory β agonists and the work that facilitated increasing β_2AR subtype selectivity. And although it should be recognized that essentially all currently used SABAs and LABAs used to treat asthma are at least to some extent selective for the β_2AR, there is considerable variability in the degree of selectivity (see Table 1). In addition the sensitivity of patients to experience cardiovascular side effects with drug usage will also depend on individual characteristics including the presence or absence of significant co-morbidities such as ischemic heart disease.

Safety Concerns over β Agonist Use in Asthma Prompted by Mortality and Morbidity Data Are Controversial, and the Mechanistic Basis for Increased Mortality/Morbidity Is Poorly Understood Mortality and morbidity are the adverse effects that have dominated the discussion of β agonist safety for the last several decades. A recent review by Ortega and Peters (2010) provides an excellent history and analysis of the various "epidemics" associated with the use of SABAs and LABAs as asthma drugs. As mentioned above, use of the SABA isoprenaline in several countries was associated with increased adverse events and mortality. Asthma-related mortality increased after the release of the SABA fenoterol in New Zealand in 1976, yet subsequently waned after the drug was removed from the market in 1989. In 1990, one of the first prospective trials of β agonist safety reported that regularly scheduled fenoterol therapy resulted in worse asthma control than did as-needed (rescue) fenoterol (Sears et al. 1990).

The safety of LABAs was also questioned shortly after their introduction, first in the Nationwide Surveillance (SNS) Study, a prospective study which suggested a trend towards asthma-related deaths associated with the use of salmeterol (Castle

et al. 1993). These results of the SNS Study appeared to be of sufficient concern to prompt numerous retrospective and prospective studies. The critical study that heightened the debate of LABA safety was SMART (Salmeterol Multicentre Asthma Research Trial), a prospective study of salmeterol initiated by Glaxo Smith Kline in 1996. In 2002 an interim analysis of the data demonstrated a 4.4-fold increase in death in those asthmatics receiving salmeterol compared to those receiving placebo (Nelson et al. 2006). A subsequent meta-analysis by Salpeter et al. analyzing results from 19 randomized placebo-controlled trials (including SMART) reported significantly increased odds ratios for both in life-threatening exacerbations and asthma-related deaths associated with LABA use (Salpeter et al. 2006).

Hotly debated since the SMART study and the Salpeter meta-analysis has been the interpretation of the statistics, and the relevance of the study design, of SMART in addressing the question of LABA safety in people with asthma. Additional prospective clinical studies of LABA safety (reviewed in Ortega and Peters 2010) have resulted in conclusions asserting LABA safety in asthmatics. Regardless of the merits of each side of the debate, the major consequence of the SMART study was to cause the US FDA to question the safety the LABAs and issue a black box warning issued for the then available LABAs (salmeterol and formoterol) in the USA. The FDA recommends that labels incorporate the following:

- Use of a LABA alone without use of a long-term asthma control medication, such as an inhaled corticosteroid, is *contraindicated* (absolutely advised against) in the treatment of asthma.
- LABAs should not be used in patients whose asthma is adequately controlled on low or medium dose inhaled corticosteroids.
- LABAs should only be used as additional therapy for patients with asthma who are currently taking but are not adequately controlled on a long-term asthma control medication, such as an inhaled corticosteroid.
- Once asthma control is achieved and maintained, patients should be assessed at regular intervals and step down therapy should begin (e.g., discontinue LABA), if possible without loss of asthma control, and the patient should continue to be treated with a long-term asthma control medication, such as an inhaled corticosteroid.
- Pediatric and adolescent patients who require the addition of a LABA to an inhaled corticosteroid should use a combination product containing both an inhaled corticosteroid and a LABA, to ensure adherence with both medications

Partly as a consequence of these concerns, in most European countries guidelines only recommend use of LABAs in conjunction with co-administration of ICS in patients with asthma.

What is the mechanistic basis for increased mortality and morbidity with β_2 agonist use in asthmatics? Several possible explanations have been offered but empirical evidence supporting them is largely lacking. The loss of drug effectiveness due to desensitization of β_2AR on ASM has been proposed often (reviewed in Walker

et al. 2011). Indeed, β_2AR desensitization as evidenced by diminished β agonist stimulated intracellular signalling and function (relaxation, inhibition of ASM proliferation and pro-inflammatory "synthetic" functions) has been seen to occur with chronic β_2 agonist treatment in ASM cell, tissue, and in vivo (mouse) models. Moreover, experimental strategies that inhibit mechanisms of β_2AR desensitization [involved GRK (Kong et al. 2008; Deshpande et al. 2014)] and arrestin molecules (Penn et al. 2001; Deshpande et al. 2008) can mitigate β_2AR desensitization (signalling and function) in these models. These findings are consistent with a loss of bronchoprotective effect (functional desensitization) with chronic β_2 agonist use in humans (Bhagat et al. 1995; Cheung et al. 1992; Grove and Lipworth 1995; Lipworth et al. 1998). Thus the loss of asthma control associated with chronic β_2 agonist use causing β_2AR desensitization represents one attractive explanation for poorer control of disease in asthmatics taking β_2 agonists.

A more recent explanation has emerged that relates to the qualitative signalling properties of β_2ARs mentioned above. β_2AR signalling can occur via both G protein-dependent and arrestin (G protein-independent) pathways. Murine studies of allergic lung inflammation implicate a pathologic role for arrestins, and β_2AR ligands capable of stimulating arrestin signalling, but not those incapable of stimulating arrestin signalling. Those ligands capable of stimulating arrestin signalling thus appear potentially important in promoting allergen-induced inflammation (including mucous production) and AHR. Circulating epinephrine appears to serve this critical permissive function, and specific β_2AR ligands (e.g., nadolol) capable of blocking epinephrine activation of the β_2AR while themselves not activating arrestin signalling are effective in blocking the development of the allergen-induced asthma phenotype. Which cell types mediate β_2AR- and arrestin-dependent inflammation are unclear, although mucin-producing airway epithelia appear to have an important role (Thanawala et al. 2013; Penn et al. 2014). Whether or not exogenous β_2 agonists (e.g., SABAs or LABAs when used therapeutically) exacerbate the facilitary pro-inflammatory effects of endogenous epinephrine is unclear, but based on accumulating evidence (reviewed in Walker et al. 2011) it does not appear that current therapeutic β_2 agonists have anti-inflammatory effects (despite early assertions to the contrary).

Interestingly, these pathogenic effects of β_2AR-mediated arrestin signalling are consistent with an early hypothesis attempting to explain the loss of asthma control and safety concerns associated with LABA use (Nelson 2006): i.e., that β agonist fails to address underlying inflammation but effectively bronchodilates via its direct actions on ASM. Ultimately, the failure to reduce (and indeed perhaps exacerbate) inflammation creates the conditions for life-threatening exacerbations.

Thus, monotherapy β_2 agonists may fall short (and lack safety) for effective asthma control in all but patients with very mild disease when as required SABA usage is acceptable. Whether concomitant treatment with ICS addresses the possible neutral or pro-inflammatory effects of β_2 agonists is not clear. Of note, the black box warnings exist for combined (LABA + ICS) therapy as well, suggesting that sufficient evidence of ICS addressing the LABA safety concern does not yet exist.

5 Pharmacogenetics and the β_2AR

Pharmacogenetics is a term referring to the study of genetic factors on efficacy and side effect profiles of drugs. The most common type of genetic variation in the human genome is single nucleotide polymorphisms (SNPs) whereby one nucleotide is different at a given position. The prevalence and functional and clinical significance of SNPs in the β_2AR have received exhaustive scrutiny over the last few decades. Nine SNPs have been found within the β_2AR gene, although only four result in amino acid substitutions because of redundancy in coding for amino acids. The most studied SNP results in a change at the 16th amino acid after the start codon of the β_2AR, Gly16Arg. This was first identified in 1993 by Liggett and colleagues (Reihsaus et al. 1993). Since 1993 there have been extensive efforts to identify all the genetic variation present at the locus of the β_2AR gene (*ADRB2*), resulting in the identification of >50 variants within close proximity to the coding region for the gene. The possible functional effects of most of these variants are unknown, although many are likely to have no functional consequences.

Of the four SNPs within the coding region of the gene which alter the amino acid sequence, one, the valine to methionine 34 substitution (Val34Met) is very rare and does not appear to alter receptor function. Another rare polymorphism is the threonine to isoleucine 164 mutation (Thr164Ile; allelic frequency around 2%). Very few individuals homozygous for this polymorphism have been identified. However, when recombinant approaches in cell based systems are studied, the Thr164Ile variant produces marked alterations in the in vitro behavior of the receptor. Cells transfected with this form of the receptor display reduced agonist binding to catechol ligands and also show altered receptor trafficking (Green et al. 1993).

In contrast, the Arg16Gly and another nearby variant, glutamine to glutamate 27 (Glu27Gln) substitutions are common in the Caucasian population; allele frequencies at each locus are between 0.3 and 0.7 (Litonjua et al. 2004). In in vitro studies, Gly 16 homozygosity results in increased receptor downregulation and homozygosity of Glu 27 in reduced receptor downregulation following agonist stimulation compared with the control "wild type" receptor. Because of linkage disequilibrium between the two SNPs, chromosomes carrying the Gly16 variant are more likely to also have Glu 27 (Dewar et al. 1998; Ramsay et al. 1999).

There have been multiple clinical studies addressing the potential contribution of *ADRB2* polymorphism to both disease risk and clinical response to treatment. In general, although some small studies have found associations between SNPs at *ADRB2* (most frequently the Arg16Gly and/or the Gln27Glu variants) and disease subphenotypes such as bronchial responsiveness, large studies have generally failed to confirm associations (Hall et al. 2006). In keeping with the probable lack of a causal role for *ADRB2* polymorphism in asthma itself was the failure to identify a signal at this locus in the large asthma genome wide association studies which have been performed (Moffatt et al. 2010; Wan et al. 2012).

However, there is more debate about the potential contribution of either individual SNPs at this locus, or combination of SNPs (haplotypes) to treatment response.

Early studies suggested an association between Arg16Gly and treatment response to regular SABA administration. Although not consistent across all studies, several studies have found that frequent or regular administering of SABAs impairs asthma control in patients with the Arg16Arg genotype (Israel et al. 2000, 2004; Taylor et al. 2000; Basu et al. 2009). Two small retrospective cohort studies also suggested that patients with the Arg16Arg genotype had a worse response to salmeterol than patients with the Gly16Gly genotype (Wechsler et al. 2006; Palmer et al. 2006); however, most larger retrospective and prospective studies have not confirmed these results (Bleecker et al. 2006, 2007, 2010; Wechsler et al. 2009).

6 Future Perspectives and Summary

β_2 agonists continue to have a major role in the treatment of airflow obstruction and, despite the concerns over their safety when administered in patients with asthma as monotherapy, when used in combination with inhaled corticosteroids they have proven effective and have a good overall safety record. Improvements in the way these agents are used clinically, and the development of novel agents with altered profiles offers potential to further refine the use of this class of drugs. Whereas at present the widespread use of genetic profiles to dictate treatment strategies for this class of drugs does not seem warranted, it is conceivable that a better understanding of the pharmacogenetics of this drug class may result in stratified approaches to treatment in the future in at least some conditions. The development of more selective agents with longer durations of action will likely continue, although safety concerns will still need to be assessed with each new drug profile, especially given the signals seen in early clinical studies involving full agonists used at relatively high dosage. Finally, opportunities exist to further modify the profile of β_2 agonists to select for biased signalling; whether or not agents with relative selectivity for a given signalling pathway will show clinical benefit over the agents in existing use remains to be explored.

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Muscarinic Receptor Antagonists

Maria Gabriella Matera and Mario Cazzola

Contents

Abstract

Parasympathetic activity is increased in patients with chronic obstructive pulmonary disease (COPD) and asthma and appears to be the major reversible component of airway obstruction. Therefore, treatment with muscarinic receptor

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antagonists is an effective bronchodilator therapy in COPD and also in asthmatic patients. In recent years, the accumulating evidence that the cholinergic system controls not only contraction by airway smooth muscle but also the functions of inflammatory cells and airway epithelial cells has suggested that muscarinic receptor antagonists could exert other effects that may be of clinical relevance when we must treat a patient suffering from COPD or asthma. There are currently six muscarinic receptor antagonists licenced for use in the treatment of COPD, the short-acting muscarinic receptor antagonists (SAMAs) ipratropium bromide and oxitropium bromide and the long-acting muscarinic receptor antagonists (LAMAs) aclidinium bromide, tiotropium bromide, glycopyrronium bromide and umeclidinium bromide. Concerns have been raised about possible associations of muscarinic receptor antagonists with cardiovascular safety, but the most advanced compounds seem to have an improved safety profile. Further beneficial effects of SAMAs and LAMAs are seen when added to existing treatments, including LABAs, inhaled corticosteroids and phosphodiesterase 4 inhibitors. The importance of tiotropium bromide in the maintenance treatment of COPD, and likely in asthma, has spurred further research to identify new LAMAs. There are a number of molecules that are being identified, but only few have reached the clinical development.

Keywords
Antimuscarinic agents • Fixed-dose combinations • Inhaled corticosteroids • β2-agonists

1 Introduction

Airway tone is mainly controlled by the vagus nerve, and the parasympathetic nerves carried in the vagus nerve are tonically active, producing a stable, readily reversible baseline tone of the airway smooth muscle (ASM). Acetylcholine (ACh) is the 'classic' neurotransmitter of the parasympathetic nervous system at both the level of ganglionic transmission and the neuroeffector junctions. ACh acts via activation of muscarinic receptors [reviewed by Cazzola et al. (2012a)].

The distribution of muscarinic receptor subtypes throughout the bronchial tree is mainly restricted to muscarinic M_1, M_2 and M_3 receptors. In humans, M_1 receptors seem to be expressed particularly in peripheral lung tissue and in the alveolar wall, but they have not been detected in larger airways where M_2 and M_3 receptors represent the major population of receptors. Under 'physiological' conditions, the ASM contraction induced by ACh is mediated primarily via the M_3 subtype. In the proximal airways, ACh is released from vagus nerve and activates M_3 receptors present on smooth muscle cells. In the peripheral airways, M_3 receptors are expressed, but there is no cholinergic innervation; these receptors can be activated by ACh released from the epithelial cells that may express choline acetyltransferase in response to inflammatory stimuli (Barnes 2004).

The M_3 receptors are the predominant receptors mediating mucus secretion. They also mediate dilation of airway blood vessels, an action that has been demonstrated to be an endothelium-dependent mechanism.

M_2 receptors couple with adenylyl cyclase via G_i in an inhibitory manner. They functionally oppose the β-adrenoceptor-mediated increase in cAMP, leading to attenuation of β-adrenoceptor-induced relaxation of ASM and prevent activation of Ca^{2+}-dependent K^+ (K_{Ca}) channels. Muscarinic M_2 receptors are expressed by neurons where they function as autoreceptors, inhibiting the release of ACh from both preganglionic nerves and parasympathetic nerve terminals. Further, M_2 receptors are widely expressed by airway fibroblasts.

Parasympathetic activity is increased in patients with chronic obstructive pulmonary disease (COPD), and this appears to be the major reversible component of airway obstruction (Gross and Skorodin 1984). Therefore, treatment with muscarinic receptor antagonists, inhibiting muscarinic receptor activation, is an effective bronchodilator therapy in COPD (Matera et al. 2011). Moreover, cholinergic parasympathetic tone contributes to contraction of bronchial smooth muscle and narrowing of the airways in asthmatic patients. The extent to which increased parasympathetic tone is a consequence of reflex to the inflammatory state or is a pathophysiological mechanism in itself is unclear. Regardless, the raised parasympathetic tone does provide a rationale for the use of antimuscarinic agents in asthma (Price et al. 2014).

2 Muscarinic Receptor Antagonists Currently Used for Treating COPD and Asthma

2.1 COPD

There are currently six muscarinic receptor antagonists licenced for use in the treatment of COPD, the short-acting muscarinic receptor antagonists (SAMAs) ipratropium bromide and oxitropium bromide and the long-acting muscarinic receptor antagonists (LAMAs) aclidinium bromide, tiotropium bromide, glycopyrronium bromide and umeclidinium bromide.

SAMAs have become orphan bronchodilators because in patients with COPD ipratropium bromide 40 μg is less effective than long-acting β_2-agonists (LABAs) (Matera et al. 1995), salmeterol 50 μg elicits a greater peak bronchodilation and longer duration of action than oxitropium bromide 200 μg (Cazzola et al. 1998a) and, even more important, tiotropium 18 μg, the first once-daily LAMA, delivered via HandiHaler is significantly more effective than ipratropium 40 μg four times daily in improving trough, average and peak forced expiratory volume in 1 s (FEV_1) (van Noord et al. 2000) and generally improves lung function to a significantly greater extent than salmeterol in patients with COPD (Donohue et al. 2002).

Although tiotropium bromide binds to all muscarinic receptors, it dissociates much faster from the M_2 mAChRs, which results in a more selective antagonist action for M_1 and M_3 muscarinic receptor subtypes. Its prolonged pharmacologic

activity is the result of its slow dissociation from M_1 and M_3 muscarinic receptors. The half-life of the tiotropium bromide M_3 muscarinic receptor complex is approximately 35 h, compared with 0.3 h for ipratropium bromide. The mechanism allowing for the long residency of tiotropium bromide at M_3 muscarinic receptors is not completely known (Cazzola et al. 2012a). Vauquelin and Charlton (2010) suppose that the complex geometry of micro-anatomic features may restrict the free diffusion of drug molecules away from the local environment where the receptors are concentrated, meaning that freshly dissociated drug is more likely to 'rebind' to the same receptor and/or receptors nearby. The process of rebinding has been suggested to occur at a local tissue level even when drug concentrations in the bulk phase have already dropped to insignificant levels and may explain how LAMAs maintain their 24-h duration of action in the lung, despite their relatively rapid kinetic off rates.

Looking beyond FEV_1, there is solid evidence that tiotropium bromide improves health-related quality of life (HRQoL) and reduces dyspnoea and rescue medication use (Keating 2012). Moreover, it reduces the risk of exacerbations, with a number needed to treat to benefit of 16 to prevent one exacerbation, and also exacerbations leading to hospitalisation (Karner et al. 2012).

The current international guidelines for the treatment of COPD do not made distinction as to which class of bronchodilators should be considered first in patients with moderate-to-very severe COPD (Global Initiative for Chronic Obstructive Lung Disease 2014). Unfortunately, there is no head-to-head randomised controlled trial (RCT) that evaluates all the different monotherapies available, and it is unlikely that such a trial will ever be performed (given the increasing number of options available) (Cope et al. 2013). However, a Cochrane analysis (Chong et al. 2012), which compared the relative clinical effects of tiotropium bromide alone versus LABAs (salmeterol, formoterol and indacaterol) alone in RCTs, reported that tiotropium reduced the number of COPD patients experiencing one or more exacerbations compared with LABAs, with no statistical difference in mortality observed between the treatment groups, and also the number of COPD exacerbations leading to hospitalisation, but not in the overall rate of all-cause hospitalisations. There was no statistically significant difference in FEV_1 or symptom score between tiotropium and LABA-treated participants, but there was a lower rate of nonfatal serious adverse events recorded with tiotropium compared with LABA and a lower rate of study withdrawals. All these findings support the opinion that tiotropium is a reasonable choice for the management of patients with stable COPD (Matera et al. 2014). In effect, LAMAs are recommended as first-line maintenance bronchodilator therapy in patients with stable COPD who have a high risk of exacerbations or more symptoms (Global Initiative for Chronic Obstructive Lung Disease 2014).

The growing evidence that tiotropium bromide is important in the maintenance treatment of COPD has promoted further research to identify new LAMAs that could share some of the beneficial characteristics of tiotropium and perhaps improve upon less desirable ones (Cazzola et al. 2012b; Cazzola et al. 2013a). Glycopyrronium bromide, umeclidinium bromide and aclidinium bromide are

already marketed in many countries for the treatment of COPD. Comparison between different LAMAs is difficult due to differences in study design and lengths.

Glycopyrronium bromide is a LAMA with a rapid onset (5 min) and 24-h duration of action. Compared with the other LAMAs, glycopyrronium has the most favourable ratio of M_3/M_2 receptor residency time, although whether the apparent advantage of glycopyrronium vs. the other agents translates into improved clinical efficacy and safety remains to be established (Cazzola et al. 2015b). Compared with tiotropium, glycopyrronium is 5-fold faster in the in vitro calcium assay and 2.5-fold faster in the rat tracheal strip assay. Simulated kinetic rate constants suggest that tiotropium would take four to five times longer than glycopyrronium to equilibrate with the M_3 receptor at equi-effective concentrations (Sykes et al. 2012). The recommended dose is 50 μg once daily, which is administered via a single capsule loaded into a dry powder inhaler (DPI) (Breezhaler); the delivered dose from this is 44 μg, although in the USA the approved dose is 12.5 μg twice daily. In clinical trials lasting 6–12 months in patients with moderate-to-severe COPD, glycopyrronium improved lung function, reduced breathlessness and improved symptoms, and it reduced moderate-to-severe exacerbations (Buhl and Banerji 2012; Compton et al. 2013). Glycopyrronium also produced immediate and significant improvement in exercise tolerance and had a similar safety profile to tiotropium (Buhl and Banerji 2012; Compton et al. 2013). Therefore, it appears to have the potential for a significant role in the management of COPD.

Umeclidinium bromide delivered once daily via the Ellipta inhaler is an effective and well-tolerated treatment for COPD (Manickam et al. 2014; Segreti et al. 2014). In vitro, umeclidinium displayed subnanomolar affinity for all the cloned human muscarinic receptors. It showed kinetic selectivity for M_3 receptors over M_2 and dissociation from the M_3 muscarinic receptors, which was slower than that for the M_2 muscarinic receptors (half-life values: 82 and 9 min, respectively). Umeclidinium dissociates from the M_2 and M_3 receptors more readily than does tiotropium (about four- and threefold, respectively).

The dose that has been approved by the US FDA and the EMA is 62.5 μg, which is equivalent to 55 μg delivered dose (emitted from the inhaler). There is clinically meaningful increase in FEV_1 at the current approved dose. Results generated by pivotal trials seem to indicate comparable effectiveness between umeclidinium and tiotropium (Manickam et al. 2014; Segreti et al. 2014). Therefore, it could be used as an alternative to LAMAs already in the market, but further trials are needed (Segreti et al. 2014).

Also aclidinium bromide could be used as an alternative to tiotropium. In vitro, aclidinium bromide displayed subnanomolar affinity for all muscarinic receptors and kinetic selectivity for M_3 receptors over M_2 and rapidly associates at recombinant M_3 receptors (2.6 times faster than tiotropium) (Cazzola et al. 2013b). Compared with other long-acting muscarinic receptor antagonists, aclidinium bromide has the advantage of being degraded rapidly and cleared from the circulation within 3 h. Therefore, it is a LAMA with low systemic bioavailability (Cazzola et al. 2013b). The dose approved for use in Europe is 400 μg delivered twice daily via the Genuair inhaler. Interestingly, maximum bronchodilation is achieved

after the first dose and persists over the time. The effect is similar to that observed with tiotropium and formoterol. Aclidinium elicits significant improvements in lung function and HRQoL and reductions in breathlessness, nighttime symptoms and hospitalisations due to severe exacerbations in patients with moderate-to-severe stable COPD compared to placebo (Jones 2013; Ni et al. 2014). Compared to tiotropium, aclidinium does not demonstrate significant differences for exacerbations requiring oral steroids or antibiotics or both exacerbation-related hospitalisations and nonfatal serious adverse events (Ni et al. 2014). Unfortunately, the available data are insufficient and of very low quality in comparisons of the efficacy of aclidinium versus tiotropium. Therefore, long-term, double-blinded phase III trials are needed to assess the real advantages of aclidinium bromide over tiotropium (Cazzola et al. 2013b). In any case, safety database meets regulatory standards and demonstrates aclidinium 400 μg twice daily is well tolerated, safe and effective with a positive benefit/risk profile (Cazzola et al. 2013b).

2.2 Asthma

SAMAs have not proved to be very effective in controlling asthma although may be useful in patients with chronic asthma who develop fixed airway obstruction (Cazzola et al. 1998b). In asthma, SAMAs are recommended as an alternative reliever for the minority of adult patients who experience side effects with SABAs, although SAMAs are not as effective as SABAs (Lougheed et al. 2010). Currently, SABAs are primarily used in the acute asthma setting. There is evidence suggesting that the inclusion of ipratropium in the initial treatment of acute severe asthma may provide greater and more rapid improvement in lung function and may avoid prolonged emergency room treatments and hospitalisation (Gross 2006).

The modest trough FEV_1 improvements following LAMA treatment do not support a therapeutic benefit of these agents in non-inhaled corticosteroid (ICS)-treated patients with asthma (Lee et al. 2015a). However, a recent systematic review that has assessed the efficacy and safety of tiotropium in symptomatic patients with asthma with various levels of severity and therapeutic protocols indicates that tiotropium is noninferior to salmeterol and superior to placebo in patients with moderate-to-severe asthma who are not adequately controlled by low-to-moderate ICS or high doses of ICS plus LABA (Rodrigo and Castro-Rodríguez 2015). Major benefits are concentrated in lung function and, in patients with severe asthma, an increase in control and a decrease in exacerbations. In particular, tiotropium add-on to medium-dose ICS has been shown to provide lung function and symptom scores measured by the seven-question Asthma Control Questionnaire (ACQ-7) improvements that were comparable with those of salmeterol, suggesting that, in patients for whom LABAs may be unsuitable, LAMAs could be a helpful alternative (Kerstjens et al. 2015). Predictors of a positive clinical response to tiotropium include a positive response to salbutamol and airway obstruction, factors that could help identify appropriate patients for this therapy (Peters et al. 2013).

3 Alternative Mechanisms for Muscarinic Receptor Antagonists

In recent years, the accumulating evidence that the cholinergic system controls not only contraction by airway smooth muscle but also the functions of inflammatory cells and airway epithelial cells (Cazzola et al. 2012a) has suggested that muscarinic receptor antagonists could exert other effects that may be of clinical relevance when we must treat a patient suffering from COPD or asthma.

3.1 Muscarinic Receptor Antagonists as Potential Anti-inflammatory and/or Anti-remodelling Therapy

ACh released from nerve terminals and airway cells contributes to inflammation and remodelling of the airways via M_3 receptors [reviewed by Kistemaker and Gosens (2015)]. In vitro studies have indicated that various structural cells in the airways, including epithelial and airway smooth muscle cells, as well as macrophages, release eosinophil and neutrophil chemotactic factors and pro-inflammatory cytokines upon muscarinic receptor stimulation [reviewed by Meurs et al. (2013a)]. Environmental factors, including cigarette smoke, allergens and bronchoconstricting agents, can induce or enhance ACh release and thereby contribute to inflammation and remodelling. Cigarette smoke exposure results in enhanced cytokine release, including interleukin (IL)-8, IL-6 and monocyte chemoattractant protein-1 (MCP-1), and transforming growth factor (TGF)-β release, mediated via M_3 receptors on structural cells. Exposure to allergens also enhances inflammation and remodelling of the airways. Intriguingly, ACh may also be involved in attraction of eosinophils to parasympathetic nerves.

Enhanced goblet cell metaplasia airway smooth muscle thickening and extracellular matrix deposition are mediated via M_3 receptors. Moreover, the muscarinic M_3 receptor appeared to be the primary receptor involved in alveolar macrophage-mediated neutrophil chemotaxis of neutrophils from COPD patients, IL-8 release from epithelial cells and the cooperative secretion of IL-8 in response to cigarette smoke and muscarinic receptor stimulation by airway smooth muscle. ACh contributes to allergen-induced remodelling and smooth muscle mass via the muscarinic M_3 receptor and not via M_1 or M_2 receptors. No stimulatory role for muscarinic M_3 receptors in allergic inflammation was observed, suggesting that the role of acetylcholine in remodelling is independent of the allergic inflammatory response and may involve bronchoconstriction. The documentation that allergen-induced inflammation is not affected by knockout of the M_3 receptor suggests that bronchoconstriction drives airway remodelling, independent of the inflammatory response. Both neuronally released ACh and non-neuronally released ACh contribute to inflammation and remodelling processes in the airways. For these reasons, potential anti-inflammatory and anti-remodelling effects of muscarinic receptor antagonists have been postulated.

Tiotropium is able to inhibit ACh-induced release of leukotriene $(LTB)_4$ from human-isolated lung alveolar macrophages and A549 cells (Buhling et al. 2007), regulate $CD4^+$ and $CD8^+$ apoptosis of peripheral blood T-cells from patients with COPD (Profita et al. 2012) and inhibit pro-inflammatory effects of ACh in neutrophils isolated from patients with COPD (Profita et al. 2005). Tiotropium and 4-DAMP have been shown to inhibit alveolar macrophage-mediated migration of neutrophils from patients with COPD (Vacca et al. 2011). Further, although glycopyrronium does not affect the lipopolysaccharide (LPS)-stimulated tumour necrosis factor (TNF)-α release by itself, it synergistically inhibits the rolipram- and budesonide-induced decrease in TNF-α release from human primary monocytes (Pahl et al. 2006). Anti-inflammatory effects of muscarinic receptor antagonists in the lung have also been reported in several animal models (Meurs et al. 2013b). Recently, it has been documented that aclidinium is associated with a reduced number of neutrophils in the alveolar septa of guinea pigs exposed to cigarette smoke (Domínguez-Fandos et al. 2014).

Tiotropium partially inhibits eosinophilia recruitment in a guinea pig model of asthma (Bos et al. 2007; Buels et al. 2012). In a murine model of airway hyperresponsiveness using ovalbumin sensitisation, IL-4, IL-5 and IL-13 levels measured in bronchoalveolar lavage (BAL) supernatant were decreased (Ohta et al. 2010). In a mouse model of *Aspergillus fumigatus*-induced asthma, aclidinium treatment completely abrogated the methacholine-induced lung resistance and reduced the numbers of eosinophils in BAL fluid with no significant changes in other cell types or in IL-4 or IL-6 levels. The treatment markedly decreased total protein levels in BAL fluid with a correlation with lung injury and capillary leakage indices (Damera et al. 2010).

These experimental findings suggest that LAMAs elicit anti-inflammatory effects, but the importance to the anti-inflammatory effects of muscarinic receptor antagonists is not yet established also because until now, methodological problems complicated the evaluation of airway inflammation in drug studies (Kistemaker and Gosens 2015). A 1-year, double-blind, randomised, placebo-controlled trial in 142 patients with severe COPD demonstrated that tiotropium did not reduce sputum or systemic markers of inflammation but increased sputum IL-8 concentration (Powrie et al. 2007). A clinical evidence for anti-inflammatory effects of tiotropium has been published, recently. A reduction of the superoxide and LTB_4 release was observed in neutrophils from COPD patients treated with tiotropium (Santus et al. 2012).

Inhibitory effects of muscarinic receptor antagonists on airway mesenchymal cell remodelling have been reported in animal models of COPD (Meurs et al. 2013b). Treatment with tiotropium inhibited the increased peribronchial collagen deposition in a guinea pig model of COPD (Pera et al. 2011). Moreover, tiotropium reduced metalloproteinase (MMP)-2 production from lung fibroblasts induced by inflammatory stimulation (Asano et al. 2008), likely through interference of TGF-β-mediated signalling pathways (Asano et al. 2010). Interestingly, it has been documented that aclidinium dose dependently inhibits human lung fibroblast to myofibroblast transition induced by carbachol and TGF-β_1 stimulation

(Milara et al. 2012) and attenuates the cigarette smoke-induced increase in the expression of the myofibroblast markers collagen type I and α-smooth muscle actin (SMA) (Milara et al. 2013). Also tiotropium concentration dependently inhibits the ACh-induced proliferation in both the fibroblasts and myofibroblasts in vitro (Pieper et al. 2007). Similar preventive effects of tiotropium against the increase in collagen synthesis have been detected in human lung fibroblasts when muscarinic receptors are stimulated (Haag et al. 2008). These observations suggest that LAMAs may have anti-remodelling properties in addition to their sustained bronchodilation.

In a guinea pig model of asthma using repeated challenges with ovalbumin, treatment with tiotropium significantly inhibited airway smooth muscle remodelling and inhibited the overexpression of the contractile protein 'myosin' (Gosens et al. 2005). It also significantly inhibited decreased goblet cell metaplasia, smooth muscle thickening and airway fibrosis, as well as decreased T_H2 cytokine levels, including TGF-β levels in BAL fluid in a murine model of asthma (Ohta et al. 2010). Tiotropium bromide treatment for 3 months in a mouse model of chronic asthma had a protective effect against parameters of airway remodelling, including peribronchial fibrosis and smooth muscle thickening (Kang et al. 2012).

3.2 Muscarinic Receptor Antagonists as Potential Mucus-Modifying Therapy

Mucus secretion can be increased predominantly via muscarinic M_3 receptors expressed on the submucosal glands (Rogers 2001). In addition, electrolytes and water secretion are regulated by muscarinic M_1 and M_3 receptors (Ishihara et al. 1992; Ramnarine et al. 1996). In response to ACh, goblet cells also produce mucus (Rogers 2001).

The expression of MUC5AC, which is the predominant mucin gene expressed in healthy human airway epithelial cells, is markedly increased in smokers and COPD and asthmatic patients (Morcillo and Cortijo 2006) and can be induced by carbachol and cigarette smoke extract while being inhibited by aclidinium or atropine (Cortijo et al. 2011). Animal studies show that tiotropium inhibits increased MUC5AC expression and mucus gland hypertrophy in a guinea pig model of COPD (Pera et al. 2011), as well as the allergen-induced mucus gland hypertrophy and MUC5AC-positive goblet cell number (Bos et al. 2007). Furthermore, it inhibits neutrophil elastase (NE)-induced goblet cell metaplasia and mucin production, probably mediated by suppression of inflammation and a direct action on epithelial cells (Arai et al. 2010).

There is evidence of mucociliary clearance impairment after administration of old antimuscarinic drugs (Annis et al. 1976). However, while tertiary ammonium compounds such as atropine may slow mucociliary clearance (Wanner 1986), quaternary ammonium compounds such as ipratropium bromide (Taylor et al. 1986), oxitropium bromide (Miyata et al. 1989) and tiotropium bromide (Hasani et al. 2004) have been reported not to do so.

3.3 Muscarinic Receptor Antagonists as Potential Anti-cough Therapy

Only a few small studies have investigated the effects of muscarinic receptor antagonists on cough and cough reflex sensitivity. Methodological differences make interpretation of these studies difficult.

One small, crossover study evaluated the effect of inhaled ipratropium bromide compared with placebo in postviral cough. A dose of 320 μg/die of ipratropium was found to be effective in suppressing subjectively described cough (Holmes et al. 1992), but a study of cough clearance of radiolabelled particles in COPD found clearance following ipratropium bromide was slower than following placebo (Bennett et al. 1993). Oxitropium bromide, which inhibited the cough response to ultrasonically nebulised distilled water (Lowry et al. 1988), did not offer an effective therapy for cough associated with an upper respiratory tract viral infection (Lowry et al. 1994).

Glycopyrronium inhibited the capsaicin-induced cough reflex in normal volunteers (van Wyk et al. 1994). However, since this inhibition was only significant from 40 min onwards, an interaction with poorly accessible peripheral or even central nervous pathways in the cough reflex was postulated. Nonetheless, Dicpinigaitis et al. (2008) showed that tiotropium is able to inhibit cough reflex sensitivity to capsaicin in subjects with acute viral upper respiratory tract infection. As there were no associated changes in lung function, the authors suggested that the antitussive effects of tiotropium might be through a mechanism other than bronchodilation. It was shown in guinea pig and human tissue that tiotropium can directly inhibit the transient receptor potential V1 (TRPV1) and thereby inhibit the cough reflex via a reduction in airway sensory nerve activity (Birrell et al. 2014). However, (Casaburi et al. 2000) were unable to document any effect of this drug on cough in patients with COPD.

4 Side Effects of Muscarinic Receptor Antagonists

All muscarinic antagonists currently used as bronchodilating agents show high affinity for all muscarinic receptor subtypes, thus increasing the likelihood of unwanted side effects. However, they have a very wide therapeutic margin and are very well tolerated, in part because they are very poorly absorbed after inhalation [reviewed by Cazzola et al. (2012a)]. Dry mouth is the most commonly reported adverse drug reaction induced by these agents that might also worsen the signs and symptoms of narrow-angle glaucoma, if drug were inadvertently deposited in the eye. In older men, who may have prostatic hyperplasia, muscarinic receptor antagonists should be used with caution because they can cause urinary retention.

Concerns have been raised about possible associations of muscarinic receptor antagonists with cardiovascular morbidity and mortality, and a body of evidence supports the possible existence of a link between the use of these agents and

cardiovascular risk, mainly among those with pre-existing arrhythmias (Lee et al. 2008). Stimulation of M_2 receptors, the predominant muscarinic receptor subtype in the heart, mediates negative chronotropic and inotropic effects, and inhibition of M_2 receptors by muscarinic receptor antagonists is responsible for the characteristic tachycardia seen with this class of compound (Cazzola et al. 2013a).

However, the Tiotropium Safety and Performance in Respimat (TIOSPIR) trial, a large international safety study that involved 17,135 patients with COPD for a median duration of 835 days to specifically elucidate the risk of mortality in patients treated with tiotropium Respimat Soft Mist Inhaler (a propellant-free inhaler that delivers a slow-moving mist of aerosolised solution) 2.5 and 5 μg, using tiotropium HandiHaler 18 μg as reference category, documented that both tiotropium Respimat and tiotropium HandiHaler do not increase risk of death or cardiac adverse events, even in patients with coexisting cardiac conditions (Wise et al. 2013). This finding confirms the results of the Understanding Potential Long-Term Impact of Tiotropium on Lung Function Trial (UPLIFT) (Tashkin et al. 2008) and those of a meta-analysis of 30 trials including UPLIFT (enrolling a total of 19,545 participants) (Celli et al. 2010), which documented that tiotropium was associated with reduced rates of death from any cause and from cardiac causes and of cardiovascular events. Pooled analysis of adverse event data from 28 tiotropium HandiHaler 18 μg and 7 tiotropium Respimat 5 μg trials, which totalled 14,909 (12,469 with HandiHaler; 2,440 with Respimat) patient-years of tiotropium exposure, indicates that tiotropium is associated with lower rates of adverse events, severe adverse events and similar rates of fatal adverse events than placebo when delivered via HandiHaler or Respimat (overall and separately) in patients with COPD (Halpin et al. 2015).

However, a systematic review and mixed treatment comparison meta-analysis of randomised controlled trials, which included 42 randomised controlled trials (52,516 patients), indicated that tiotropium Respimat Soft Mist Inhaler is associated with a universally increased risk of overall death compared with any comparator and demonstrates an increased risk of death from cardiovascular causes in comparison with LABA-ICS (Dong et al. 2013).

No issues associated with cardiovascular safety have been identified with the most advanced compounds (aclidinium bromide, glycopyrronium bromide and umeclidinium bromide), suggesting that these compounds may have an improved safety profile (Cazzola et al. 2013a).

5 Combination Therapy of Muscarinic Receptor Antagonists with Other Drugs in COPD and Asthma

Further beneficial effects of SAMAs and LAMAs are seen when added to existing treatments, including LABAs, ICSs and phosphodiesterase (PDE)4 inhibitors.

5.1 LAMA/LABA Combination

Children with an asthma exacerbation experience a lower risk of admission to hospital if they are treated with the combination of inhaled SABAs plus SAMA versus SABA alone (Griffiths and Ducharme 2013). They also experience a greater improvement in lung function and less risk of nausea and tremor. However, no evidence of benefit for length of hospital stay and other markers of response to therapy is noted when SAMAs were added to SABAs (Vézina et al. 2014). Pooled analysis of studies, albeit ones with significant limitations, found little benefit in regard to most physiologic and clinical measures arguing against routine use of combination SAMA and SABA in asthma (Westby et al. 2004). Nonetheless, in the context of asthma, there will be no place for dual LAMA/LABA combination inhalers unless given in conjunction with a separate ICS inhaler (Lipworth 2014).

The regular addition of a LABA to LAMA not only induces a larger bronchodilation than that obtained with only the LAMA in patients with COPD but also significantly improves many patient-reported outcomes (Mahler et al. 2012; ZuWallack et al. 2014).

The main pathways involved in LABA/LAMA interaction are localised at the level of postganglionic parasympathetic neurons and airway smooth muscle cells (Cazzola and Molimard 2010; Meurs et al. 2013b; Pera and Penn 2014). The pharmacological mechanisms that justify the combination of bronchodilators include the activation of postsynaptic β_2-adrenoceptors and presynaptic β_2-adrenoceptors by LABAs and the inhibition of postsynaptic M_2 and M_3 muscarinic receptors by LAMAs. The activation of presynaptic β_2-adrenoceptors and the inhibition of presynaptic M_2 muscarinic receptor may increase the release of acetylcholine into the synaptic space. The crosstalk between the intracellular pathways stimulated by LABAs and those inhibited by LAMAs at the level of airway smooth muscle leads to synergistic bronchorelaxant response. Combining a LABA and a LAMA provides synergistic benefit on airway smooth muscle relaxation of both medium and small human airways (Cazzola et al. 2014, 2015a), which, in turn, may have major implications for the use of LABA/LAMA combinations in the treatment COPD (Cazzola et al. 2015a).

However, the dissimilarities in the onset and duration of action of LABA and LAMA and, in any case, the differences in the devices used for the delivery of these drugs make free combinations uncomfortable and therefore unpredictable, especially if focused on adherence to prescribed treatment (Cazzola and Matera 2014a). It is therefore obvious that there is the need for fixed-dose combinations (FDCs) of bronchodilators in a single inhaler. Several once-daily LABA/LAMA FDCs, including QVA149 (combination of indacaterol and glycopyrronium bromide), vilanterol plus umeclidinium bromide and olodaterol plus tiotropium bromide, have been developed or are in clinical development (Cazzola and Matera 2014a; Matera et al. 2015a, b). Also two twice-daily LABA/LAMA FDCs, aclidinium bromide/formoterol and glycopyrronium bromide/formoterol, are under development (Cazzola and Matera 2014a; Cazzola et al. 2015c).

5.2 LAMA/ICS Combination

Experimental evidence suggests an influence of corticosteroids on muscarinic receptors, signifying the potential of such a type of combination for the treatment of COPD and asthma (Cazzola et al. 2012a). Dexamethasone decreases airway responsiveness to vagus nerve stimulation via two mechanisms: increased M_2 receptor function that results in decreased acetylcholine release and increased degradation of acetylcholine by cholinesterases (Jacoby et al. 2001). Various corticosteroids used in pharmacotherapy of asthma inhibit ACh release by directly binding to organic cation transporters (OCT) 1 and 2, which are members of solute carrier family SLC22, so that part of the acute action of these drugs might be due to interference with this epithelial cholinergic system (Lips et al. 2005).

Unfortunately, studies designed to specifically evaluate the effect of LAMA/ICS combinations on clinical outcomes in asthma and, mainly, COPD are very rare. A small study has shown that in subjects with mild-to-moderate COPD and airway hyperresponsiveness to mannitol, HRQoL and airway responsiveness improved after treatment with ICSs in combination with tiotropium therapy (Scherr et al. 2012). In another study, treatment with tiotropium and budesonide in COPD patients led to significant improvements in the HRQoL status according to SGRQ, dyspnea and lung function, and a reduction in the number of exacerbations over 6 months of treatment (Choi et al. 2007; Um et al. 2007). In any case, a real-life retrospective analysis has shown that compared with controls taking only long-acting bronchodilators either alone or in combination, all-cause mortality was reduced in patients taking LAMA + ICS as dual therapy but not by LABA + ICS (Manoharan et al. 2014).

An increasing body of evidence is supporting the case for a LAMA to be added as an alternative controller to low or moderate doses of ICSs in patients with moderate symptomatic asthma (Chung 2015), particularly in those who have fixed airflow obstruction (Lee et al. 2015b).

5.3 LAMA/LABA/ICS Combination

LAMAs as part of the triple combination (LAMA + LABA + ICS) are widely prescribed in real-life management of COPD, even in patients with mild or moderate COPD severity, although a growing body of evidence suggests that triple therapy is efficacious in patients with more severe COPD, such as those with frequent exacerbations (Cazzola and Matera 2014b). Interestingly, it has been documented that tiotropium plus fluticasone propionate/salmeterol therapy is more effective than tiotropium, salmeterol and fluticasone propionate/salmeterol alone for reducing airway wall thickness in COPD (Hoshino and Ohtawa 2013).

In patients, with severe asthma poorly controlled, on an ICS–LABA combination, the addition of a LAMA provides a modest improvement in lung function and prolongs the time to the first asthma exacerbation (Kerstjens et al. 2012). It has been hypothesised that a contributing factor to exacerbations might be an increase in

afferent sensory nerve activity, resulting in an increase in parasympathetic tone and subsequent bronchoconstriction. If this were the case, treatment with long-acting anticholinergic therapies may attenuate such autonomic effects and provide additional bronchodilation (Price et al. 2014).

All these findings make triple therapy an attractive combination in both COPD and asthma. Therefore, a variety of triple combinations (budesonide/glycopyrronium/formoterol fumarate pMDI, PT010; beclomethasone/formoterol 100/6 μg plus glycopyrronium at dosage of 12.5 or 25 μg, CHF-5993; umeclidinium/vilanterol/fluticasone furoate 62.5/25/100 μg; and glycopyrronium/indacaterol/mometasone) are currently under development (Cazzola and Matera 2014b).

6 LAMA/PDE4 Inhibitors

Combination of a LAMA and a PDE4 inhibitor is suggested for patients with more symptoms, high risk of exacerbation and severe or very severe airflow limitation (Global Initiative for Chronic Obstructive Lung Disease 2014). In effect, roflumilast, a PDE4 inhibitor, was efficacious when used concomitantly with tiotropium bromide over a 6-month treatment period in a RCT, indicating that the benefits of roflumilast plus tiotropium were over and above those achieved with tiotropium alone (Fabbri et al. 2009).

7 LAMA Compounds in Development

The importance of tiotropium bromide in the maintenance treatment of COPD, and likely in asthma, has spurred further research to identify new LAMAs. There are number of molecules that are being identified, but only few have reached the clinical development.

Some other glycopyrronium bromide products are under clinical investigation in several different formulations by several pharmaceutical companies (Cazzola et al. 2012b). A metered-dose inhaler (MDI) of glycopyrronium (GP-MDI; PT001), using a porous phospholipid microparticle technology that has been developed to provide respirable particles in which microcrystals of the drugs are associated with the particles, has been formulated and manufactured (Vehring et al. 2012). It is currently in development as a twice-daily monotherapy for COPD. GP-MDI 36 μg twice daily provided improvements in lung function that were similar to those induced by tiotropium bromide (Orevillo et al. 2011).

SUN-101, formerly EP-101, is a LAMA formulation of glycopyrronium in development for the treatment of COPD. It is delivered using an investigational high-efficiency nebuliser (eFlow). SUN-101 is the first nebulised LAMA in phase 3 development. It has demonstrated comparable efficacy and safety to tiotropium in a randomised, double-blind, crossover study in patients with moderate-to-very severe COPD (Singh et al. 2011).

CHF-5259 is another inhaled formulation of glycopyrronium bromide also delivered using a pMDI (Cazzola et al. 2012b). No data are available to date, but it is likely being developed as twice-daily bronchodilator.

AZD9164 has potential as an inhaled, once-daily LAMA. In a dose-ranging study, AZD9164 100, 400 and 1200 μg caused increases in FEV_1 to peak effects of 12, 17 and 12 % above baseline, respectively, following an initial transient and dose-related fall in FEV_1 and associated increase in mild respiratory symptoms, such as cough (Bjermer et al. 2013). The bronchodilator effect elicited by 400 μg was shown to be superior to tiotropium 18 μg. However, the transient initial, dose-dependent drop in FEV_1 observed with AZD9164, which likely is due to a localised irritant action that is greater in the inflamed lung tissue of patients with COPD than in healthy subjects, makes this molecule unfit for its intended purpose (Jorup et al. 2014), and no further studies have been reported to date.

TD-4208 is under development as a once-daily LAMA. It has a high affinity and long residence time at the M_3 receptor and demonstrates in vitro kinetic selectivity for M_3 over M_2 muscarinic receptor subtype (Steinfeld et al. 2009). TD-4208 is well tolerated and demonstrates significant peak bronchodilation with rapid onset that is sustained over 24 h suggesting a once-daily dosing regimen (Potgieter et al. 2012).

V-0162, TRN-157, CHF-5407, trospium chloride (ALKS27) and QAX028 have been reported to be in clinical development for COPD, but the available data are still too limited (Cazzola and Matera 2014a).

Unfortunately, all muscarinic receptor antagonists under clinical development in pulmonary medicine also retain high affinity for M_2 receptors whose blockade may lead to enhanced ACh release in the airways and trigger bronchoconstriction (Buels and Fryer 2012). Thus, the development of novel muscarinic drugs that inhibit M_3 receptor activity with high efficacy but do not interfere with M_2 receptors function appears to be a very attractive goal. Recent X-ray crystallographic studies with two muscarinic receptor subtypes (M_2 receptor, M_3 receptor) have offered detailed insights into the structural configuration of the orthosteric and allosteric muscarinic binding sites (Kruse et al. 2014). This new structural information should guide the development of novel muscarinic receptor antagonists endowed with a high degree of selectivity for distinct muscarinic receptor subtypes. Such agents could include allosteric (ectopic) agonists and positive or negative allosteric modulators (PAMs or NAMs, respectively) of agonist-induced signalling via specific muscarinic receptor subtypes or bitopic muscarinic ligands which can interact with both allosteric and orthosteric muscarinic receptor sites simultaneously (Kruse et al. 2014).

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Xanthines and Phosphodiesterase Inhibitors

D. Spina and C.P. Page

Contents

Abstract

Theophylline is an orally acting xanthine that has been used since 1937 for the treatment of respiratory diseases including asthma and chronic obstructive pulmonary disease (COPD). However, in most treatment guidelines, xanthines have now been consigned to third-line therapy because of their narrow therapeutic window and propensity for drug–drug interactions. However, lower than

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conventional doses of theophylline considered to be bronchodilator are now known to have anti-inflammatory actions of relevance to the treatment of respiratory disease. The molecular mechanism(s) of action of theophylline are not well understood, but several potential targets have been suggested including non-selective inhibition of phosphodiesterases (PDE), inhibition of phosphoinositide 3-kinase, adenosine receptor antagonism and increased activity of certain histone deacetylases. Although theophylline has a narrow therapeutic window, other xanthines are in clinical use that are claimed to have a better tolerability such as doxofylline and bamifylline. Nonetheless, xanthines still play an important role in the treatment of asthma and COPD as they can show clinical benefit in patients who are refractory to glucocorticosteroid therapy, and withdrawal of xanthines from patients causes worsening of disease, even in patients taking concomitant glucocorticosteroids.

More recently the orally active selective PDE4 inhibitor, roflumilast, has been introduced into clinical practice for the treatment of severe COPD on top of gold standard treatment. This drug has been shown to improve lung function in patients with severe COPD and to reduce exacerbations, but is dose limited by a range side effect, particularly gastrointestinal side effects.

Keywords
Adenosine receptor antagonism • Bamifylline • Doxofylline • HDAC • Phosphodiesterases • PI3δ-kinase • Theophylline

1 Introduction

Theophylline has been in clinical use for more than a century, although it is only during the last 50 years that this drug has been in regular use for the treatment of respiratory diseases. In 1886, Henry Hyde Salter described the efficacious use of strong coffee taken on an empty stomach as a treatment for asthma (Persson and Pauwels 1991). The principal agent in coffee producing the bronchodilator effect observed is the xanthine, caffeine. Theophylline, which is structurally related to caffeine, was first used in 1937 for the treatment of acute asthma by the intravenous route and then in 1940 in combination with ephedrine by the oral route. There are now many studies in the literature describing the effects of theophylline in the treatment of both asthma (Hendeles et al. 1985; Weinberger and Hendeles 1996) and COPD (Ashutosh et al. 1997). Theophylline is presently used in various slow-release formulations to overcome rapid metabolism and maintain constant plasma levels. However, over the last decade, the number of prescriptions being written for theophylline has declined as newer medications have been introduced for the treatment of respiratory disease. This decline has mainly arisen due to concerns raised over the narrow therapeutic window of theophylline, which has typically been classified as being 10–20 μg/ml in plasma to obtain bronchodilation (Hendeles et al. 1985; Weinberger and Hendeles 1996). However, it is now recognised that

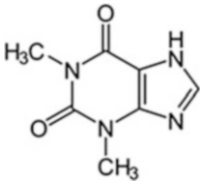
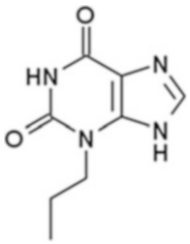
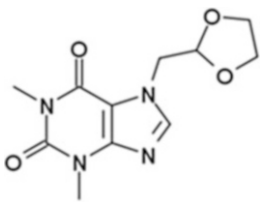
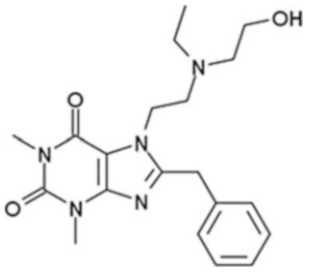
xanthines have other clinically relevant activities beyond bronchodilation, including anti-inflammatory activity and beneficial effects on diaphragm contractility. However, the mechanisms underlying these clinical effects remain poorly understood, although a number of mechanisms have been suggested, including adenosine receptor antagonism (Feoktistov and Biaggioni 1995), activation of ryanodine receptors (Rousseau et al. 1988), non-selective PDE inhibition (Nicholson and Shahid 1994), activation of the cystic fibrosis transmembrane conductance regulator (CFTR) (Chappe et al. 1998), inhibition of phosphoinositide 3-kinase (Foukas et al. 2002), inhibition of poly(ADP-ribose)polymerase-1 (PARP-1) (Moonen et al. 2005) and activation of histone deacetylase 2 activity (Ito et al. 2002). This chapter will provide an update on the pharmacology of xanthines and selective PDE inhibitors in the treatment of asthma and COPD.

2 Mechanism of Action of Theophylline

2.1 Bronchodilation

Theophylline has traditionally been classified as a bronchodilator drug, and concentrations greater than 9–18 μg/ml (50–100 μM) are required to induce a 50% reversal of contraction of isolated bronchial preparations in vitro obtained from otherwise healthy subjects and individuals who died of severe asthma (Finney et al. 1985; Goldie et al. 1986; Cortijo et al. 1993; Rabe et al. 1993). This concentration range is consistent with clinical studies reporting bronchodilation in asthmatic subjects in response to intravenously administered theophylline achieving plasma levels of approximately 10 μg/ml (Clarke et al. 1989). This beneficial action associated with theophylline could potentially be a consequence of a direct relaxation of airway smooth muscle, although the concentrations of theophylline that are required to inhibit known phosphodiesterases are some 1–2 orders of magnitude greater than is achievable safely in the clinic, suggesting that inhibition of any particular isoform of PDE is unlikely to be the primary mechanism of action of xanthines (Table 1). Theophylline is also an antagonist for adenosine receptors, although the potency of this drug for adenosine A_1 and A_{2B} receptors is modest although it is possible that submaximal inhibition of PDE is required to exert this functional effect (see Sect. 3.2) (Table 1). Patients with asthma undergo airway obstruction in response to inhaled adenosine monophosphate (AMP), something not observed in healthy subjects, and we have shown an upregulation of A1 receptors on human airway smooth muscle obtained from biopsies of allergic asthmatic subjects (Brown et al. 2008) and that the A1 receptor is important for adenosine-induced contraction of human airway smooth muscle (Calzetta et al. 2011). Others have suggested that A_{2B} receptors expressed on mast cells may also be a target for xanthines, and in particular, enprofylline has been claimed to be an A_{2B} selective antagonist. In one study, it was demonstrated that theophylline was more effective than enprofylline as a bronchoprotective agent against inhaled AMP, but equally effective against histamine-induced obstruction, thereby ruling out a major role for

Table 1 Examples of xanthines and drug targets

Name	Structure	Known drug targets[a]
Theophylline		A1 receptor (10–30 μM) A2A receptor (2–10 μM) A2B receptor (10–30 μM) A3 receptor (20–100 μM) PDE3 (98 μM) PDE4 (150 μM) PI-3 kinase (100 μM) HDAC1-11 (no effect)
Enprofylline		A1 receptor (42/156 μM) A2A receptor (32/81 μM) A2B receptor (5/10/20 μM) A3 receptor (65/93 μM) PDE1, 2, 3, 4, 5 (>100 μM)
Doxofylline		A1 receptor (>100 μM) A2A receptor (>100 μM) A2B receptor (>100 μM) A3 receptor (>100 μM) PDE2A (50% inhibition at 100 μM) All other PDEs (modest effect > 100 μM) HDAC1-11 (no effect)
Bamifylline		A1 > A2 (562 times)

[a]Values represent affinity constants and inhibitory potency for xanthines against adenosine receptors, phosphodiesterase, PI-3 kinase and HDAC, respectively. The therapeutic window for theophylline is around 10–20 μg/ml which is equivalent to 56–111 μM

adenosine A1 receptor antagonism to explain the antiasthmatic action of this xanthine (Clarke et al. 1989). The suggestion that xanthines might block the stimulatory effect of endogenously released adenosine on mast cell adenosine A_{2B} receptors leading to a reduction of mast cell activation and subsequent beneficial effects on airways obstruction seems unlikely given that neither theophylline nor enprofylline can inhibit acute allergen-induced bronchospasm in subjects with asthma (see Sect. 3.2). Moreover, we have demonstrated that theophylline at clinically effective doses does not inhibit the allergen-induced release of mast cell-derived mediators recovered from

bronchoalveolar lavage fluid (Jaffar et al. 1996). Another possibility is that theophylline might impair afferent neuronal activity thereby reducing airway calibre indirectly (Barlinski et al. 1992) and increasing respiratory drive (Ashutosh et al. 1997).

2.2 Bronchoprotection

The development of the late asthmatic response to antigen challenge was suppressed by theophylline with plasma levels in the range of 8–16 μg/ml following acute intravenous administration (Pauwels et al. 1985) or chronic oral dosing (Crescioli et al. 1991; Ward et al. 1993; Hendeles et al. 1995). In contrast, other studies reported little if any demonstrable protective effect against the late phase response (Cockcroft et al. 1989; Kraft et al. 1996a). These discrepancies could relate to the length of drug exposure and trough plasma levels at the time of the development of the late asthmatic response. Clearly sufficient plasma levels of theophylline were present prior to antigen provocation because baseline responsiveness to methacholine was reduced (Cockcroft et al. 1989; Ward et al. 1993), and modest protection against the immediate antigen-induced bronchoconstriction was observed (Pauwels et al. 1985; Cockcroft et al. 1989; Crescioli et al. 1991) in subjects administered with theophylline. However, not every study has documented protection against antigen-induced increases in airway sensitivity to methacholine (Cockcroft et al. 1989; Crescioli et al. 1991; Hendeles et al. 1995).

In other circumstances, susceptible individuals exposed for long periods of time to certain industrial chemicals also develop asthma-like symptoms that can be duplicated in the clinical laboratory following aerosol challenge with the inciting agent. Following inhalation of toluene di-isocyanate (TDI), an immediate followed by a late asthmatic, response is observed (Mapp et al. 1987). The inflammatory nature of this response has been confirmed by its sensitivity to inhibition by the glucocorticosteroid, beclomethasone dipropionate. In such patients, theophylline partly modified the acute response, yet attenuated the late asthmatic response (Mapp et al. 1987; Crescioli et al. 1992), similar to the observations reported following allergen challenge of allergic asthmatic subjects.

It is recognised that asthmatic subjects are sensitive to various indirect and directly acting contractile stimuli. Adenosine and histamine are commonly used as a provocative agent, and both theophylline and enprofylline when administered acutely produce bronchoprotection against these substances (Clarke et al. 1989). In terms of acute airway obstruction, both xanthines were equally effective against histamine, but it appeared that theophylline was more effective than enprofylline when adenosine was used as the inciting agent. This study shows that adenosine receptor antagonism is unlikely to play a role in bronchoprotection afforded by xanthines in view of the fact that enprofylline has a similar affinity for A_1 and A_{2B} receptors as theophylline (Table 1). However, the degree of bronchoprotection afforded by theophylline is dependent on the nature of the bronchoconstrictor agonist. For example, intravenous infusion of theophylline leading to plasma levels

as low as 6 μg/ml significantly attenuated bronchoconstriction induced by histamine (1.15 doubling doses; DD), but not methacholine (0.8 doubling doses; DD). Consequently, a doubling of the plasma concentration of theophylline afforded a greater degree of protection for histamine (2.6 doubling doses; DD) compared with methacholine (1.9 doubling doses; DD) (Magnussen et al. 1987). Similarly, intravenous infusion with theophylline giving rise to peak plasma levels as low as 6 μg/ml was also sufficient to protect asthmatic subjects from exercise-induced bronchoconstriction (Magnussen et al. 1988).

Chronic treatment with theophylline has been reported to reduce the slope of methacholine dose–response curves in subjects with asthma versus placebo treatment (Page et al. 1998), a change also seen with glucocorticosteroids (Bel et al. 1991a), but not with beta$_2$-adrenoceptor agonists (Bel et al. 1991b), which actually steepen the curve. Thus, airway reactivity to indirectly or directly acting substances either released within the airway wall or inhaled from the environment (e.g. pollutants, fog, cold air) can be suppressed following chronic treatment with theophylline. This beneficial action of theophylline is unlikely to be explained solely by a direct action on airway smooth muscle, and additional mechanisms including inhibition of afferent neuronal activity (Barlinski et al. 1992), thereby leading to inhibition of bronchospasm mediated by reflex activation of cholinergic pathways, are likely to contribute to the clinical benefit afforded by theophylline. Additionally, theophylline has a range of other pharmacological effects of potential therapeutic value in the treatment of respiratory diseases that occur independently of its bronchodilator actions, including anti-inflammatory and immunomodulatory actions (Spina et al. 1998; Page and Spina 2011).

2.3 Anti-inflammatory Actions

The late asthmatic response is often associated with the recruitment of inflammatory cells to the airways including eosinophils (De Monchy et al. 1985) which can be significantly reduced following 4–6 weeks of treatment with theophylline (Sullivan et al. 1994; Lim et al. 2001). Importantly, this anti-inflammatory effect occurred at plasma levels of theophylline below the conventional 10 μg/ml plasma levels required for bronchodilation. Not only was the number but also the activation status of eosinophils reduced following 6 weeks of treatment with theophylline, as evidenced by a reduction in the number of cells expressing EG2, a marker used to indicate that cells were actively secreting eosinophil-derived cationic protein (Sullivan et al. 1994). A significant reduction in the number of eosinophils present in the airways of mild stable asthmatic subjects was observed at plasma levels of theophylline (6 μg/ml) that was insufficient to alter pulmonary lung mechanics (Lim et al. 2001). Similarly, various surrogate markers of nasal inflammation in response to allergen challenge including the late phase response and the accumulation/activation of eosinophils in the nose were significantly attenuated in allergic rhinitis subjects following chronic treatment with theophylline (Aubier et al. 1998).

Regular theophylline treatment has also been demonstrated to produce anti-inflammatory activity in subjects with natural exacerbations of their asthma, in the form of nocturnal asthma. Theophylline treatment significantly improved the overnight deterioration in lung function associated with nocturnal asthma compared with placebo treatment (Kraft et al. 1996b), a finding consistent with previous studies using theophylline for the treatment of asthma (D'Alonzo et al. 1990). Chronic treatment with theophylline also protected individuals from nocturnal falls in baseline FEV1 and was associated with a reduction in the number of neutrophils migrating into the airways of these subjects (Kraft et al. 1996b). The magnitude of inhibition of neutrophil recruitment to the airways was correlated with plasma concentrations of theophylline (12–24 μg/ml), and moreover, the ability of these neutrophils to release leukotriene B_4 stimulated with a calcium ionophore in culture ex vivo was suppressed in these patients. This indicated that theophylline could inhibit not only the migration of cells to the airways but also their ability to be activated once recruited to the lung. This is consistent with an anti-inflammatory action of theophylline (Spina et al. 1998; Page and Spina 2011) and supports earlier work that regular treatment with theophylline can reduce neutrophil activation (Nielson et al. 1986, 1988). In contrast, withdrawing theophylline from asthmatics who were taking glucocorticosteroids resulted in a significant deterioration of their disease together with a concomitant rise in the number of $CD4^+$ and $CD8^+$ T lymphocytes in bronchial biopsies (Brenner et al. 1988; Kidney et al. 1995). The anti-inflammatory effects of theophylline have also been documented in respiratory diseases like COPD. Various indices of inflammation were significantly reduced, including sputum number of neutrophils (40%), IL-8 (24%) and myeloperoxidase (31%) at plasma levels of theophylline between 9 and 11 μg/ml (Culpitt et al. 2002).

Studies in paediatric asthma have shown that there is a clear effect of theophylline in the treatment of asthma that is comparable to low doses of glucocorticosteroids following 1 year of treatment (Tinkelman et al. 1993). This observation is of particular interest given that theophylline is an orally active drug that has been shown to have a better compliance than inhaled medications (Kelloway et al. 1994). Given the low cost of theophylline, relative to other antiasthma medications (Barnes et al. 1996) and the fact that it is still one of the few drugs available for use orally in the treatment of this common disease, the growing body of evidence suggesting that theophylline is anti-inflammatory and immunomodulatory at lower than conventional plasma levels suggests that this drug should still have a greater role in the treatment of asthma and COPD than it currently enjoys.

Numerous studies have documented the immunomodulatory activity of theophylline on T lymphocytes which might explain the beneficial action of this drug in allergic asthma in view of the central role T lymphocytes play in this disease (Lloyd and Hessel 2010). Indeed, regular treatment with theophylline has also been reported to inhibit the recruitment of T lymphocytes into the airway following antigen challenge (Jaffar et al. 1996). Theophylline also inhibited the activation status of T lymphocytes as evident by a reduction in the expression of various

markers on $CD4^+$ T lymphocytes including HLA-DR and VLA-1 (Jaffar et al. 1996). Similarly, the number of $CD4^+$ T lymphocytes and IL-4- and IL-5-containing cells was lower in bronchial biopsies from asthmatic subjects who were prescribed theophylline over a 6-week period compared with placebo (Finnerty et al. 1996), and a fall in circulating levels of the Th2 cytokines, IL-4 and IL-5, was observed following a single low dose of theophylline (Kosmas et al. 1999), and a fall in circulating levels of Th2 cytokines, IL-4 and IL-5, was observed following a single low dose of theophylline (Kosmas et al. 1999).

2.4 Diaphragmatic Contractility

It has been suggested that xanthines can increase diaphragmatic contractility and reduce the effect of diaphragmatic fatigue which is associated with improvement in symptoms in patients administered these drugs via an action at this extrapulmonary site. However, the effect of intravenously administered aminophylline (theophylline ethylenediamine) at plasma levels achieved during oral treatment with theophylline (10–20 μg/mL) on diaphragmatic contractility is controversial with some studies demonstrating modest improvement (Aubier et al. 1981; Supinski et al. 1984; Murciano et al. 1987; Wanke et al. 1994; Gauthier et al. 1995), whilst others have demonstrated no effect (Moxham et al. 1985; DeGarmo et al. 1988; Levy et al. 1990). It is possible that this discordance might be a consequence of the different methods used to stimulate and record diaphragm contractility, the small number of subjects recruited and perhaps the bias introduced by lack of blinding and randomisation in some of these studies. It is likely that the modest effects reported in these healthy subjects may underestimate any potential beneficial action observed in disease. For example, the improvement in diaphragmatic activity in healthy subjects was best observed in situations when functional residual capacity was intentionally increased coupled with the induction of diaphragmatic fatigue in these subjects via strenuous exercise (Wanke et al. 1994; Gauthier et al. 1995). Very few studies have addressed this issue in subjects with respiratory diseases. For example, in subjects with severe COPD, treatment with theophylline resulted in a 16% increased trans-diaphragmatic pressure and decreased respiratory muscle fatigue after resistive load breathing (Murciano et al. 1984), and imaging techniques have also provided evidence of increased diaphragmatic activity in COPD subjects treated with theophylline (Etlik et al. 2004). In contrast, there was no significant increase in diaphragmatic activity, nor any improvement in diaphragmatic fatigue during periods of severe dyspnea in patients with moderate to severe COPD who were predominantly emphysematous (Kongragunta et al. 1988), and no dose–response relationship was established in patients with mild to moderate COPD treated with theophylline on diaphragmatic contractility (Foxworth et al. 1988).

The mechanisms of any purported increase in contractility were suggested to be due to increased calcium mobilisation within the diaphragm skeletal muscle that was sensitive to verapamil and was proposed to involve an A1 receptor-dependent

mechanism because enprofylline was without effect on diaphragmatic activity in healthy subjects (Aubier and Roussos 1985; Murciano et al. 1987).

2.5 Dyspnea and Gas Trapping

Theophylline can improve baseline measures of lung function in subjects with airway obstruction (Mahler 1987) and in addition have other actions which may contribute to improvement in symptoms in these patients. For example, acute administration of intravenous aminophylline in healthy subjects increases ventilatory drive (Gorini et al. 1994), although this was not observed in a study of subjects with mild to moderate airflow obstruction (Gigliotti et al. 1987). The effect of theophylline on respiratory drive appears to be mediated by a direct action on the brainstem and via antagonism of adenosine A1 receptors (Eldridge et al. 1985). There is consistent evidence showing that treatment with theophylline can lead to a reduction in gas trapping (Mulloy and McNicholas 1993; Waterhouse et al. 1993) and dyspnea (Mahler 1987; Murciano et al. 1989).

3 Molecular Targets for Xanthines

3.1 Adenosine Receptor Antagonism

Theophylline and enprofylline are antagonists of adenosine receptors with affinities against the human cloned adenosine A_1, A_{2B} and A_3 receptors in the micromolar range (Table 1), levels that can be achieved clinically. It has been argued that the similarity in clinical effectiveness between theophylline and enprofylline against early and late phase asthmatic responses (Pauwels et al. 1985), and against histamine, but not adenosine-induced bronchoconstriction (Clarke et al. 1989), suggested that adenosine receptor antagonism could not account for the bronchoprotective action exhibited by the xanthines. It was reported that both theophylline and enprofylline inhibited mediator secretion from human lung mast by acting as a selective adenosine A_{2B} receptor antagonist and with affinities in the order of 1.8 μg/ml (Feoktistov and Biaggioni 1995). However, whilst asthmatic subjects are very sensitive to inhaled adenosine, an effect that is blocked by theophylline and enprofylline, it appeared that theophylline was more effective than enprofylline in causing bronchoprotection against inhaled adenosine at plasma levels which caused a similar degree of bronchodilation (Clarke et al. 1989).

The possibility that theophylline might interfere with adenosine A_1 receptor signalling has largely been ignored. Experimental animals suggest that it is the adenosine A_1 receptor that is upregulated following allergic sensitisation (El Hashim et al. 1996) and is consistent with the ability of an antisense oligonucleotide to adenosine A_1 receptors to inhibit eosinophilia and BHR in allergic rabbits (Nyce and Metzger 1997) and inhibit vagal reflex activation of tracheal smooth muscle in response to adenosine (Reynolds et al. 2008). Human studies

have revealed that isolated bronchial airways from asthmatic subjects (Bjorck et al. 1992) and passively sensitised human bronchial tissue (Calzetta et al. 2011) contract in response to adenosine via an adenosine A_1 receptor-dependent mechanism. This is consistent with the increased expression of these receptors in the epithelium and airway smooth muscle in biopsies obtained from mild asthmatic subjects (Brown et al. 2008). Anecdotally, bamifylline, another xanthine that has been used clinically in Europe for the treatment of respiratory disease, is 562 times more selective at adenosine A_1 vs. A_2 receptors (Table 1). However, in contrast, another clinically effective xanthine, doxofylline, appears to lack adenosine receptor antagonism (van Mastbergen et al. 2012) (Table 1).

Paradoxically, inhibition of adenosine A_{2A} receptor signalling could potentially worsen inflammation, but current evidence supports the view that theophylline is predominantly anti-inflammatory and so any clinically relevant effect of xanthines on adenosine receptors remains inconclusive.

3.2 PDE Inhibition

Theophylline has long been recognised as a non-selective PDE inhibitor, and this molecular action has been suggested to contribute to the effectiveness of this drug clinically and to account for many of the side effects of xanthines (Nicholson et al. 1991). However, it is now recognised that there is an ever-growing family of PDE enzymes, most of which are not sensitive to inhibition by theophylline at plasma levels that can be safely achieved clinically. Nonetheless, the pharmaceutical industry has expended considerable effort in developing selective PDE inhibitors because of the narrow therapeutic window of theophylline, and these newer selective inhibitors are discussed below (see Sect. 4) and the comparison with theophylline has been reviewed elsewhere (Boswell-Smith et al. 2006a). Currently, the main focus is on developing PDE4 inhibitors which target inflammatory cells (Abbott-Banner and Page 2014; Page 2014), PDE3 inhibitors which primarily target airway smooth muscle (Abbott-Banner and Page 2014; Page 2014) or dual PDE3/4 inhibitors such as RPL 554 which achieve both bronchodilation and anti-inflammatory activity in the same molecule (Franciosi et al. 2013) (see Sect. 4).

Plasma concentrations of theophylline required to achieve 50% inhibition of PDE enzymes only occurs above 18 μg/ml, and theophylline is a relatively poor inhibitor of the known PDEs. There are now known to be at least 11 gene families of PDEs that are capable of metabolising the intracellular signalling molecules, cyclic AMP and cyclic GMP, thereby finely controlling their intracellular levels. Of particular relevance to respiratory diseases is the presence of PDE3 in airway and vascular smooth muscle and PDE4 in inflammatory cells. In homogenates of human bronchus, PDE3, PDE4 and PDE5 are present in equal abundance, and theophylline has been demonstrated to inhibit cyclic AMP PDE activity by about 40% at a plasma levels equivalent to 18 μg/ml and, at this concentration, caused relaxation of spontaneously contracted human bronchial tissue 60% below baseline tone (Rabe et al. 1995). This is consistent with clinical data showing a 10% improvement in

baseline FEV1 with plasma levels of theophylline around 12 μg/ml in atopic asthmatic subjects (Clarke et al. 1989). However, the potency of theophylline diminishes depending on the spasmogen used to contract tissue. For example, the relaxant potency of theophylline in tissues obtained from non-diseased or severe asthmatic subjects and precontracted with the muscarinic agonist, carbachol (50% maximum response), was 50 μg/ml (Goldie et al. 1986) which is consistent with a greater degree of protection seen with intravenous theophylline in asthmatic subjects undergoing airway obstruction in response to histamine compared with methacholine (Magnussen et al. 1987). Similarly, the contraction of passively sensitised human bronchial tissue to antigen was reduced by an order of magnitude with a concentration equivalent to 54 μg/ml (Schmidt et al. 2000) and is consistent with the relatively modest protection observed in clinical studies against the early asthmatic response (see above). The functional antagonism of antigen-induced contractions of airway smooth muscle was not observed with the adenosine receptor antagonist 8-phenyltheophylline which lacks PDE inhibitory activity at the concentrations employed, thereby ruling out a role for endogenously released adenosine in mediating contractions of passively sensitised human airway smooth muscle in vitro in response to antigen (Schmidt et al. 2000).

Whilst many of the biological effects of theophylline have been attributed to inhibition of the PDE family of enzymes, the effect of theophylline on apoptosis of eosinophils was not shared by the selective PDE4 inhibitor rolipram, suggesting that this anti-inflammatory action of theophylline may be independent of PDE4 inhibition (Yasui et al. 1997), although this is not a universally accepted finding (Ohta and Yamashita 1999; Takeuchi et al. 2002). Nevertheless, this observation supports other recent work carried out in mononuclear cells obtained from asthmatic subjects since theophylline was able to inhibit mononuclear cell proliferation via mechanisms distinct from selective PDE4 inhibitors (Banner et al. 1999), and other data with the related xanthine, pentoxifylline, has demonstrated that this drug can inhibit proliferation of fibroblasts via a mechanism unrelated to the generation of cyclic AMP (Peterson et al. 1998). To what extent these non-PDE-mediated effects contribute towards the clinical efficacy of theophylline remains to be established.

3.3 Phosphoinositide 3-Kinase

Phosphoinositide 3-kinase (PI 3-kinase) belongs to a family of lipid kinase enzymes of which there are four classes (IA, IB, II and III) based on their in vitro substrate specificities and their activation by various receptors (e.g. cytokine, growth hormone, Toll, G-protein coupled). Class IA and IB kinases phosphorylate phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P2) in the 3 position to give rise to PtdIns(3,4,5)P3 which triggers a cascade of intracellular events involved in metabolism, cell survival, migration, growth and proliferation which are characteristics of diseases like cancer and inflammation. As an example, the generation of PtdIns(3,4,5)-triphosphate (PIP3) leads to the translocation of

Akt/PKB and subsequent binding by virtue of the plekstrin homology (PH) domain of PIP3 on the inner leaflet of cell membranes. A similar interaction between 3′-phosphoinositide-dependent kinase-1 (PDK1) results in the phosphorylation and subsequent activation of Akt/PKB. As mentioned previously, one consequence of the activation of PI-3 kinase is the formation of PIP3, resulting in the translocation of Akt/PKB to the cell membrane and subsequent phosphorylation of Akt/PKB on threonine^{308} and serine^{473} by PDK1 and PDK2, respectively. Theophylline inhibited insulin-induced phosphorylation of Akt/PKB in rat soleus muscle and CHO cells with an IC50 value of 100 and 500 μM, respectively, showing that natively expressed PI-3 kinase can be inhibited by theophylline (Foukas et al. 2002).

An example of a downstream target of Akt/PKB which is of relevance to airway inflammatory disease includes IkB kinase which subsequently phosphorylates NF-kB, a pro-inflammatory transcription factor (Wymann et al. 2003). Inactivation of either the δ or γ isoform of PI-3 kinase did not affect inflammation in the airways in mice exposed to cigarette smoke (Marwick et al. 2009), although these enzymes appeared to contribute to the inflammatory response in other models (Hirsch et al. 2000; Ali et al. 2004; Puri et al. 2004; Lee et al. 2006).

Theophylline inhibits the enzyme activity of human recombinant PI-3 kinase with an IC50 value of 75, 300 and 800 μM for the class IA heterodimers p85α/p110δ, p85α/p110α and p85α/p110β, respectively, and 800 μM for the class IB monomeric p110γ PI-3 kinase. Whether the IC50 value against PI-3 δ isoform would be achieved with plasma levels of theophylline between 10 and 20 μg/ml remains a subject of debate; however, the sensitivity of this enzyme to theophylline may be increased by an order of magnitude under inflammatory conditions and therefore might well be a target for theophylline (To et al. 2010). The inhibitory effect of theophylline against PI-3 kinase is not observed with enprofylline and suggests clear structure–activity relationship amongst xanthines for this target (Foukas et al. 2002).

3.3.1 HDAC

A significant improvement in a number of clinical variables was observed in asthmatic subjects who were poorly controlled on existing glucocorticosteroid therapy, following concomitant treatment with theophylline compared with increasing the dose of the glucocorticosteroid (Evans et al. 1997; Ukena et al. 1997). In both studies, the plasma levels of theophylline were unlikely to be sufficient to induce bronchodilation. In contrast, withdrawing theophylline from asthmatics who were taking glucocorticosteroids resulted in a significant deterioration of their disease together with a concomitant rise in the number of $CD4^+$ and $CD8^+$ T lymphocytes in bronchial biopsies (Brenner et al. 1988; Kidney et al. 1995). These results suggest that theophylline may offer additional benefit to glucocorticosteroids as has been previously suggested from other clinical studies by the use of different types of protocol (Brenner et al. 1988; Kidney et al. 1995; Ukena et al. 1997).

A novel mechanism was proposed that might explain the apparent beneficial effect of theophylline in severe asthmatic subjects taking glucocorticosteroids and for the relative ineffectiveness of glucocorticosteroids in the treatment of COPD, which involved the activation of a family of nuclear proteins regulating gene transcription (Ito et al. 2002). At a molecular level, the rate of gene transcription can be controlled by transcriptional coactivators (e.g. p300, CBP) which link transcription factors to RNA polymerase II complex via protein–protein interactions. Both p300 and CBP possess intrinsic histone acetyltransferase (HAT) activity resulting in the acetylation of lysine on the N-terminal regions of histone leading to a neutralisation of positive charge, resulting in the remodelling of chromatin and unwinding of DNA resulting in a more favourable environment for gene transcription. Conversely, gene transcription can be silenced by a family of proteins called histone deacetylases (HDACs) which deacetylate lysine residues in chromatin, thereby silencing gene transcription (Barnes 2011; Shakespear et al. 2011). Theophylline and enprofylline at concentrations between 1 and 10 μM increased the activity of HDAC, whilst concentrations above this value inhibited the activity of this enzyme (Ito et al. 2002). A functional consequence of these low doses of theophylline was a suppression of IL-8 release when administered together with low concentrations of dexamethasone and may explain the clinical observations of the deterioration in asthma symptoms following removal of theophylline from patients taking glucocorticosteroids (Ito et al. 2002). It was proposed that theophylline might act as an allosteric activator of HDAC, although this is unlikely as there is no evidence to support direct interaction with this protein (van Mastbergen et al. 2012) (Table 1), or by inhibition of the activity of PI-3 kinase as this coincides with an increase in HDAC2 activity and restoration of glucocorticosteroid sensitivity. Adenosine receptor antagonism and inhibition of cyclic AMP metabolism did not account for the ability of theophylline to increase HDAC activity (Ito et al. 2002). Further studies showed that the ability of theophylline to restore glucocorticosteroid sensitivity was dependent on the activation of the p110δ isoform of PI-3 kinase (Marwick et al. 2009; To et al. 2010). It appears that the inhibitory activity of theophylline is increased by 65-fold in cells exposed to oxidant stress which would explain why low concentrations of theophylline both in vitro and in vivo can augment HDAC2 activity (To et al. 2010).

To summarise, it is proposed that oxidant stress activates PI-3 kinase, a cell membrane localising protein which leads to the subsequent phosphorylation of downstream signalling molecules (e.g. Akt/PKB), although the precise molecular details have yet to be established. For example, Akt/PKB has been shown to translocate to the nucleus and promote phosphorylation of p300 with subsequent increased gene transcription (e.g. adhesion protein, ICAM-1 in response to TNF alpha) (Huang and Chen 2005), although it is unclear whether Akt or PI-3 kinase can phosphorylate HDAC2. Casein-dependent kinase II (CK2) is also activated during oxidant stress and directly phosphorylates HDAC2 which is located in the nucleus. Activation of CK2 promotes loss of deacetylase activity and reduces

transrepression, loss of HDAC2 protein and steroid resistance (Tsai and Seto 2002; Adenuga et al. 2009; Adenuga and Rahman 2010).

3.4 Other Potential Mechanisms of Action of Xanthines

Oxidative stress is also known to cause the activation of the enzyme poly (ADP-ribose) polymerase (PARP-1) resulting in the metabolism of NAD^+, and a reduction in the level of this cofactor can lead to cell death. It appears that theophylline can inhibit the activity of this enzyme with an IC50 value of 200 μM (36 μg/ml), but whether this nuclear enzyme is the target for the anti-inflammatory action of theophylline remains to be established (Moonen et al. 2005).

Another target that has been proposed is the activation of small and intermediate conductance calcium-activated potassium channels at concentrations as low as 100 nM of theophylline have been shown to affect the former channel subtype (Dubuis et al. 2014). The activation of these channels leads to membrane hyperpolarisation and has been suggested to be a mechanism contributing to the antitussive properties of theophylline. Whether this mechanism also contributes to the ability of theophylline to suppress BHR or for the anti-inflammatory actions of this drug is not yet known. However, it is unlikely that this mechanism accounts for the anti-inflammatory activity of theophylline on T cells since it has been demonstrated that the opening of these channels leads to T-cell activation (Feske et al. 2012).

4 Selective Phosphodiesterase Inhibitors

Phosphodiesterase (PDE4) enzymes are a subclass of the wider PDE gene family which specifically metabolise the cyclic nucleotide, cyclic AMP. It is now recognised that there are four PDE4 subtypes (PDE4A-D) and over 25 splice variants. The expression of some of these isoforms has been demonstrated in many of the inflammatory cells of relevance to asthma and COPD, and the cell and molecular biology of the PDE4 family has been extensively reviewed elsewhere (Page and Spina 2012; Maurice et al. 2014).

There are many preclinical reports that PDE4 inhibitors are able to suppress the activation of a variety of inflammatory cells and to exhibit anti-inflammatory actions in vivo. As a consequence a number of highly potent PDE4 inhibitors have entered into clinical development for the treatment of asthma and COPD. However, despite their potency, a number of selective PDE4 inhibitors including RP 43701 (piclamilast) failed to suppress bronchospasm caused by allergen challenge in asthmatic subjects when administered as a single oral inhaled dose (Jonker et al. 1996), whilst multiple dosing of oral inhaled PDE4 inhibitors including GSK256066 (Singh et al. 2010; Watz et al. 2013), UK-500,501 (Phillips et al. 2007; Vestbo et al. 2007) and CHF6001 (Singh et al. 2016) has demonstrated

only modest and in some case no beneficial effects in various clinical studies in patients with asthma and COPD. This is unlikely to be due to a failure of achieving the appropriate degree of local pharmacodynamically active drug concentrations, since in one study specifically designed to explore this, the investigators found that there was evidence of local changes in cyclic AMP signalling within the airways (Watz et al. 2013). Furthermore, these newer inhaled PDE4 inhibitors were designed with long duration of action in mind had retention within the airways, with minimal systemic exposure in an attempt to reduce the side effects associated with earlier oral formulations of PDE4 inhibitors.

Why these highly potent PDE4 inhibitors were not more effective when inhaled is not understood as these observations stand in contrast to the evidence of anti-inflammatory activity as assessed by various indices including suppression in the magnitude of the late phase response or diminution in the number of inflammatory cells within the microenvironment of the lung following treatment with oral formulations of PDE4 inhibitors. Thus, a number of orally active PDE4 inhibitors including CDP840 (Harbinson et al. 1997) and cilomilast (Compton et al. 2001; Gamble et al. 2001; Gamble et al. 2003; Rennard et al. 2008) demonstrated positive anti-inflammatory effects, but development of these drugs was halted either due to perceived lack of clinical effectiveness or tolerability issues. As with other PDE4 inhibitors, however, roflumilast was able to suppress inflammation in the lungs of patients with COPD (Grootendorst et al. 2007) and to suppress the late asthmatic response (Gauvreau et al. 2011). These early clinical studies were followed by a number of phase III clinical trials that demonstrated improvements in lung function, even in those patients with moderate to severe COPD prescribed bronchodilators as standard care (Rabe et al. 2005; Fabbri et al. 2009). These and other studies have also suggested that roflumilast is able to reduce rates of exacerbation in patients with COPD, and the drug has now been approved in Europe and the USA. A summary of the clinical studies with roflumilast has recently been reviewed (Cazzola et al. 2016). The REACT trial specifically designed to investigate the effect of roflumilast on exacerbations and hospitalisation in patients treated with roflumilast for 1 year confirmed a reduction in the frequency of severe exacerbation of 13% and of hospitalisation of 26%, the latter despite patients receiving maintenance bronchodilators and glucocorticosteroid treatment (Martinez et al. 2015).

It is not understood why PDE4 inhibitors administered by the oral inhaled route despite being highly potent and with long effect duration produce such modest beneficial effects in respiratory disease patients that with the exception of CHF6001 (Singh et al. 2016) has led to the abandonment of inhaled PDE4 inhibitors for the treatment of asthma and COPD. The reason why oral administration of the PDE4 inhibitor roflumilast demonstrates clinical effectiveness might be due to several reasons. Firstly, an oral drug may reach sites of the diseased lung which are not accessible by the inhaled route, and a recent study has shown that the beneficial action of roflumilast against lung dynamic hyperinflation is likely due to an anti-inflammatory action in the distal airways as measured using functional respiratory imaging (De Backer et al. 2014; Vos et al. 2016). Secondly, roflumilast has a particularly interesting pharmacokinetic profile which gives rise to long duration

of PDE4 inhibition. Roflumilast is 79% orally bioavailable and subject to first-pass metabolism by CYP3A4 and CYP1A2 to the active N-oxide and both exhibit high plasma protein binding (99 and 97%, respectively). Roflumilast is approximately three times more potent than the N-oxide. The plasma median half-life of roflumilast and the N-oxide is approximately 23 and 25 h with the AUC of the metabolite 12 times greater than roflumilast following a single oral dose of roflumilast in healthy volunteers (Tenor et al. 2011). This favourable pharmacokinetic profile would be anticipated to produce long periods of PDE4 inhibition. It has been calculated that 90% of the total inhibitory PDE4 activity is accounted by the N-oxide (Tenor et al. 2011). Hence, these non-PDE inhibitory factors probably explain to a greater extent the reason for the clinical beneficial action of roflumilast over other PDE inhibitors (oral or inhaled). It is also possible that roflumilast may be exerting its beneficial actions via a systemic effect in suppressing cytokine production and or cell activity in the plasma before these mediators and cells reach the lung. Indeed, it has been shown that roflumilast can reduce the incidence of major adverse cardiovascular events which include death from a cardiovascular event, nonfatal myocardial infarction and nonfatal stroke (White et al. 2013). Of interest is that these major adverse events are likely caused by systemic inflammation (e.g. elevated levels of IL-6, C-reactive protein and TNF alpha), and whilst these systemic markers were not measured, one might speculate that roflumilast is having an impact on vascular inflammation which would otherwise spill over into the lung and contribute to the pathophysiology of COPD (White et al. 2013). Other evidence for a systemic action of roflumilast arises from studies showing a beneficial effect of roflumilast on hyperglycaemic control (Wouters et al. 2010).

However, despite the selectivity of roflumilast for PDE4, this has not resulted in loss of side effects associated with the non-selective PDE inhibitors such as xanthines (see later). Based on a large clinical database, it is now clear that the use of roflumilast is also associated with a number of unwanted effects including nausea and gastrointestinal discomfort and, in some patients, significant weight loss, although the cause of this problem is not yet understood. The use of roflumilast though is not associated with cardiovascular side effects or increased risk of infection, and a number of reviews have discussed the tolerability of roflumilast in more detail (Page and Spina 2012; Page and Cazzola 2014; Wedzicha et al. 2016). It is perhaps surprising that an anti-inflammatory drug like roflumilast is not associated with increased risk of infection given its inhibitory effect on numerous cell types responsible for innate defence. Several conflicting reports in preclinical models of bacterial infection reported that PDE4 inhibitors either increased *Pseudomonas aeruginosa* burden (Kasetty et al. 2016) or reduced damage of airway epithelium in cell cultures with this bacterium (Dowling et al. 1997). Other studies reported that roflumilast had no effect on *Streptococcus pneumoniae* burden, but did improve clearance when combined with an antibiotic (Tavares et al. 2016) and reduced clearance of *Klebsiella pneumoniae* (Soares et al. 2003). Irrespective of these preclinical findings, it is reassuring that PDE4 inhibitors are shown to reduce the incidence of exacerbations and hospitalisations and reduce

Table 2 Examples of different theophylline formulations and absorption characteristics

Formulation	Dose (mg)	Comment
Slo-bid Gyrocap[a,c]	50–300	Cmax ~ 5–9 h every 12 h. For children can be swallowed whole or granules sprinkled on food. Rapid and complete absorption in the absence or presence of food
Slo-Phyllin[b,c]	60–250	Cmax ~ 4–8 h every 12 h. For children can be swallowed whole or granules sprinkled on food. Rapid and complete absorption in the absence or presence of food
Theo-24[a,c]	100–400	Cmax ~ 6–14 h every 24 h. Absorption is variable in mornings, after meals, or in the evening
Theo-Dur[a]	100, 200, 300	Cmax ~ 4–10 h every 12 h. Tablets (100, 300 mg) are scored. Complete absorption in the absence or presence of food
Uni-Dur[a]	400, 600	Cmax ~ 8–12 h once-daily evening dose. Complete absorption in the absence or presence of food
Uniphyl[a]	200, 400	Cmax ~ 8–12 h every 12 h. Incomplete absorption after overnight fast, more complete when taken after food
Uniphyllin Continus[b]	200, 300, 400	200, 300, 400
Nuelin SA[b]	175, 250	Cmax ~ 4–8 h every 12 h
Phyllocontin Continus[b]	225	Aminophylline hydrate. Cmax ~ 4–8 h. Taken twice daily to two tablets bid after a week

Data obtained from previously published articles (Weinberger et al. 1981; Hendeles et al. 1985; Weinberger and Hendeles 1996)
[a]Marketed in the USA
[b]Marketed in the UK
[c]Tablet unless otherwise indicated (capsule)

cardiovascular mortality in patients with COPD (White et al. 2013; Martinez et al. 2015).

More recently a dual PDE3/4 inhibitor RPL554 has been shown to be both bronchodilator and anti-inflammatory in both preclinical (Boswell-Smith et al. 2006b) and clinical studies (Franciosi et al. 2013). This drug is under development as an inhaled "bifunctional" drug by inhalation for the treatment of asthma and COPD which is currently undergoing phase II clinical trials. Of particular interest is the ability of RPL 554 to show synergistic interactions with other classes of bronchodilator to elicit airway smooth muscle relaxation in both large (Calzetta et al. 2013) and small human airways (Calzetta et al. 2015). This inhaled dual phosphodiesterase inhibitor may also be advantageous over inhaled PDE4 inhibitors because it would target cells expressing both PDE3 and PDE4 within the airways and, hence, could lead to a greater reduction in function of structural, innate and inflammatory cell function with the lung.

5 Dosages and Routes of Administration of Xanthines

Theophylline is rapidly and completely absorbed when administered as a capsule or solution but delayed when given as a coated tablet, although absorption is nonetheless complete. Absorption may be delayed when taken with a meal or when administered in the evening. There is some degree of variability between the absorption of theophylline from different formulations which are manufactured for slow release. Examples of different formulations and their absorption characteristics are listed in Table 2 (Weinberger et al. 1981; Hendeles et al. 1985; Weinberger and Hendeles 1996).

Dosing is usually commenced at 10 mg/kg for children over 6 months and adults to a maximum of 300 mg/day and subsequently increased by 13–16 mg/kg/day (maximum 450–600 mg/kg). If the dose achieves plasma concentrations less than 10 μg/ml, then the dose is increased by 25%. The dose is maintained if plasma levels of 10–15 μg/ml are achieved and the patient tolerates the dose. A dose reduction of 10% should be considered if plasma levels between 15–20 μg/ml are observed, and if plasma levels between 20–25 μg/ml and >25 μg/ml are observed, then the next dose and two doses should be withheld, respectively, and treatment resumed on the next lower dose increment (Weinberger et al. 1981; Hendeles et al. 1985; Weinberger and Hendeles 1996).

Theophylline rapidly distributes into non-adipose tissue and body water (Vd approximately 0.5 l/kg), and this value can increase in subjects who have liver disease, elderly patients and factors which alter albumin binding (40% plasma protein binding). Theophylline is metabolised extensively in the liver (up to 70%) where it undergoes N-demethylation to 1- and 3-methylxanthine via cytochrome P450 1A2 and 8-hydroxylation by CYP 2E1 (Weinberger and Hendeles 1996). The metabolism of theophylline is influenced by environmental factors (e.g. CYP 1A2 activity is increased by cigarette smoking), hepatic disease, genetic factors and important drug–drug interactions which increase, decrease or interfere with CYP 1A2 activity (see Table 3) (Hendeles et al. 1985; Weinberger and Hendeles 1996). The elimination half-life of theophylline is approximately 8 h in adults and 4 h in children and 10% is excreted unchanged by the kidney in adults. Theophylline does cross the placenta, but there have not been any reports of teratogenicity in the neonate when theophylline is administered during the first trimester. However, if the dose is not adjusted during pregnancy, then potentially harmful plasma levels can be achieved particularly as the clearance of theophylline during the third trimester is delayed (Pregnancy Category C). It is advisable to monitor the neonate for signs of theophylline toxicity (Weinberger et al. 1981; Hendeles et al. 1985; Weinberger and Hendeles 1996).

Table 3 Some examples of drug–drug interactions with theophylline

Effect	Pharmacological agents
Reduced clearance	Allopurinol; propafenone; antibacterials (erythromycin, ciprofloxacin, clarithromycin), antifungals (fluconazole, ketoconazole); antivirals (acyclovir); beta-blocker (propranolol); calcium channel blockers (diltiazem, verapamil); deferasirox; interferon; leukotriene receptor antagonist (zafirlukast); oestrogens; pentoxifylline; tuberculosis (isoniazid); ulcer drugs (cimetidine); tobacco; alcohol
Increased clearance	Antibacterial (rifampicin); antidepressants (fluvoxamine, St John's wort); antiepileptics (phenobarbital, phenytoin); antiviral (ritonavir); sulfinpyrazone; ulcer drugs (sucralfate)
Other drug interactions	Antagonists antiarrhythmic action of adenosine; increased risk of convulsions with quinolones; increased metabolism of phenytoin; reduces the effect of benzodiazepines; increased risk of hypokalaemia (beta2-agonists, glucocorticosteroids, diuretics); increase excretion of lithium; caffeine prolongs half-life

Examples sourced from various review articles for theophylline (Hendeles et al. 1985; Upton 1991a, b; Weinberger and Hendeles 1996)

6 Side Effects of Xanthines

The major side effects associated with theophylline occur when plasma concentrations rise above 20 μg/ml which include gastrointestinal side effects like vomiting, diarrhoea and nausea. Other side effects above this dose include insomnia, irritability and headache. At concentrations greater than 30 μg/ml, the potential for cardiac arrhythmia (A1 receptor antagonism), hypotension, hypokalaemia and hyperglycaemia is more likely. Seizures, brain damage and death occur at levels greater than 40 μg/ml. The side effects associated with high plasma levels of theophylline are only likely to be achieved following rapid intravenous administration and are unlikely to occur following oral ingestion with proper monitoring. The propensity for these side effects will be exacerbated in the elderly with comorbidities, impaired renal and liver function, in patients with cardiac failure and in patients on other medications that could give rise to drug–drug interactions particularly if chronic overdosing occurs. Consequently, monitoring of plasma levels is important particularly during pregnancy when neonatal levels of theophylline can achieve toxic levels because of reduced clearance as described earlier.

The numerous side effects associated with theophylline, drug–drug interactions and requirement for plasma monitoring limit the utility of this drug, although its demonstrable anti-inflammatory effect in asthma and COPD and at doses below the therapeutic window and relatively continued ease of administration provide a justification for its use (Hendeles et al. 1985; Weinberger and Hendeles 1996).

7 Indications and Contraindications of Xanthines

Reviews of randomised clinical trials investigating the effectiveness of theophylline in asthma (Tee et al. 2007; Dennis and Solarte 2011) and COPD (Sin et al. 2003; McIvor et al. 2011) have concluded that this drug has modest clinical effectiveness. Theophylline can improve lung function and reduce exacerbations in mild to moderate asthmatic subjects who are taking inhaled glucocorticosteroids or in whom asthma is poorly controlled with glucocorticosteroids, although these effects are relatively modest (Dennis and Solarte 2011). It has also been suggested that theophylline may restore glucocorticosteroid responsiveness in COPD patients (Ford et al. 2010).

A Cochrane report concluded that theophylline was as effective as long-acting beta2-adrenoceptor agonists in the control of nocturnal asthma in adolescents and adults with persistent asthma (Tee et al. 2007). An earlier report concluded that whilst there is some evidence that theophylline has beneficial actions in terms of reducing symptoms and the need for rescue medication in children with mild to moderate asthma, theophylline was less effective when compared to inhaled glucocorticosteroids (Seddon et al. 2006). One potential problem in treating children with theophylline is the possibility of deterioration in cognitive and behavioural scores, but no significant changes were found (Seddon et al. 2006). Oral versus inhaled treatment is clearly advantageous in the paediatric asthmatic population, and there is some evidence that they may be beneficial as add-on therapy in severe asthmatic children not controlled with inhaled glucocorticosteroids, although large randomised clinical trials are required to address this issue.

Randomised clinical trials have highlighted that long-term treatment with theophylline significantly improved lung function (0–120 ml) and reduced exacerbations (50% reduction) and worsening of symptoms (12 vs. 4 days without symptoms), although no study has investigated whether theophylline can reduce the decline in lung function and mortality and improve quality of life. However, it was concluded that the utility of this treatment is compromised by numerous side effects (e.g. risk of nausea was approximately sevenfold greater than placebo) even within the normal therapeutic window for theophylline (e.g. headache, irritability, arrhythmia and seizures) (Sin et al. 2003; McIvor et al. 2011). The risk of these adverse effects is clearly an issue for COPD patients as they tend to be older with systemic comorbidities and who may be prescribed other medications that could give rise to drug–drug interactions (Table 3), and the need for monitoring plasma levels makes this treatment far from ideal.

8 Summary

Theophylline has been used in the treatment of respiratory disease for nearly a century, but its precise mechanism of action remains to be established. Theophylline is a bronchodilator drug but its anti-inflammatory activity has gained increasing

acceptance. However, the anti-inflammatory effectiveness is modest compared with the gold standard, glucocorticosteroids, for the treatment of asthma. Theophylline has found a niche as add-on therapy in patients with severe asthma. In COPD, the bronchodilator action of theophylline is accepted although it remains to be established whether this drug is anti-inflammatory in this disease, although there is some evidence that theophylline might facilitate glucocorticosteroid activity in this disease. However, theophylline has significant potential for side effects, and drug–drug interaction demands the need for monitoring of plasma levels and therefore it is a disadvantage for this oral treatment. More recently, selective PDE4 inhibitors have been evaluated in asthma and COPD, with the first of these, roflumilast, introduced into clinical practice for the treatment of severe COPD. The inhaled "bifunctional" drug, RPL 554, is in clinical development which appears to be both bronchodilator and anti-inflammatory that has an excellent side effect profile. It is anticipated that future research will help us better understand how theophylline induces its wide-ranging clinical benefits and that this may lead to new potential targets for the development of novel therapeutic agents for the treatment of asthma and COPD, as well as improved PDE inhibitors.

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Glucocorticosteroids

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Contents

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Abstract

Glucocorticosteroids are the most effective anti-inflammatory therapy for asthma but are relatively ineffective in COPD. Glucocorticoids are broad-spectrum anti-inflammatory drugs that suppress inflammation via several molecular mechanisms. Glucocorticoids suppress the multiple inflammatory genes that are activated in asthma by reversing histone acetylation of activated inflammatory genes through binding of ligand-bound glucocorticoid receptors (GR) to coactivator molecules and recruitment of histone deacetylase-2 (HDAC2) to the activated inflammatory gene transcription complex (trans-repression). At higher concentrations of glucocorticoids GR homodimers interact with DNA recognition sites to activate transcription through increased histone acetylation of anti-inflammatory genes and transcription of several genes linked to glucocorticoid side effects (trans-activation). Glucocorticoids also have post-transcriptional effects and decrease stability of some proinflammatory mRNAs. Decreased glucocorticoid responsiveness is found in patients with severe asthma and asthmatics who smoke, as well as in all patients with COPD. Several molecular mechanisms of glucocorticoid resistance have now been identified which involve phosphorylation and other post-translational modifications of GR. HDAC2 is markedly reduced in activity and expression as a result of oxidative/nitrative stress and pi3 kinase-δ inhibition, so that inflammation is resistant to the anti-inflammatory actions of glucocorticoids. Dissociated glucocorticoids and selective GR modulators which show improved trans-repression over trans-activation effects have been developed to reduce side effects, but so far it has been difficult to dissociate anti-inflammatory effects from adverse effects. In patients with glucocorticoid resistance alternative anti-inflammatory treatments are being investigated as well as drugs that may reverse the molecular mechanisms of glucocorticoid resistance.

keywords

Anti-inflammatory • Corticosteroid resistance • Glucocorticoid receptor • Glucocorticoid receptor-beta • Histone deacetylase-2 • p38 MAP kinase

1 Introduction

Glucocorticosteroids (also called glucocorticoids, corticosteroids or steroids) are the most effective anti-inflammatory drugs available for the treatment of many chronic inflammatory and immune diseases, including asthma (Barnes 2011). However, a minority of patients with these diseases show little or no response even to high doses of glucocorticoids. Several other inflammatory diseases, including chronic obstructive pulmonary disease (COPD), interstitial pulmonary fibrosis and cystic fibrosis, appear to be largely glucocorticoid-resistant (Barnes and Adcock 2009). Both asthma and COPD involve chronic inflammation of the respiratory tract, with the activation and recruitment of many inflammatory cells and orchestrated by a complex network of inflammatory mediators (Barnes 2008).

However, there are differences in the nature of this inflammation and its inflammatory consequences between these diseases and this is demonstrated best by the differing response to glucocorticoids, which is excellent in most patients with asthma but very poor in most patients with COPD. There is now a much better understanding of how glucocorticoids suppress chronic inflammation in asthma and also why they fail to work in some patients with asthma and most patients with COPD, despite the fact that inflammatory genes are activated in these two diseases by similar molecular mechanisms. This has given insights into how glucocorticoids might be improved in the future and how glucocorticoid resistance may be overcome with new classes of therapy.

2 Clinical Use in Asthma

The widespread use of inhaled corticosteroids (ICS) has revolutionised the management of asthma, with marked reductions in asthma morbidity and mortality in patients of all severity. ICS are now recommended as first-line therapy for all patients with persistent asthma, including children (Reddel et al. 2015). Several topically acting glucocorticoids are now available for inhalation and all have similar efficacy, but there are pharmacokinetic differences that account for differences in therapeutic ratio between these drugs. ICS are very effective in controlling asthma symptoms in asthmatic patients of all ages and severity. ICS improve the quality of life of patients with asthma and allow many patients to lead normal lives, improve lung function, reduce the frequency of exacerbations and may prevent irreversible airway changes with long-term use (Barnes et al. 1998; O'Byrne et al. 2006). ICS were initially introduced to reduce the dose of oral glucocorticoids in patients with severe asthma and many studies have confirmed that the great majority of patients can be weaned off oral glucocorticoids. Only about 1% of asthmatic patients now require maintenance treatment with oral glucocorticoids for control of asthma ("steroid-dependent" asthmatics), but short courses of oral glucocorticoids are still needed to treat exacerbations of asthma. There are local side effects of ICS, including increased oral candidiasis and dysphonia, but these are rarely a major problem. Systemic side effects, largely arising from absorption of ICS from the lung, are not a problem in patients treated with the usually required doses, but may become a problem in patients with severe asthma who require larger doses for asthma control.

3 Clinical Use in COPD

Most patients with COPD have a poor response to glucocorticoids in comparison to asthma with little improvement in lung function or symptoms (Barnes 2010a). High doses of ICS have shown a reduction (20–25%) in exacerbations in patients with severe disease and this is the main clinical indication for their use (Vestbo et al. 2013). However, even the effect on exacerbations has been questioned as it may be explained by an artefact in trial design (Suissa and Barnes 2009). Several

large studies have shown that glucocorticoids fail to reduce the progression in COPD (measured by annual fall in FEV_1) or its mortality (Yang et al. 2012). This is likely to reflect the resistance of pulmonary inflammation to glucocorticoids in COPD patients (as discussed below). Current guidelines suggest that high doses of ICS should be used only in patients with severe disease ($FEV_1 < 50\%$ predicted) who have frequent exacerbations ($\leq$2/year) which would comprise about 10% of patients, whereas currently high dose ICS are used in approximately 80% of patients with a clinical diagnosis of COPD. This overuse of glucocorticoids is likely to produce several long-term side effects, such as osteoporosis, diabetes, cataracts and hypertension. In addition there is now considerable evidence that high doses of ICS in COPD increase the risk of pneumonia (Finney et al. 2014). Oral glucocorticoids are used to treat acute exacerbations, although they are poorly effective. Some patients with COPD, who also have concomitant asthma (termed "overlap syndrome"), benefit from ICS and these patients may be recognised by increased sputum and blood eosinophils and exhaled nitric oxide and by a greater bronchodilator reversibility (Postma and Rabe 2015).

4 Anti-Inflammatory Mechanisms of Glucocorticoids

There have been major advances in understanding the molecular mechanisms whereby glucocorticoids suppress inflammation in asthma (Kadmiel and Cidlowski 2013; Barnes 2010b). Glucocorticoids activate many anti-inflammatory genes, and repress many proinflammatory genes that have been activated in inflammation (Table 1), as well as having several post-transcriptional effects. Understanding the molecular mechanisms of glucocorticoid action has also provided new insights into understanding molecular mechanisms involved in glucocorticoid resistance (Barnes and Adcock 2009; Barnes 2010b).

4.1 Glucocorticoid Receptors

Glucocorticoids diffuse across the cell membrane and bind to glucocorticoid receptors (GR) in the cytoplasm (Nicolaides et al. 2010). Upon ligand binding, GR are activated and released from chaperone proteins (heat shock protein-90 and others) and rapidly translocate to the nucleus where they exert their molecular effects. The mechanism of nuclear translocation involves the nuclear import proteins importin-α (karyopherin-β), importin-7 and importin-13 (Goldfarb et al. 2004; Hakim et al. 2013; Tao et al. 2006). There is only one form of GR that binds glucocorticoids termed GRα. GRβ is an alternatively spliced form of GR that interacts with DNA but not with glucocorticoids, so may theoretically act as a dominant-negative inhibitor of glucocorticoid action by interfering with the binding of GR to DNA (Kino et al. 2009). In addition, there is evidence that multiple GR isoforms are generated by alternative splicing and alternative translation initiation (Kadmiel and Cidlowski 2013). These isoforms have unique tissue distribution patterns and transcriptional regulatory profiles. Furthermore, each is subject to

Table 1 Effect of glucocorticoids on transcription of genes relevant to asthma

Increased transcription (*trans-activation*)
• Lipocortin-1
• β_2-Adrenoceptor
• Secretory leukocyte inhibitory protein
• IkB-α (inhibitor of NF-kB)
• MKP-1 (inhibits MAP kinase pathways)
• Glucocorticoid inducible leucine zipper (GILZ)
• Anti-inflammatory or inhibitory cytokines
IL-10, IL-12, IL-1 receptor antagonist
Decreased transcription (*trans-repression*)
• Inflammatory cytokines
IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IL-15, TNFα, GM-CSF, SCF, TSLP
• Chemokines
CCL1, CCL5, CCL11, CXCL8
• Inflammatory enzymes
Inducible nitric oxide synthase (iNOS), inducible cyclo-oxygenase (COX-2)
Inducible phospholipase A_2 ($cPLA_2$)
• Inflammatory peptides
Endothelin-1
• Inflammatory mediator receptors
Neurokinin (NK_1)-, bradykinin (B_2)-receptors
• Adhesion molecules
ICAM-1,VCAM-1

various post-translational modifications that may affect receptor function, which determine the cell-specific response to glucocorticoids.

4.2 Gene Activation

GR homodimerise and bind to glucocorticoid response elements (GRE) usually in the promoter region of glucocorticoid-responsive genes and this interaction switches on (or occasionally switches off) gene transcription. Activation of glucocorticoid-responsive genes occurs via an interaction between the DNA-bound GR and transcriptional coactivator molecules such as CREB-binding protein (CBP), which have intrinsic histone acetyltransferase activity and cause acetylation of core histones (particularly histone-4) (Fig. 1). This tags histones to recruit chromatin remodelling engines such as SWI/SNF and subsequent association of RNA polymerase II resulting in gene activation (Ito et al. 2000; John et al. 2008). Genes that are switched on by glucocorticoids include genes encoding β_2-adrenergic receptors and the anti-inflammatory proteins secretory leukoprotease inhibitor and mitogen-activated protein kinase phosphatase-1 (MKP-1), also known as dual specificity phosphatase-1 (DUSP-1) which inhibits MAP kinase pathways. These effects may contribute to the anti-inflammatory actions of glucocorticoids. GR interaction with negative GREs, or to GREs that cross the transcriptional start

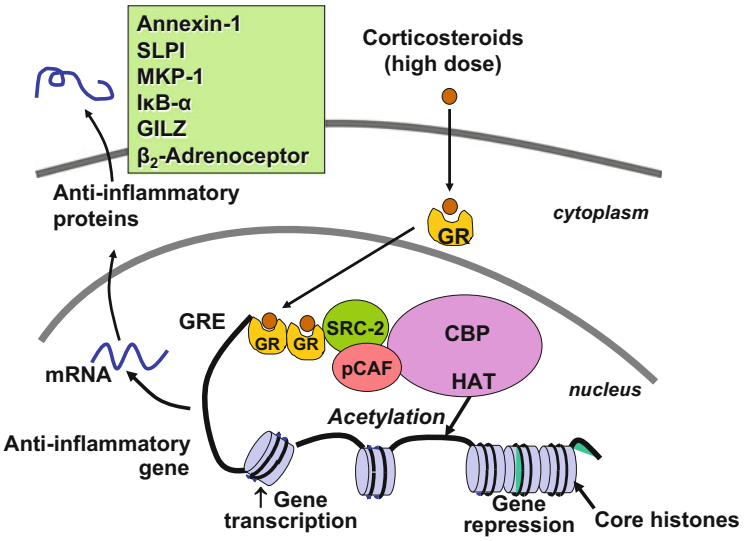

Fig. 1 Glucocorticoid activation of anti-inflammatory gene expression. Glucocorticoids bind to cytoplasmic glucocorticoid receptors (GR) which translocate to the nucleus where they bind to glucocorticoid response elements (GRE) in the promoter region of steroid-sensitive genes and also directly or indirectly to coactivator molecules such as CREB-binding protein (CBP), p300/CBP-activating factor (pCAF) or steroid receptor coactivator-2 (SRC-2), which have intrinsic histone acetyltransferase (HAT) activity, causing acetylation of lysines on histone H4, which leads to activation of genes encoding anti-inflammatory proteins, such as secretory leukoprotease inhibitor (SLPI), mitogen-activated kinase phosphatase-1 (MKP-1), inhibitor of NF-kB (IkB-α) and glucocorticoid-induced leucine zipper protein (GILZ)

site, may suppress gene transcription and this may be important in mediating many of the side effects of glucocorticoids, such as inhibition of osteocalcin that is involved in bone synthesis (Dostert and Heinzel 2004) (Fig. 2).

4.3 Switching Off Activated Inflammatory Genes

The major action of glucocorticoids is to switch off multiple activated inflammatory genes that encode for cytokines, chemokines, adhesion molecules inflammatory enzymes and receptors (Barnes 2011). These genes are switched on in the airways by proinflammatory transcription factors, such as nuclear factor-kB (NF-kB) and activator protein-1 (AP-1), both of which are usually activated at sites of inflammation in asthma and COPD, resulting in the switching on of multiple inflammatory genes. These genes are activated through interactions with transcriptional coactivator molecules in a similar manner to that described above for GR-mediated gene transcription.

Activated GR interact with co-repressor molecules to attenuate NF-kB-associated coactivator activity, thus reducing histone acetylation, chromatin

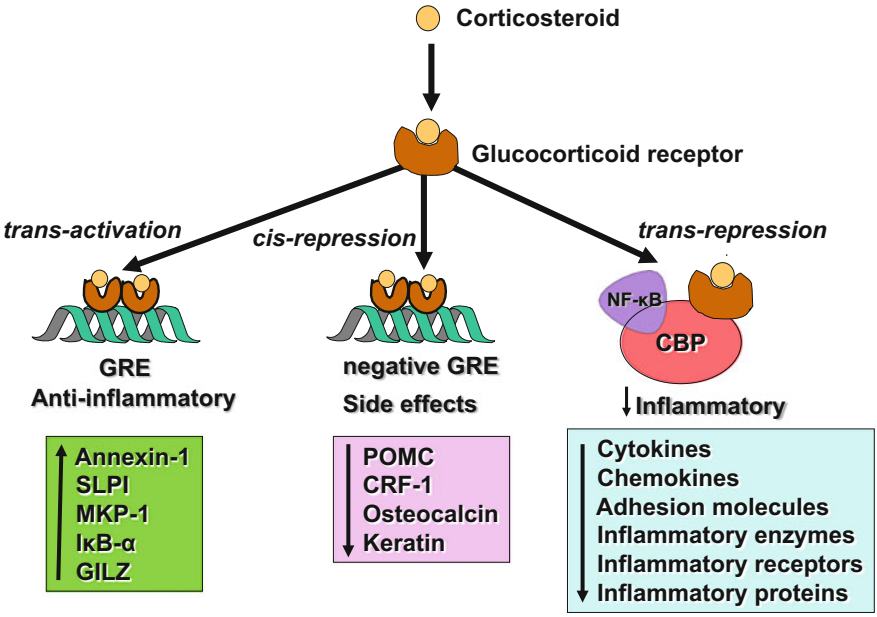

Fig. 2 Glucocorticoids regulate gene expression in several ways. Glucocorticoids enter the cell to bind to glucocorticoid receptors (GR) in the cytoplasm that translocate to the nucleus. GR homodimers bind to glucocorticoid response elements (GRE) in the promoter region of steroid-sensitive genes, which may encode anti-inflammatory proteins. Less commonly, GR homodimers interact with negative GREs to suppress genes, particularly those linked t. Nuclear GR also interact with coactivator molecules, such as CREB-binding protein (CBP), which is activated by proinflammatory transcription factors, such as nuclear factor-kB (NF-kB), thus switching off the inflammatory genes that are activated by these transcription factors. *Other abbreviations*: *SLPI* secretory leukoprotease inhibitor, *MKP-1* mitogen-activated kinase phosphatase-1, *IkB-α* inhibitor of NF-kB, *GILZ* glucocorticoid-induced leucine zipper protein, *POMC* proopiomelanocortin, *CRH* corticotrophin releasing factor

remodelling and RNA polymerase II actions. Reduction of histone acetylation more importantly occurs through the specific recruitment of histone deacetylase-2 (HDAC2) to the activated inflammatory gene complex by activated GR, thereby resulting in effective suppression of activated inflammatory genes within the nucleus (Ito et al. 2000) (Fig. 3). This may account for why glucocorticoids are so effective in the control of inflammation, but also why they are relatively safe, since genes other than those that encode inflammatory proteins are not affected. GR becomes acetylated upon ligand binding allowing it to bind to GREs and HDAC2 can target acetylated GR thereby allowing it to associate with the NF-kB complex (Ito et al. 2006). The site of acetylation of GR is the lysine rich region – 492–495 with the sequence KKTK, which is analogous to the acetylation sites identified on other nuclear hormone receptors. Site-directed mutagenesis of the lysine residues K494 and K495 prevents GR acetylation and reduces the activation of the SLPI gene by glucocorticoids, whereas repression of NF-kB is unaffected.

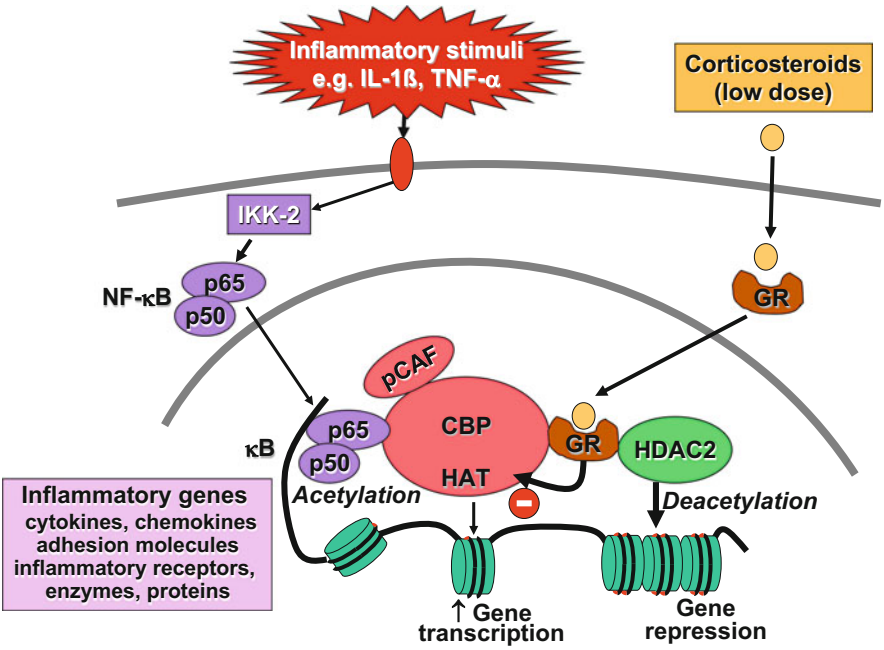

Fig. 3 Corticosteroid suppression of activated inflammatory genes. Inflammatory genes are activated by inflammatory stimuli, such as interleukin-1β (IL-1β) or tumour necrosis factor-α (TNF-α), resulting in activation of IKK2 (inhibitor of I-kB kinase-2), which activates the transcription factor nuclear factor kB (NF-kB). A dimer of p50 and p65 NF-kB proteins translocates to the nucleus and binds to specific kB recognition sites and also to coactivators, such as CREB-binding protein (CBP) or p300/CBP-activating factor (pCAF), which have intrinsic histone acetyltransferase (HAT) activity. This results in acetylation of core histone H4, resulting in increased expression of genes encoding multiple inflammatory proteins. Glucocorticoid receptors (GR) after activation by glucocorticoids translocate to the nucleus and bind to coactivators to inhibit HAT activity directly and recruiting histone deacetylase-2 (HDAC2), which reverses histone acetylation leading in suppression of these activated inflammatory genes

Additional mechanisms are also important in the anti-inflammatory actions of glucocorticoids. Glucocorticoids have potent inhibitory effects on mitogen-activated protein kinase (MAPK) signalling pathways through the induction of MKP-1/DUSP-1 as discussed above. An important effect of glucocorticoids in the treatment of allergic diseases is through suppression of Th2 cells and Th2 cytokines (IL4, IL-5, and IL-13) and this may be mediated via inhibition of the transcription factor GATA3 which regulates the transcription of Th2 cytokine genes. This is controlled by translocation of GATA3 from the cytoplasm to the nucleus via importin-α after phosphorylation by p38 MAPK. Glucocorticoids potently inhibit GATA3 nuclear translocation as GR competes for nuclear import via importin-α and also induces MKP-1 to reverse the phosphorylation of GATA3 by p38 MAPK (Maneechotesuwan et al. 2009). A further immunosuppressive effect of glucocorticoids is through enhanced activity and expression of indoleamine-2,3-

dioxygenase (IDO), a tryptophan-degrading enzyme that plays a key role in the regulation of T-lymphocyte function in allergic diseases through increased secretion of the anti-inflammatory cytokine IL-10 (Maneechotesuwan et al. 2008).

4.4 Post-Transcriptional Effects

Some proinflammatory genes, such as TNF-α, have unstable messenger RNA that is rapidly degraded by certain RNAses but stabilised when cells are stimulated by inflammatory mediators. Glucocorticoids reverse this effect, resulting in rapid degradation of mRNA and reduced inflammatory protein secretion (Bergmann et al. 2004). This may be mediated through the increased gene expression of proteins that destabilize mRNAs of inflammatory proteins, such as the zinc finger protein tristetraprolin, which binds to the 3' AU-rich untranslated region of mRNAs (Prabhala and Ammit 2015).

5 Cellular Effects in Asthma and COPD

At a cellular level glucocorticoids reduce the numbers of inflammatory cells in the airways of asthmatic patients, including eosinophils, T-lymphocytes, mast cells and dendritic cells (Barnes et al 1998) (Fig. 4). These effects of glucocorticoids are produced through inhibiting the recruitment of inflammatory cells into the airway by suppressing the production of chemotactic mediators and adhesion molecules

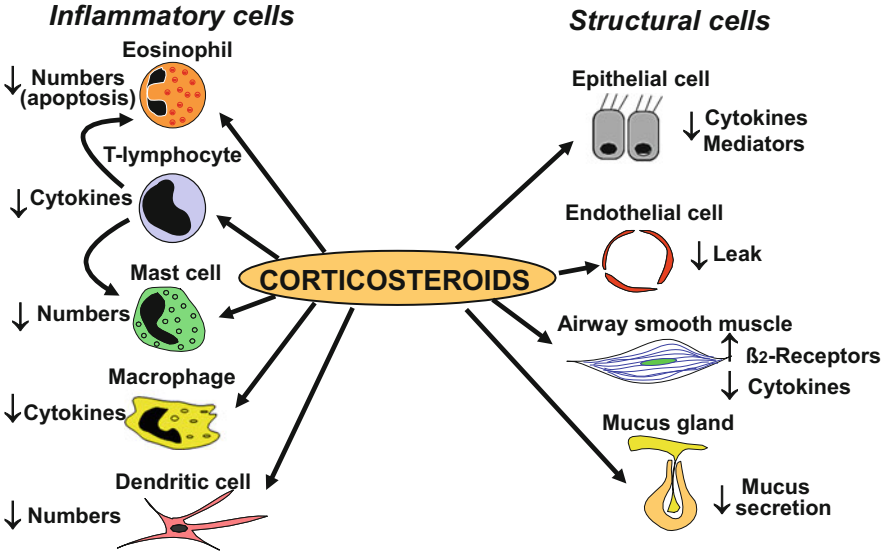

Fig. 4 Cellular effect of corticosteroids

and by inhibiting the survival in the airways of inflammatory cells, such as eosinophils, T-lymphocytes, mast cells and dendritic cells. Epithelial cells may be the major cellular target for ICS, which are the mainstay of modern asthma management. ICS suppress many activated inflammatory genes in airway epithelial cells. Epithelial integrity is restored by regular ICS. The suppression of mucosal inflammation is relatively rapid with a significant reduction in eosinophils detectable within 3 h and associated with reduced airway hyperresponsiveness (Erin et al. 2008). This almost certainly accounts for the clinical benefits seen with inhalation of budesonide and formoterol combination inhaler as a rescue therapy in asthma, as this is likely to stop the evolution of an exacerbation (Barnes 2007). However, reversal of airway hyperresponsiveness may take several months to reach a plateau, probably reflecting recovery of structural changes in the airway.

In COPD patients even high doses of ICS fail to reduce airway inflammation. This glucocorticoid resistance has been demonstrated by the failure of high doses of ICS to reduce inflammatory markers in sputum or bronchial biopsies of COPD patients (Keatings et al. 1997; Culpitt et al. 1999). The reason why ICS fail to suppress inflammation cannot be explained by impaired access of the inhaled drug to sites of inflammation as an oral glucocorticoid is equally ineffective.

6 Interaction with β_2-Adrenergic Agonists

Inhaled β_2-agonists and glucocorticoids are frequently used together, usually as a fixed combination inhaler containing a glucocorticoid with a long-acting β_2-agonist (LABA) in the control of asthma and it is now recognized that there are important molecular interactions between these two classes of drug (Barnes 2002; Black et al. 2009) (Fig. 5). Glucocorticoids increase the transcription of the β_2-receptor gene, resulting in increased expression of cell surface receptors. This has been demonstrated in human lung in vitro and nasal mucosa in vivo after topical application of a glucocorticoid. In this way glucocorticoids protect against the down-regulation of β_2-receptors after long-term administration. This may be important for the non-bronchodilator effects of β_2-agonists, such as mast cell stabilisation. Glucocorticoids may also enhance the coupling of β_2-receptors to G-protein (G_s), thus enhancing β_2-agonist effects and reversing the uncoupling of β_2-receptors that may occur in response to inflammatory mediators, such as IL-1β through a stimulatory effect on a G-protein coupled receptor kinase (Mak et al. 1995). Glucocorticoids may increase responses to β2-agonists in COPD patients and this may account for the effects of adding ICS to LABA in COPD patients (Nannini et al. 2013).

There is now increasing evidence that β_2-agonists may affect GR function and thus enhance the anti-inflammatory effects of glucocorticoids. LABA increase the translocation of GR from cytoplasm to the nucleus after activation by glucocorticoids (Roth et al. 2002). This effect has now been demonstrated in sputum macrophages of asthmatic patients after an ICS and inhaled LABA in asthma and COPD (Usmani et al. 2005; Haque et al. 2013). This suggests that

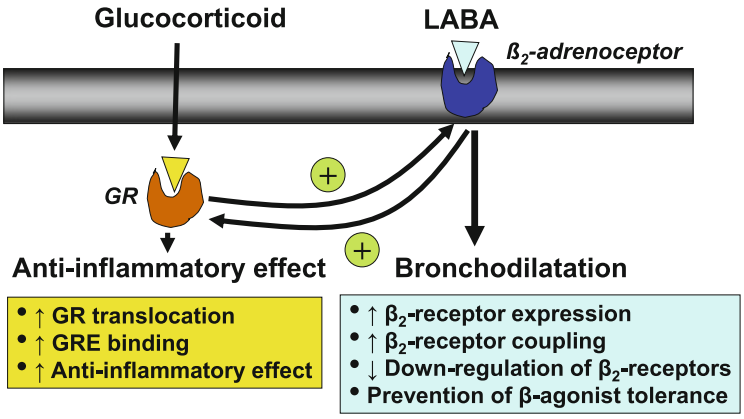

Fig. 5 Interaction between glucocorticosteroids and long-acting β_2-agonists (LABA). Glucocorticoids increase the numbers of β_2-receptors, whereas β_2-agonists, as well as inducing direct bronchodilatation, act on glucocorticoid receptors (GR) to increase the anti-inflammatory effects of glucocorticoids

LABA and glucocorticoids enhance each other's beneficial effects in asthma therapy and this may contribute to the greater efficacy of combination inhalers compared to increased doses of ICS in clinical trials in asthma. LABA also enhance GR translocation in COPD macrophages and to reduce the corticosteroid resistance found in these cells in COPD patients (Haque et al. 2013).

7 Glucocorticoid Resistance

Patients with severe asthma have a poor response to glucocorticoids, which necessitates the need for high doses and a very small number of patients are completely resistant. These patients are difficult to manage as they get side effects from high doses of glucocorticoids, despite their lack of clinical benefit. All patients with COPD show a degree of glucocorticoid resistance (Barnes 2010a). Asthmatics who smoke are also relatively glucocorticoid-resistant and require increased doses of glucocorticoids for asthma control (Polosa and Thomson 2012). Several molecular mechanisms have now been identified to account for glucocorticoid resistance in severe asthma and COPD (Table 2) (Barnes 2013a).

7.1 Genetic Susceptibility

Glucocorticoid-resistant asthma suggested that it was more commonly found within families, indicating that there may genetic factors may determine glucocorticoid responsiveness. Microarray studies of peripheral blood mononuclear cells (PBMC) from glucocorticoid-sensitive and glucocorticoid-insensitive asthma patients have

Table 2 Molecular mechanisms of steroid resistance in asthma and COPD

• Familial glucocorticoid resistance
• Glucocorticoid receptor modification ↑ Phosphorylation: ↓ nuclear translocation ↑ p38MAPKα due to IL-2 + IL-4 or IL-13 in severe asthma due to MIF in severe asthma ↑ p38MAPKγ in severe asthma ↑ JNK1 due to proinflammatory cytokines in severe asthma ↑ ERK due to microbial superantigens in severe non-allergic asthma ↓ MKP-1 in severe asthma ↓ PP2A in severe asthm Nitrosylation: ↑ NO from inducible NO synthase Ubiquitination: ↑ degradation by proteasome
• Increased GRβ expression
• Increased proinflammatory transcription factors Activator protein-1, JNK
• Defective histone acetylation ↓ Acetylation of lysine-5 on histone 4 in severe asthma ↓ Histone deacetylase-2 in COPD, severe asthma. smoking asthma ↑ Oxidative stress ↑ Phosphoinositide-3-kinase-δ activation

GR glucocorticoid receptor, *IL* interleukin, *MAP* mitogen-activated protein, *MIF* macrophage migration inhibitory factor, *MKP* MAP kinase phosphatase, *JNK* c-Jun N-terminal kinase, *ERK* extracellular signal-regulated kinase, *NO* nitric oxide, *PP* protein phosphatase

identified several genes that discriminated between these patients (Hakonarson et al. 2005), suggesting that it might be possible to develop a genomic test for glucocorticoid resistance. However, in normal subjects differential gene expression between the 10% with the greatest and least glucocorticoid responsiveness of circulating genes identified 24 genes of which the most discriminant was bone morphogenetic protein receptor type II (BMPR2), which enhanced glucocorticoid responsiveness when transfected into cells (Donn et al. 2007).

The very rare inherited syndrome familial glucocorticoid resistance (FGR) is characterised by high circulating levels of cortisol without signs or symptoms of Cushing's syndrome (Charmandari et al. 2013). Clinical manifestations are due to an excess of non-glucocorticoid adrenal steroids, stimulated by high adrenocorticotropin levels, resulting in hypertension with hypokalaemia and/or signs of androgen excess. Inheritance appears to be dominant with variable expression, but only about a few cases have so far been reported. Sporadic cases have also been described. Several mutations in GR have been described in FGR, with impaired GR function in PBMC or fibroblasts, including decreased binding for cortisol, reduced numbers and abnormal binding to DNA. These patients are clearly different from patients with glucocorticoid-resistant inflammatory diseases and in patients with glucocorticoid-resistant asthma mutational analysis demonstrated no obvious abnormality in GR structure (Lane et al. 1994).

7.2 Defective GR Binding and Translocation

There is increased expression of interleukin(IL)-2 and IL-4 in the airways of patients with glucocorticoid-resistant asthma and in vitro these cytokines in combination reduce GR nuclear translocation and binding affinity within the nucleus of T-cells IL-13 alone mimics this effect in monocytes (Sher et al. 1994; Irusen et al. 2002). The mechanism whereby these cytokines reduce GR function may be mediated via phosphorylation of GR by p38 MAPK and their effect is blocked by a p38 MAPK-α inhibitor (Irusen et al. 2002). In support of this p38 MAPK shows a greater degree of activation in alveolar macrophages from asthmatics with a poor response to glucocorticoids than patients who show a normal response (Bhavsar et al. 2008) and a p38 MAPK inhibitor increases glucocorticoid sensitivity in PBMC from patients with severe asthma (Mercado et al. 2012). The γ-isoform of p38 MAPK is also involved in phosphorylation of GR and reduced GR nuclear translocation (Mercado et al. 2011). GR may be phosphorylated by several kinases that may alter its binding, stability, translocation to the nucleus, binding to DNA and interaction with other proteins, such as transcription factors and molecular chaperones (Weigel and Moore 2007). In patients with glucocorticoid-resistant asthma a large proportion show reduced nuclear translocation of GR and reduced GRE binding in PBMC following glucocorticoid exposure and this may be explained by GR phosphorylation (Matthews et al. 2004). Another MAPK c-Jun N-terminal kinase (JNK), which is activated by TNF-α and other proinflammatory cytokines, also directly phosphorylates GR at Ser^{226} and inhibits GRE binding (Ismaili and Garabedian 2004). Activation of PI3 kinase signalling through oxidative stress leads to activation of mTOR and which in turn activates JNK, resulting in corticosteroid resistance (Mitani et al. 2016). Microbial superantigens induce glucocorticoid resistance in T cells in vitro via activation of extracellular receptor kinase (ERK) pathways, resulting in GR phosphorylation (Li et al. 2004). Macrophages from MKP-1 gene knock-down mice show reduced anti-inflammatory responses to glucocorticoids in vitro due to increased MAPK activation (Abraham et al. 2006). In asthmatic patients with glucocorticoid insensitivity there is a significant reduction in MKP-1 expression in alveolar macrophages after glucocorticoid exposure and this is correlated with increased p38 MAPK activity (Bhavsar et al. 2008). Another phosphatase PP2A plays an important role in dephosphorylating phosphorylated GR and thus reversing corticosteroid resistance and there is a defect in PP2A expression in patients with severe asthma (Kobayashi et al. 2011) (Fig. 6).

In vitro GR may be nitrosylated by NO donors resulting in reduced binding affinity for glucocorticoids (Galigniana et al. 1999). In severe asthma and COPD there is increased expression of inducible NO synthase (iNOS) which produces large amounts of NO that could reduce glucocorticoid responsiveness, although this mechanism has not yet been demonstrated in asthma and COPD.

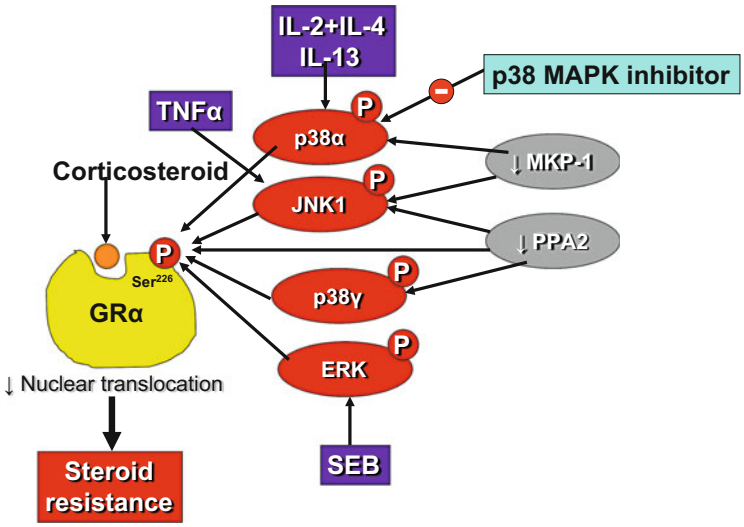

Fig. 6 Glucocorticoid receptor phosphorylation. GRα phosphorylation at serine-226 (Ser^{226}) impedes nuclear translocation, leading to steroid resistance. GR may be phosphorylated by several kinases: p38 mitogen-activated kinase (MAPK)-α (p38α), which is activated by interleukin(IL)-2 and IL-4 or IL-13 and inhibited by p38MAPK inhibitors; C-terminal N-terminal kinase (JNK), which is activated by tumour necrosis factor(TNF)-α; p38MAPK-γ (p38γ), which is also activated by IL-2 + IL-4; extracellular signal-regulated kinase (ERK), which is activated by staphylococcal enterotoxin B (SEB). These kinases are dephosphorylated by the phosphatases MAP kinase phosphatase-1 (MKP-1) and protein phosphatase(PP)2A, both of which are defective in cells from severe asthma patients

7.3 Increased GRβ

Increased expression of GRβ has been reported in glucocorticoid-resistant patients of several diseases, including asthma but this has not been confirmed in several other studies (Pujols et al. 2007). GRβ is induced by proinflammatory cytokines and has the capacity to compete for the binding of GRα to GRE, thus acting as a dominant-negative inhibitor. GRβ expression is also increased by microbial superantigens, such as staphylococcal enterotoxins, which may account for glucocorticoid resistance in atopic dermatitis (Fakhri et al. 2004). However, in most cell types the expression of GRβ is much lower than GRα, making this mechanism unlikely. Another mechanism may be through interference with GRα nuclear translocation, since knockdown of GRβ in alveolar macrophages from glucocorticoid-resistant asthma patients results in increased GRα nuclear localisation and increased glucocorticoid responsiveness (Goleva et al. 2006).

7.4 Transcription Factor Activation

Excessive activation of AP-1 has been identified as a mechanism of glucocorticoid resistance in asthma as AP-1 binds GR and thus prevents its interaction with GRE and other transcription factors (Adcock et al. 1995). AP-1 is a heterodimer of Fos and Jun proteins and may be activated by proinflammatory cytokines such as TNF-α, acting through the JNK pathway.

7.5 Abnormal Histone Acetylation

Histone acetylation plays a critical role in the regulation of inflammatory genes and the mechanism of action of glucocorticoids. Glucocorticoids switch on glucocorticoid-responsive genes, such as MKP-1, via acetylation of specific lysine residues (K5 and K16) on histone-4 (Ito et al. 2000). In a small proportion of patients with glucocorticoid-resistant asthma, GR translocates normally to the nucleus after glucocorticoid exposure but fails to acetylate K5 so that transactivation of genes does not occur (Matthews et al. 2004). These patients show a poor response to high dose inhaled glucocorticoids but unlike most patients with glucocorticoid resistance seem to have fewer side effects as many of these are mediated via GRE binding.

7.6 Decreased HDAC2

Recruitment of HDAC2 to activated inflammatory genes is a major mechanism of inflammatory gene repression by glucocorticoids and reduced HDAC2 activity and expression is reduced in some diseases where patients respond poorly (Barnes 2009) (Fig. 7). For example, HDAC2 is markedly reduced in alveolar macrophages, airways and peripheral lung in patients with COPD (Ito et al. 2005), and similar changes are found in PBMCs and alveolar macrophages of patients with refractory asthma and in the airways of smoking asthmatics (Hew et al. 2006). The glucocorticoid resistance of COPD bronchoalveolar macrophages is reversed by overexpressing HDAC2 (using a plasmid vector) to the level seen in control subjects (Ito et al. 2006). The mechanisms for HDAC2 reduction in COPD involve nitrates tyrosine residues on HDAC2 resulting in its inactivation, ubiquitination and degradation (Osoata et al. 2009). Oxidative stress also activates PI3Kδ, which leads to phosphorylation and inactivation of HDAC2 (To et al. 2010). This is confirmed in mice exposed to cigarette smoke that develop glucocorticoid-resistant pulmonary inflammation. This glucocorticoid-resistance is completely absent in mice where the PI3Kδ gene is inactivated (Marwick et al. 2009). This suggests that oxidative stress may be an important mechanism of glucocorticoid resistance and is increased in most severe and glucocorticoid-resistant inflammatory diseases.

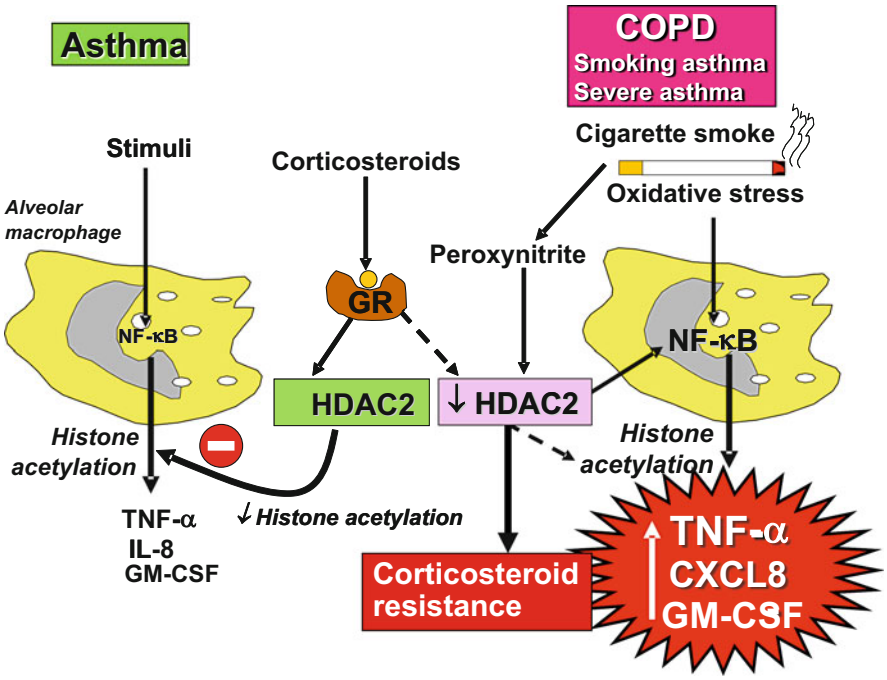

Fig. 7 Mechanism of corticosteroid resistance in COPD, smoking asthma and severe asthma. Stimulation of mild asthmatic alveolar macrophages activates nuclear factor-kB (NF-kB) and other transcription factors to switch on histone acetyltransferase leading to histone acetylation and subsequently to transcription of genes encoding inflammatory proteins, such as tumour necrosis factor-α (TNF-α), interleukin-8 (IL-8) and granulocyte-macrophage colony stimulating factor (GM-CSF). Corticosteroids reverse this by binding to glucocorticoid receptors (GR) and recruiting histone deacetylase-2 (HDAC2). This reverses the histone acetylation induced by NF-kB and switches off the activated inflammatory genes. In COPD patients and smoking asthmatics cigarette smoke generates oxidative stress (acting through the formation of peroxynitrite) and in severe asthma and COPD intense inflammation generates oxidative stress to impair the activity of HDAC2. This amplifies the inflammatory response to NF-kB activation, but also reduces the anti-inflammatory effect of corticosteroids, as HDAC2 is now unable to reverse histone acetylation

8 Therapeutic Implications

ICS are highly effective in treating most patients with asthma. Patients with severe asthma may require high doses and this has a risk of systemic side effects, which has led to a search for ICS with even greater therapeutic ratios. A few patients with asthma and most patients with COPD are poorly responsive to glucocorticoids and are at risk of side effects, so that alternative anti-inflammatory treatments are needed, or the mechanisms of glucocorticoid resistance need to be reversed. Resistance to the anti-inflammatory effects of glucocorticoids is a major barrier

to effective control of many common diseases and enormously increases their morbidity and medical costs.

8.1 Dissociated Steroids

There has been a major effort to develop glucocorticoids that have reduced side effects, while retaining anti-inflammatory efficacy. Selective glucocorticoid receptor agonists (SEGRAs or dissociated steroids) are more effective in trans-repression than trans-activation so theoretically have less side effects (Belvisi et al. 2001). Several dissociated steroids have now been developed, including non-glucocorticoid GR modulators, but there is uncertainly about the efficacy of these drugs as anti-inflammatory therapies. In a mouse knock-in strain with dimerization-deficient GR some inflammatory processes can be suppressed by glucocorticoids, whereas others cannot which may reflect the anti-inflammatory effects of glucocorticoid mediated through trans-activation of genes, such as MKP-1 (Kleiman and Tuckermann 2007). While several inhaled non-steroidal GR modulators are currently in clinical development for asthma, there are no studies demonstrating any clinical advantage. For example, inhaled GW870086X is effective in asthma but no safety advantage has been demonstrated (Leaker et al. 2015).

8.2 Alternative Anti-Inflammatory Treatments

There are several therapeutic strategies to manage glucocorticoid-resistant diseases, but the most important general approaches are to use alternative anti-inflammatory ("steroid-sparing") treatments or to reverse the molecular mechanisms of glucocorticoid resistance if these are identified. Several non-steroidal anti-inflammatory drugs currently available to treat certain glucocorticoid-resistant diseases, but these may have a toxicity of their own. Calcineurin inhibitors, such as cyclosporin A and tacrolimus, may be effective in some patients with glucocorticoid-resistant inflammation, but have not been found to be very effective in glucocorticoid-resistant asthma (Evans et al. 2001). This has led to a search for novel anti-inflammatory treatments, particularly for diseases with marked glucocorticoid resistance, such as COPD, where no effective and safe anti-inflammatory treatments are available (Barnes 2013b).

Phosphodiesterase-4 inhibitors are broad-spectrum anti-inflammatory treatments that are now in clinical development for several inflammatory diseases, such as COPD (Hatzelmann et al. 2010). However, systemic doses have been limited by side effects, such as nausea, diarrhoea and headaches. Roflumilast is the first PDE4 inhibitor licensed for the treatment of inflammation in COPD patients and reduces neutrophilic inflammation with some improvement in lung function and reduction in exacerbations (Garnock-Jones 2015).

Several p38 MAPK inhibitors have been in clinical development and theoretically could be particularly effective in asthma with glucocorticoid resistance due to IL-2 and IL-4, as this is reversed in vitro by selective p38 MAPK inhibitors. These drugs may also be useful in other glucocorticoid-insensitive inflammatory diseases such as COPD where p38 MAPK is activated and they have been shown to have efficacy in glucocorticoid-resistant animal models of these diseases (Medicherla et al. 2007). However these drugs have had problems with toxicity and side effects. Blocking NF-kB by selective inhibitors of inhibitor of NF-kB kinase (IKKβ, IKK2) is another way of treating glucocorticoid-resistant inflammation, but it is likely that these drugs will also have toxicity and side effects so may only be suitable for topical application.

8.3 Reversing Glucocorticoid Resistance

Another therapeutic option for treating glucocorticoid resistance is to reverse the cause of resistance if it can be identified. This is possible with smoking cessation in smoking asthmatics and might be possible for some patients with glucocorticoid-resistant asthma with p38 MAPK or JNK inhibitors in the future. Selective activation of HDAC2 can be achieved with theophylline, which restores HDAC2 activity in COPD macrophages back to normal and reverses glucocorticoid resistance (Cosio et al. 2004). In COPD patients the combination of theophylline and ICS is more effective in reducing airway inflammation than either drug alone (Ford et al. 2010). There are now therapeutic trials in COPD with low doses of theophylline (Devereux et al. 2015). Low dose theophylline also improves asthma control in smoking asthmatic patients who show no response to ICS alone (Spears et al. 2009). The molecular mechanism of action of theophylline in restoring HDAC2 is through selective inhibition of PI3Kδ, which is activated by oxidative stress in COPD patients (To et al. 2010). This suggests that selective PI3Kδ inhibitors may also be effective and these drugs are currently in clinical trials in COPD and severe asthma. Since oxidative stress appears to be an important mechanism in reducing HDAC2 and leads to glucocorticoid resistance, antioxidants should also be effective. Unfortunately currently available antioxidants are not very effective and several more potent antioxidants are in clinical development. In the future novel drugs which increase HDAC2 may be developed when the molecular signalling pathways that regulate HDAC2 are better understood (Barnes 2005).

9 Conclusions

Glucocorticoids remain by far the most effective therapy for controlling asthma and suppress airway inflammation mainly through repression of activated inflammatory genes, but also by increasing the transcription of anti-inflammatory genes, such as MKP-1 (Barnes 2006). It is unlikely that it will be possible to develop more effective anti-inflammatory treatments for asthma in the future as glucocorticoids

have such a broad-spectrum of anti-inflammatory actions, reflecting their ability to switch off all activated inflammatory genes. ICS are now amongst the most widely used drugs in the world and there has been considerable effort expended in trying to improve their therapeutic ratio. Addition of LABA in the form of fixed combination inhalers improves asthma control to a greater extent than increasing the dose of ICS and this has become the standard approach for controlling patients with moderate to severe asthma. This is, at least in part, explained by the favourable molecular interactions between glucocorticoids and β_2-agonists. Selective GR modulators which favour trans-repression over trans-activation mechanisms were designed to reduce side effects that are largely due to gene activation, but so far have proved difficult to develop clinically. Furthermore, it is now clear that some anti-inflammatory effects of corticosteroids are due to trans-activation of anti-inflammatory genes, whereas some adverse effects may be due to trans-repression.

The major area of research interest is now focussed on understanding glucocorticoid resistance as it is a major barrier to the effective treatment of COPD patients and asthmatic patients with severe disease or who smoke. The recognition that there are different molecular mechanisms of glucocorticoid resistance has identified several new therapeutic targets. A major mechanism for reduced glucocorticoid responsiveness in COPD, severe and smoking asthma is reduction in HDAC2 activity and expression as a result of oxidative stress via activation of PI3Kδ. This pathway may be blocked by low concentrations of theophylline as well as selective PI3Kδ inhibitors, suggesting new therapeutic approaches to the treatment of severe asthma and COPD in the future. Other drugs that target this pathway are also in development and may lead to a new therapeutic strategy whereby drugs are able to reverse glucocorticoid resistance in airway diseases and perhaps other glucocorticoid-resistant inflammatory diseases, such as atherosclerosis and multiple sclerosis.

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Fixed-Dose Combination Inhalers

Mario Cazzola and Maria Gabriella Matera

Contents

Abstract

In asthma and chronic obstructive pulmonary disease (COPD), an important step in simplifying management and improving adherence with prescribed therapy is to reduce the dose frequency to the minimum necessary to maintain disease control. Fixed-dose combination (FDC) therapy might enhance compliance by decreasing the number of medications and/or the number of daily doses. Furthermore, they have the potential for enhancing, sensitizing, and prolonging the effects of monocomponents. Combination therapy with an inhaled corticosteroid (ICS) and a long-acting β-agonist (LABA) is considered an important approach for treating patients with asthma and patients with severe COPD who have frequent exacerbations. Several ICS/LABA FDCs are now commercially available or will become available within the next few years for the treatment of COPD and/or asthma. Several studies demonstrate that there are a number of

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added benefits in using combinations of β_2-agonists and antimuscarinic agents. In particular, LABA/long-acting antimuscarinic agent (LAMA) combination seems to play an important role in optimizing bronchodilation. Several once-daily and twice-daily LABA/LAMA FDCs have been developed or are in clinical development. LAMA/ICS FDCs seem to be useful in COPD and mainly in asthma, in patients with severe asthma and persistent airflow limitation. The rationale behind the ICS/LABA/LAMA FDCs seems logical because all three agents work via different mechanisms on different targets, potentially allowing for lower doses of the individual agents to be used, accompanied by improved side effect profiles. In effect, in clinical practice, concomitant use of all three compounds is common, especially in more severe COPD but also in the treatment of adults with poorly controlled asthma despite maintenance treatment with high-dose ICS and a LABA.

Keywords
Antimuscarinic agents • Fixed-dose combinations • Inhaled corticosteroids • β2-agonists

1 Introduction

The inverse correlation between the complexity of a drug regimen and medication adherence is well established (Pan et al. 2008). Moreover, it is generally accepted that patient compliance is far better if the dosage frequency is reduced (Bjerrum et al. 2013). For diseases that require treatment with multiple drugs, safe and efficacious fixed-dose combination (FDC) therapy, that is a drug product in which two or more separate active substances are combined in a single dosage form (Bjerrum et al. 2013), offers help in addressing some of the problems of adherence (Bangalore et al. 2007). However, the benefits of combining agents are not merely additive and range from increased compliance via simple convenience to complex receptor-level synergies (Ehrick et al. 2014).

Also in asthma and chronic obstructive pulmonary disease (COPD), an important step in simplifying management and improving adherence with prescribed therapy is to reduce the dose frequency to the minimum necessary to maintain disease control (Tamura and Ohta 2007). In effect, some investigators have reported that adherence to treatment with inhalants is poor because of the complex procedures required to use them, as well as the tedious, frequent dosing (Jones et al. 2003). FDC inhalers are hypothesized to enhance compliance by decreasing the number of medications and/or the number of daily doses (Marceau et al. 2006). Furthermore, they have the potential for enhancing, sensitizing, and prolonging the effects of monocomponents (Cazzola et al. 2012a).

2 Inhaled Corticosteroid/Long-Acting β-Agonist Combinations

Inflammation plays a major role in the pathology of asthma and has an important role in COPD. ICS therapy forms the basis for treatment of asthma of all severities, improving asthma control and lung function and preventing exacerbations of disease [Global Initiative for Asthma (GINA) 2015]. Use of ICS has also been increasingly established in the treatment of COPD, particularly in symptomatic patients, who experience useful gains in quality of life (likely from an improvement in symptoms such as breathlessness and in reduction in exacerbations) and an attenuation of the yearly rate of deterioration in lung function (Cazzola et al. 2013).

The development of combinations of inhaled corticosteroids (ICSs) with long-acting β-agonists (LABAs) has led to FDC inhalers constituting the largest therapeutic segment of the respiratory market. Basically, the National Asthma Education and Prevention Program's Expert Panel Report-3 (EPR-3) (2007), the British Thoracic Society/Scottish Intercollegiate Guidelines Network (2014), and Global Initiative for Asthma (GINA) (2015) guidelines recommend using combination therapy of ICS and LABA for those patients whose asthma is not well controlled on ICS monotherapy. The use of combinations of LABAs and ICSs is also recommended for patients with COPD because, in general, the addition of LABA to ICS provides additional benefits (O'Reilly et al. 2010; Qaseem et al. 2011; Vestbo et al. 2013).

There is an exciting possibility that the observed benefit from combining these two classes of drugs might be due to a synergistic interaction, with the resulting synergetic effect being greater than the sum of responses achieved from each drug alone (Cazzola and Dahl 2004). However, the basic molecular mechanism of such an interaction is still to be fully identified although there are some mechanisms that have been documented (Barnes 2011; Chung et al. 2009). Corticosteroids increase the transcription of the β_2-adrenoceptor (β_2-AR) gene, resulting in increased expression of cell surface receptors. They may also enhance the coupling of β_2-AR to G-protein (G_s), thus enhancing β_2-agonist effects and reversing the uncoupling of β_2-AR that may occur in response to inflammatory mediators, such as IL-1β through a stimulatory effect on a G-protein-coupled receptor kinase. On the other hand, LABAs increase the translocation of glucocorticoid receptor (GR) from cytoplasm to the nucleus after activation by corticosteroids and thus enhance the anti-inflammatory effects of corticosteroids, activate CAAT/enhancer binding protein (C/EBP)α together with corticosteroids, or alter GR phosphorylation. The combination of ICSs and LABAs potentiates inhibition of CXCL8 (IL-8) and CCL11 (eotaxin) release from human airway smooth muscle cells and their proliferation and has additive effects on granulocyte-macrophage colony-stimulating factor (GM-CSF) release from epithelial cells. There are differences between LABAs that must always be considered when using an ICS/LABA combination (Cazzola et al. 2013). In fact, formoterol, but not salmeterol, reverses oxidative stress-induced corticosteroid insensitivity and decreases β_2-AR-dependent cAMP production via inhibition of PI3K-δ signaling (Rossios et al. 2012).

Moreover, it has been shown that budesonide prevents the inhibitory effects of cytokines on formoterol but not salmeterol-induced tracheal relaxation and cyclic adenosine monophosphate (cAMP) signaling (Adner et al. 2010). Formoterol increases corticosteroid sensitivity also via the activation of the serine/threonine protein phosphatase 2A (PP2A) in receptor-independent manner (Kobayashi et al. 2012).

ICS/LABA FDC therapy is the preferred treatment for the long-term treatment of persistent asthma when a medium dose of ICS alone fails to achieve control of asthma [National Asthma Education and Prevention Program's Expert Panel Report-3 (EPR-3) 2007; British Thoracic Society/Scottish Intercollegiate Guidelines Network 2014; Global Initiative for Asthma (GINA) 2015]. Systematic reviews have shown that adding a LABA to low-dose ICS in poorly controlled asthma patients is more effective in reducing the risk of asthma exacerbations than using higher doses of ICS (Ducharme et al. 2010). The protective effect of ICS/LABA combination therapy appeared particularly effective in the following clinically relevant subgroups: individuals 18 years or older, males, African American individuals, and individuals with either moderate-to-severe or severe asthma at baseline (Wells et al. 2012). Obviously, a combination inhaler must always be used, because in many patients, the use of separate inhalers will inevitably result in periods of LABA monotherapy because of poor compliance with ICSs in standard clinical practice. In any case, ICS/LABA combination therapy results in a more rapid improvement in asthma symptoms, lung function, and airway inflammation compared to ICS monotherapy in steroid-naive patients with asthma (Matsunaga et al. 2013). Moreover, there are cost savings when using the combined products compared to the use of individual LABA and ICS inhalers (Shepherd et al. 2008).

Unfortunately, there is still a lack of knowledge regarding safety of LABAs with concomitant ICSs use, with both theoretical arguments and limited empirical evidence that ICSs may mitigate LABA-associated risks (Beasley et al. 2010). However, the current evidence from non-randomized studies shows that combined treatment of LABAs and ICSs is not associated with higher risk of serious adverse events (Hernández et al. 2014).

Nonetheless, since there are no studies showing that LABAs (alone or in conjunction with ICSs) increase survival or positively affect severe asthma exacerbations (those necessitating intubation or hospital-based care), and their serious risks, the FDA recommended that use of the LABA must be stopped, if possible, once asthma control is achieved and the use of an asthma-controller medication, such as an ICS, then be maintained (Chowdhury and Dal Pan 2010). LABAs should be reserved only for patients whose asthma cannot be adequately managed with asthma-controller medication such as an ICS (Chowdhury and Dal Pan 2010).

In COPD, therapy with ICS/LABA is associated with slower progression of lung function loss, decreased exacerbation rate, and improved health-related quality of life compared with treatment with LABAs alone, at least in a subset of patients with a favorable response to treatment with ICSs (Cazzola et al. 2013). Combination therapy is associated with significant difference in mortality when compared to

placebo alone but not with LABA alone (Nannini et al. 2012). Intriguingly, ICSs in combination with LABAs might also reduce cardiovascular disease and all-cause mortality (Zervas et al. 2013). Withdrawal from treatment with ICS on patients with ICS/LABA combination therapy may lead to exacerbation of COPD in some patients (van der Valk et al. 2002). Nonetheless, the risk of pneumonia with ICS/LABA is increased compared with either LABA or placebo and is dose dependent (Crim et al. 2009). In the Investigating New Standards for Prophylaxis in Reduction of Exacerbations (INSPIRE) study, the excess of pneumonia events in patients treated with an ICS/LABA combination treatment was mainly caused by exacerbations that failed to resolve (Calverley et al. 2011). Patients at greater risk of pneumonia with ICS/LABA have more severe obstruction and either a body mass index <19 kg/m^2 or a pneumonia history and comorbidities (DiSantostefano et al. 2014). Multiple comorbidities and use of psychoanaleptics also contribute to an increased risk of pneumonia in more obstructed patients. Patients treated with ICS have a higher airway bacterial load (Garcha et al. 2012), although whether this is a causal association and relates to the greater number of pneumonia events remains to be determined.

The benefits and, but even more, drawbacks of ICSs in COPD explain why all national and international COPD guidelines recommend ICS/LABA FDCs only for patients with severe impairment and high risk of exacerbations (O'Reilly et al. 2010; Qaseem et al. 2011; Vestbo et al. 2013). Actually, ICSs are recommended in combination with LABAs for those patients who have few symptoms but are at a high risk of exacerbations, for those patients who have many symptoms and a high risk of exacerbations [Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015], and also for those suffering from for the asthma–COPD overlap syndrome (Miravitlles et al. 2014).

Several ICS/LABA FDCs (fluticasone propionate/salmeterol, budesonide/formoterol, beclomethasone/formoterol, fluticasone propionate/formoterol, mometasone/formoterol, fluticasone furoate/vilanterol, mometasone/indacaterol, ciclesonide/formoterol) are now commercially available or will become available within the next few years for the treatment of COPD and/or asthma.

Despite sharing a similar basic mechanism of action, ICSs differ in terms of pharmacokinetic characteristics, and this may determine important difference in their efficacy and safety as a result of the different chemical structures of individual agents. This is the case also for LABAs. Unfortunately, there are insufficient clinical data to determine whether there are clinically important differences in efficacy between the various ICS/LABA FDCs. Pharmacological characteristics that could theoretically optimize ICS effectiveness include a low oral and a high pulmonary bioavailability, high receptor-binding affinity, high protein-binding capacity, and a long pulmonary retention time (Papi et al. 2014). Important properties for a LABA include speed of onset of action, duration of action, and agonist activity at the β_2-adrenoceptor (Papi et al. 2014).

The PATHOS study (An Investigation of the Past 10 Years Health Care for Primary Care Patients with Chronic Obstructive Pulmonary Disease), a population-based, retrospective, observational registry study conducted in Sweden, found that

long-term budesonide/formoterol treatment was associated with fewer moderate and severe COPD exacerbations than long-term treatment with fluticasone propionate/salmeterol (Larsson et al. 2013). The findings were robust irrespective of the exacerbation definition used and were not affected by several sensitivity analyses. Compared with budesonide/formoterol, rates of pneumonia, admission to hospital, and mortality related to pneumonia were higher in patients treated with fluticasone propionate/salmeterol (Janson et al. 2013). It has been suggested that since the immunosuppressant potency of fluticasone is reported to be up to tenfold higher than that of budesonide with regard to ex vivo inhibition of human alveolar macrophage innate immune response to bacterial triggers (Ek et al. 1999), this factor alone could explain these findings (Janson et al. 2013). Also differences in pharmacokinetic and pharmacodynamic properties related to differences in lipophilicity and hydrophilicity profiles between fluticasone propionate and budesonide might explain the greater risk of pneumonia with fluticasone. The highly lipophilic fluticasone molecule can remain in the mucosa and epithelial lining fluid of the bronchi longer than budesonide (Dalby et al. 2009), inducing a more potent and longer suppression of local immunity than budesonide, thereby causing an increased risk of local bacterial proliferation and a pneumonia outbreak in patients with severe COPD (Janson et al. 2013).

3 Long-Acting Anti-muscarinic Agent/Long-Acting β-Agonist Combinations

Targeting one bronchodilatory pathway alone may not 'optimize' bronchodilation because airway smooth muscle tone is influenced by more than one nerve system (Cazzola et al. 2012a). Therefore it is not surprising that reversibility (defined as $\geq$15 % increase in post-bronchodilator FEV_1) can be shown with combination salbutamol plus ipratropium in up to two-thirds of COPD patients (Tashkin et al. 2008).

The combination of two bronchodilators with different mechanisms of action to treat patients with COPD is an established medical practice (COMBIVENT Inhalation Aerosol Study Group 1994) and, in any case, several trials have documented that the free combination of a long-acting antimuscarinic agent (LAMA) and a LABA provides significant and sustained improvement in bronchodilation versus alone from day 1, with significant improvements in patient-centered outcomes (Mahler et al. 2012; Vincken et al. 2014; ZuWallack et al. 2014).

The pharmacological mechanism that justifies the combinations of bronchodilators is complex and lies also in the reciprocal influences of cholinergic and adrenergic systems at presynaptic and postsynaptic level (Belmonte 2005; Cazzola and Molimard 2010; Meurs et al. 2013; Pera and Penn 2014). It includes the activation of β_2-ARs and the block of M_3 muscarinic receptors at postsynaptic level. At postsynaptic level β_2-AR signaling limits M_3 mAChR-mediated inositol triphosphate (IP_3) production by several distinct mechanisms, most presumed to involve protein kinase A (PKA). In the other side, the M_3 mAChR blockade seems

to have a great influence on the relaxation induced by β-agonists, presumably blocking the resulting activation of protein kinase C (PKC) and the subsequent phosphorylation of β2-AR and/or G_s protein. Anti-muscarinic agents, inhibiting the postsynaptic G_i-coupled M_2 muscarinic receptors, maintain the ASM relaxation induced by β_2-agonists and sustain adenylyl cyclase (AC) activity. The inhibition of presynaptic M_2 muscarinic receptor may increase the release of acetylcholine into the synaptic space. At presynaptic level, β_2-agonists can decrease the release of acetylcholine (ACh) via a modulation of cholinergic neurotransmission that involves calcium-activated potassium (K_{Ca}) channels. Activation of K_{Ca} channels is thought to hyperpolarize the cell membrane, thus causing reductions in the concentration of intracellular Ca^{2+} and ACh release in prejunctional cholinergic nerves. By contrast, activation of AC enhances ACh release.

Some data seem to indicate that LABA/LAMA combination is able to elicit synergistic effects on isolated human bronchi (Cazzola et al. 2014). A modest synergistic effect in vivo, in patients with COPD, has also been demonstrated (Cazzola et al. 2015a).

The dissimilarities in the onset and duration of action of LABA and LAMA and, in any case, the differences in the devices used for the delivery of these drugs make free combinations uncomfortable and therefore unpredictable, especially if focused on adherence to prescribed treatment (Matera et al. 2015a). It is therefore obvious that there is the need for FDCs of bronchodilators in a single inhaler.

Since it is hoped that FDCs could offer advantages of better compliance, adherence, and cost-efficacy in addition to synergistic action of the components in free combinations in separate devices, several once-daily LABA/LAMA combinations, including QVA149 (combination of indacaterol and glycopyrronium bromide), vilanterol plus umeclidinium bromide, and olodaterol plus tiotropium bromide, have been developed or are in clinical development as FDCs (Cazzola and Matera 2014a).

The results of the pivotal Phase III IGNITE and EXPEDITION programs show that indacaterol/glycopyrronium (QVA149) is able to elicit a significant improvement in lung function and patient-reported outcomes, including breathlessness and rescue medication use, reduced rates of COPD exacerbations, and HRQoL when compared with current standard of care. Moreover, QVA149 is generally well tolerated, with most adverse events being of mild-to-moderate severity (Matera et al. 2015a). Several pivotal clinical trials have documented that also vilanterol/umeclidinium bromide (Matera et al. 2015b) and olodaterol/tiotropium bromide (Buhl et al. 2015) FDCs impact favorably on lung function and other outcome measures such as quality of life, dyspnea, rescue medication use, and exercise capacity, with no clinically meaningful treatment-related changes in vital signs or clinical laboratory parameters.

As there is a progressive attempt to shift attention toward controlling nocturnal symptoms and those present on awakening, which are indicated by epidemiologic studies to be the most troublesome for COPD patients (Kessler et al. 2011), the twice-daily dosing of bronchodilators is still considered a useful approach at least for the symptomatic treatment of COPD. Therefore, two twice-daily LABA/LAMA

FDCs, formoterol/aclidinium bromide and formoterol/glycopyrronium bromide, are under development. Formoterol/aclidinium bromide FDC has been evaluated in COPD patients and evidence suggests that it is efficacious and safe, has a quick onset of action, and is well tolerated (Cazzola et al. 2015b). Formoterol/glycopyrronium bromide (PT003), which is delivered via the eFlow Nebulizer System, is in an earlier stage of development.

4 Inhaled Corticosteroid/Long-Acting Antimuscarinic Agent Combinations

Very few studies published to date have been designed specifically to evaluate the effect of LAMA/ICS combinations on clinical outcomes, and this is an area that warrants future study. Nevertheless, there is evidence that COPD patients demonstrate not only superior spirometric responses but also improved clinically important end points such as dyspnea, health status, and frequency of exacerbations with a LAMA plus an ICS compared with a LABA plus an ICS (Hodder et al. 2007). No LAMA has received regulatory approval for asthma treatment, but there is a growing body of evidence to support the efficacy the addition of tiotropium to an ICS in patients with severe asthma and persistent airflow limitation, with reported improvements in symptoms and/or lung function, an extended time to first asthma exacerbation, and a reduced risk of severe exacerbations (Beeh et al. 2014; Kerstjens et al. 2012; Peters et al. 2010), leading to inclusion of these data in current asthma guidelines [Global Initiative for Asthma (GINA) 2015].

Experimental evidence suggests an influence of corticosteroids on muscarinic receptors. It has been shown that treatment of rats with dexamethasone for 1 week resulted in decreased acetylcholine concentration in the surface epithelium of trachea and intestine, which was accompanied by a reduction in choline acetyltransferase activity (Reinheimer et al. 1998). Moreover, at least in dogs, a treatment with methylprednisolone led to a decreased expression of both M_2 and M_3 muscarinic receptors in airway smooth muscle (Emala et al. 1997) by attenuation of a factor-controlling receptor gene expression. It has also been documented that dexamethasone decreases airway responsiveness to vagus nerve stimulation via two mechanisms: increased M_2 receptor function that results in decreased acetylcholine release and increased degradation of acetylcholine by cholinesterases (Jacoby et al. 2001). It has also been reported that dexamethasone protects against virally induced or antigen-induced M_2-receptor dysfunction and associated hyperresponsiveness (Moreno et al. 2003). Interestingly, glycopyrronium acts synergistically with budesonide in inhibiting inflammatory mediators (Pahl et al. 2006).

Fluticasone furoate/umeclidinium bromide FDC is under development. An initial study demonstrated convincing evidence of a bronchodilating effect of umeclidinium in patients with asthma uncontrolled on ICS and further showed that the effect was greater in patients with fixed versus non-fixed obstruction (Lee et al. 2015).

A phase I study that aimed to compare the systemic exposure to tiotropium and CD 1857 after treatment with the FDC of tiotropium plus BI 54903, a nonsteroidal selective glucocorticoid receptor agonist, when administered once-daily over 21 days via Respimat in healthy volunteers, has been completed. However, results have not been disclosed yet (Cazzola et al. 2012b).

5 Inhaled Corticosteroid/Long-Acting β-Agonist/ Long-Acting Antimuscarinic Agent Combinations

The growing body of evidence suggests that triple therapy with ICS, LABAs, and LAMAs is efficacious, making it an attractive combination in COPD (Cazzola and Matera 2014b). For patients who remain symptomatic despite LABA-ICS combination, GOLD recommends triple therapy with LAMA, LABA, and ICS [Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2015]. The rationale behind this seems logical because all three agents work via different mechanisms on different targets, potentially allowing for lower doses of the individual agents to be used, accompanied by improved side effect profiles. In effect, in clinical practice concomitant use of all three compounds is common, especially in more severe COPD (Ross and Hansel 2014).

Similarly, an add-on LAMA is effective and well tolerated in the treatment of adults with poorly controlled asthma despite maintenance treatment with high-dose ICS and a LABA (McKeage 2015). Thus, LAMAs can provide a valuable option in this difficult-to-treat patient group. Accordingly, a variety of triple combinations are currently under development (Cazzola and Matera 2014b). These inhalers may well improve compliance, but titration of individual component drug doses may prove difficult, and disease severity seems to affect the drug dose–response curve (Ross and Hansel 2014).

Triohale pressurized Metered-Dose Inhaler (pMDI) has been marketed as the world's first triple-combination inhaler to be taken only once a day (ciclesonide 200 μg, formoterol fumarate 6 μg, tiotropium 9 μg,) and is already available in India. This formulation is a suspension-based product and is the only pMDI to contain three therapeutics in one device.

Budesonide, formoterol fumarate, and glycopyrronium pMDI (PT010), a ICS/LAMA/LABA FDC in Pearl Therapeutics' cosuspension technology, the FDC beclomethasone/formoterol plus glycopyrronium (CHF5993) taken twice daily, and fluticasone furoate/vilanterol/umeclidinium are developed once on a daily basis are under clinical evaluation. It is likely that also a FDC with mometasone + indacaterol + glycopyrronium will be developed on a once-daily basis. However, to date there is still no information available regarding it.

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Anti-IgE and Biologic Approaches for the Treatment of Asthma

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Contents

Abstract

Current asthma treatments are effective for the majority of patients with mild-to-moderate disease. However, in those with more severe refractory asthma, agents other than inhaled corticosteroids and beta-agonists are needed both to better manage this group of patients and to avoid the side effects of high-dose corticosteroids and the social and personal hardship endured. Several biological pathways have been targeted over the last 20 years, and this research has resulted in pharmacological approaches to attempt to better treat patients with severe refractory asthma. The flagship of the biologics, the anti-IgE monoclonal antibody, omalizumab, has proven efficacious in selected subgroups of asthma patients. Tailoring asthma treatments to suit specific subtypes of asthma patients is in

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keeping with ideals of personalized medicine. Research in the complex interplay of allergens, epithelial host defenses, cytokines, and innate and adaptive immunity interactions has allowed better understanding of the mechanics of allergy and inflammation in asthma. As a result, new biologic treatments have been developed that target several different phenotypes and endotypes in asthma. As knowledge of the efficacy of these biological agents in asthma emerges, as well as the type of patients in whom they are most beneficial, the movement toward personalized asthma treatment will follow.

Keywords
Anti-IgE • Biologics • Monoclonal antibodies • Severe refractory asthma

1 Introduction

The pathophysiological mechanisms underlying asthma are characterized by discordant responses among various cell types, including airway epithelial and smooth muscle cells, mesenchymal cells, and the hematopoietic cells of the adaptive and innate immune systems. The interplay among these cell groups involves a complex pattern of cytokine-driven processes, resulting in cell migration and recruitment, pro-inflammation, and antiapoptotic and proliferative states.

The significant majority of patients with a diagnosis of asthma respond well to conventional inhaled treatments. However, about 5% of asthmatics have severe refractory asthma that fails to achieve an adequate response to both high-dose inhaled corticosteroids (ICS) and systemic corticosteroid treatment. These patients also account for 50% of the health expenditure on asthma (Turner et al. 2011), and the more severe the disease, the greater the healthcare cost (Jacob et al. 2016). The pathophysiology of severe refractory asthma differs from milder asthma with more persistent chronic airway inflammation, steroid resistance, and airway remodeling (Bergeron et al. 2006). Asthma treatments used in the severe asthma population, especially systemic corticosteroids and xanthine derivatives, have well-established side effect profiles (Pandya et al. 2014). Other therapies with better or equivocal clinical efficacy and fewer side effects are required.

Asthma is not a single disease entity and represents a heterogeneous mix of several overlapping phenotypes, characterized by varying cellular immunogenic and inflammatory pathways (Anderson 2008). Atopic asthma is an immune-inflammatory response mediated by T-helper-type (Th2) lymphocytes. The atopic asthma phenotype arises from a complex interplay between the innate and adaptive immune system (Woodruff et al. 2009). Multiple allergens such as pollens, house-dust mite, and animal dander have proteolytic properties. Trace amounts of bacterial constituents such as lipopolysaccharide (LPS) and viral RNA or DNA cause activation of the innate and adaptive immune system. These pathways of the innate and adaptive immune system are stimulated by these environmental and/or endogenous pathogen-associated molecular patterns (PAMPs) and danger-associated

molecular patterns (DAMPs) that influence the activation and trafficking of dendritic cells (DCs), cytokine production, and the hematopoietic system (Holgate 2012).

Airway epithelium exposed to inhaled allergens can prime Toll-like receptor (TLR) class of pattern recognition receptors involved in innate immunity. Activation of TLR generates the production of innate epithelial-derived cytokines such as thymic stromal lymphopoietin (TSLP) and interleukin-25 (IL-25) and interleukin-33 (IL-33) (known collectively as the epithelial alarmins) which elicit the development of Th2 adaptive responses (Gregory and Lloyd 2011). The "phenotyping" of asthma by its distinct functional and pathophysiological contrivances has allowed a more targeted and personalized approach to treat asthma. T-helper (Th) cell subsets secrete specific cytokines, which, in turn, influence the development and perpetuation of systemic inflammation. Th cells and their cytokines can be categorized into two general subsets, termed Th1 and Th2. The cytokines generated by Th1 or Th2 cells inhibit the cellular function of the other phenotype. As examples, interferon gamma and interleukin (IL)-12, which are produced by Th1 cells, inhibit Th2 cell proliferation, and IL-4 and IL-10, which are produced by Th2 cells, inhibit Th1 cytokine production. The concept that specific types of immune response are dominated exclusively by Th1 or Th2 response is now recognized as too simplistic to explain allergic or inflammatory disease in their entirety. However, many biologic therapies have been developed for the purpose of targeting the production of either Th1 or Th2 pro-inflammatory cytokines. Novel therapies have been developed that are more specific to targeting these phenotypes. The content of this chapter will discuss biologic therapies. Biologics, also known as "biologicals," are by definition an array of protein-based therapies (Cook and Bochner 2010). The majority of biologics studied or used in asthma have been monoclonal antibodies (Table 1).

2 Anti-IgE Biologic Treatments

The anti-IgE monoclonal antibody omalizumab was the first biologic treatment approved for the treatment of allergic asthma. In 1921, Prausnitz and Küstner described the passive transfer of an allergen-specific response in the skin with serum (Frankland 2004). Forty-five years later, Ishizaka and Ishizaka and Johansson and Bennich identified the protein as being immunoglobulin IgE (Ishizaka et al. 1967). Further studies determined the mechanism of cell activation through cross-linkage of cell-bound IgE on mast cells and basophils. This cross-linkage was shown to involve dimerization of the high-affinity IgE receptor, FcεR1. Cross-linkage leads to noncytotoxic degranulation and production of newly formed lipid mediators. These lipid mediators are responsible for the allergic responses. The discovery that both mast cells and basophils can also release preformed and newly generated cytokines, chemokines, and growth factors helps explain how the acute allergic response transits into late phase and the more chronic responses associated with leukocyte recruitment and activation of tissue remodeling

Table 1 Biologicals evaluated for the treatment of asthma

Target cytokine/ receptor or antibody	Drug name	Mechanism of action	Developmental status	Reference
IgE	Omalizumab	Blocks IgE to FcεRI–Ig fusion protein and membrane FcεRI	Approved by the FDA and EMA	Johansson et al. (2002)
	Ligelizumab	Binds Cε3 domain of IgE	Phase IIa trial 2014 completed	Arm et al. (2014)
	CMAB007	Blocks IgE to FcεRI–Ig fusion protein and membrane FcεRI	Phase I study 2012 completed	Zhou et al. (2012)
IL-4/IL-13	Dupilumab	Binds IL-4Rα inhibiting both IL-4 and IL-13 signaling	Phase I study 2014 completed	Wenzel et al. (2013)
	AMG 317	AMG 317 is a fully human monoclonal IgG2 antibody to IL-4R	Phase II study 2010 completed[a]	Corren et al. (2010)
	Pitrakinra	Recombinant human IL-4 variant that inhibits both IL-4R and IL-13R	Phase IIb study 2011 completed[a]	Wenzel et al. (2007)
	Altrakincept	Soluble recombinant human IL-4R	Phase III trial completed[a]	Borish et al. (1999)
	Pascolizumab	Humanized mAb blocking IL-4	Phase II trial completed[a]	Hart et al. (2002)
IL-13	Lebrikizumab	IgG4-humanized monoclonal antibody that binds IL-13	Phase IIb trial completed. Ongoing trials	Scheerens et al. (2014)
	Tralokinumab	Human monoclonal antibody that neutralizes IL-13	Phase III trial ongoing	Piper et al. (2013)
IL-5	Mepolizumab	Anti-IL-5 humanized IgG1 monoclonal antibody	EBM approval 2014	Nair et al. (2009)
	Reslizumab	Humanized monoclonal anti-IL-5 antibody	Phase III trial completed	Lim and Nair (2015)
	Benralizumab	Humanized, afucosylated monoclonal antibody against IL-5Rα	Phase IIb trial completed	Nowak et al. (2015)
IL-9	MEDI-528	Humanized IgG1 monoclonal antibody that binds to IL-9	Phase IIb completed[a]	White et al. (2009)
IL-17 and IL-23	Brodalumab	Human IL-17RA-specific monoclonal antibody	Phase II ongoing 2015	Busse et al. (2013)

(continued)

Table 1 (continued)

Target cytokine/ receptor or antibody	Drug name	Mechanism of action	Developmental status	Reference
	Secukinumab	Monoclonal antibody that neutralizes IL-17A	Phase II, ongoing 2015	Wakashin et al. (2008)
TSLP	AMG 157	Human anti-TSLP monoclonal antibody that binds TSLP	Phase I completed	Gauvreau et al. (2014)
TNF	Infliximab	Human–murine chimeric anti-TNFα monoclonal antibody	Phase II completed[a]	Erin et al. (2006)
	Etanercept	Soluble TNFα receptor fusion protein	Phase II completed[a]	Holgate et al. (2011)
	Golimumab	Fully human TNFα-blocking antibody	Phase II trial (withdrawn)[a]	Wenzel et al. (2009)
CCR4	Mogamulizumab	Defucosylated	Phase I trial 2011[a]	Subramaniam et al. (2012)
C5	Eculizumab	Humanized monoclonal antibody cleaves and deactivates C5	Phase II 2008 completed[a]	Smith et al. (2012)

EMA European Medicines Agency
[a]No further studies ongoing or yet planned (www.clinicaltrials.gov)

pathways. FcεR1 has also been found on antigen-presenting dendritic cells (DCs) where they function to facilitate the uptake and processing of allergens that augment sensitization (Sharquie et al. 2013).

Blocking IgE, without causing membrane-bound cross-linkage, had been a target for asthma drug development for many years (Holgate 2014). During the allergic immune response, allergen-specific B cells are stimulated by IL-4 and IL-13, provided by Th2 cells, and begin IgE production. IgE antibodies act by arming cells bearing either high-affinity IgE receptors (FceRI) such as mast cells, basophils, and dendritic cells or low-affinity IgE receptors (FceRII, CD23) such as B cells, monocytes, and other inflammatory cells (Heusser and Jardieu 1997). A breakthrough came about when an αFceRI-IgG fusion protein was shown to inhibit passive cutaneous anaphylactic responses (Haak-Frendscho et al. 1993). A further discovery was the identification that the Cε3 region of the Fc fragment of IgE binds very selectively to a particular component of the α-chain of the tetrameric FcεR1 (α1β1γ2); C epsilon4 is not required for binding (Baird et al. 1986). This feature of IgE allows the Cε3 region to be targeted and blocked without causing cross-linking of IgE bound to its high-affinity receptor. A chimeric IgG monoclonal antibody that was active in humans was produced in mice. This antibody was shown to be

effective in reducing circulating IgE and most importantly without causing an anaphylactic response (Corne and Holgate 1997). Omalizumab was created by humanizing a non-anaphylactogenic IgG1 antihuman IgE mAb that contained only the antigen-binding site as mouse sequences. Omalizumab is a recombinant humanized monoclonal antibody, which specifically binds to the C epsilon3 domain of IgE that is the site of high-affinity IgE receptor. It is therefore able to reduce free IgE levels and avoid binding IgE to Fcϵ RI (this binding induces consequent cross-linking of IgE triggering degranulation and synthesis of new-generated chemical mediators) (Easthope and Jarvis 2001).

Omalizumab expresses a high degree of isotype specificity and can neutralize serum-free IgE without affecting other antibody classes. Omalizumab is administered via a subcutaneous injection. Its bioavailability is 62% with an estimated half-life elimination of 26 days. The time to peak levels is 7–8 days. Excretion is primarily via hepatic degradation and intact IgG may be secreted in the bile (Hayashi et al. 2007; Lowe et al. 2009).

Initial studies with omalizumab showed attenuation of allergen-induced asthmatic responses (Fahy et al. 1997), followed by studies demonstrating improvements in asthma symptoms and health-related quality of life (Busse 2001) and a significant reduction in the frequency of asthma exacerbations in allergic asthmatic patients treated with omalizumab (Sorkness et al. 2013). Furthermore, omalizumab was also used in the treatment of children with allergic asthma, demonstrating improvements in health-related quality of life as well as a significant dosage reduction of ICS (Busse et al. 2011). Omalizumab given to patients with allergic rhinitis resulted in a rapid, but importantly dose dependent, suppression of circulating serum-free IgE levels. Omalizumab significantly improves health-related quality of life and nasal symptoms in patients with seasonal allergic rhinitis (Stokes and Casale 2015). Omalizumab has been evaluated in patients with severe refractory asthma. The INNOVATE study demonstrated that treatment with omalizumab significantly reduced the rate of severe asthma exacerbations by 50% and the rate of total emergency visits for asthma. A greater proportion of patients receiving omalizumab achieved a clinically meaningful Asthma Quality of Life Questionnaire (AQLQ) score (greater than a 0.5-point) improvement from baseline compared with placebo recipients (Humbert et al. 2005).

Omalizumab has been shown to have a beneficial effect on airway remodeling in asthmatic patients. One study investigated the effect of long-term anti-IgE on the thickening of the reticular basement membrane (RBM) and eosinophil infiltration in bronchial biopsies from patients with severe persistent allergic asthma before and after 12 months of treatment with omalizumab. The study showed that a substantial proportion of severe asthmatics had reduced the levels of airway eosinophil and RBM thickness after treatment with omalizumab (Riccio et al. 2012). In another study omalizumab demonstrated reduced airway wall thickness and airway inflammation that correlated to a lowering of the sputum eosinophil count and increase in the FEV_1 (Hoshino and Ohtawa 2012).

The optimal duration of treatment of omalizumab is not known. One study suggested a reemergence of asthma symptoms with increasing serum-free IgE

levels when omalizumab is stopped (Slavin et al. 2009). Another study followed 18 patients for 3 years after withdrawal of omalizumab reported improved or unchanged asthma compared with ongoing omalizumab treatment (Nopp et al. 2010).

The safety profile of omalizumab has been extensively studied. Pooled data from 7,500 subjects from all randomized trials indicate an overall incidence of adverse events with omalizumab similar to that in the placebo or control groups (Corren et al. 2009). No cases of serum sickness were reported. None of the omalizumab recipients developed measurable anti-omalizumab antibodies (Corren et al. 2009). Anaphylaxis was reported in 124 patients treated with omalizumab from June 2003 to Dec 2006, equating to a rate of 2 per 1,000 patients treated. Some of these cases of anaphylaxis occurred more than 2 h after omalizumab administration (Limb et al. 2007).

Anti-parasite IgE has been associated with immunity against a range of helminth infections, and IgE and its receptors have evolved to help defend from parasite infection (Fitzsimmons et al. 2014). A 52-week, randomized, double-blind, parallel-group, placebo-controlled study in patients deemed at high risk of geohelminth infection was found to have a slightly, but not significantly, higher number of patients with one or more geohelminth infections during treatment in the omalizumab group (odds ratio 1.47, 95% CI 0.74–2.95) (Cruz et al. 2007). Clinically, the severity of infection and response to anthelmintic treatment was unaltered by omalizumab therapy (Cruz et al. 2007).

Initial comparative analysis from clinical trials revealed a worryingly higher incidence of malignancy in the omalizumab group (0.5%) when compared to the placebo group (0.2%) (Busse et al. 2012). However, when compared to the incidence of malignancy in the general population, the incidence of malignancy in the omalizumab group was found to be nonsignificant (Corren et al. 2009). The incidence of primary malignancy per 1,000 patient years was 4.14 in the omalizumab group compared with 4.45 in the placebo group (Busse et al. 2012). To establish the potential risk for malignancy during routine clinical practice, a prospective post-marketing safety evaluation was requested by the FDA (Long et al. 2014). Both the omalizumab and the non-omalizumab cohorts were followed for approximately 5 years. Malignancy rates were similar in the omalizumab and non-omalizumab cohorts, with a hazard ratio, adjusted for confounders and risk factors, of 1.09 (95% CI, 0.87–1.38) for all malignancies and 1.15 (95% CI, 0.83–1.59) for all malignancies excluding nonmelanoma skin cancer (Long et al. 2014). It is worthwhile noting that the protocol excluded persons with any increased risk of malignancy or cancer history (Long et al. 2014). The FDA has advised that they are continuing to monitor as data emerges on omalizumab.

Omalizumab does cross the placental barrier and this suggests that the potential for fetal harm exists. Omalizumab is therefore not recommended for use during pregnancy unless absolutely necessary. The omalizumab pregnancy registry was a prospective observational study of pregnant women exposed to one or more doses of omalizumab within 8 weeks prior to conception or at any time during pregnancy. The prevalence of major congenital defects was no higher than that reported in the

general population with asthma. In addition, omalizumab does not appear to increase the risk of preterm birth or small for gestational age infants beyond that seen in the general asthma population (Namazy et al. 2015).

Maximizing control of free unbound IgE has been suggested to offer superior asthma symptom control (Lowe et al. 2009). Ligelizumab is another humanized IgG1 monoclonal antibody that binds with very high affinity to the Cε3 domain of IgE. Ligelizumab may achieve superior IgE suppression than omalizumab and lead to better clinical outcomes. Two small trials have been conducted with ligelizumab; one administered ligelizumab intravenously and the other subcutaneously (Arm et al. 2014). Ligelizumab demonstrated higher-affinity binding for human IgE compared with omalizumab, with an equilibrium KD of 139 pm vs. 6.98 nm, respectively. These trials concluded that treatment with ligelizumab provided greater and longer suppression of free IgE and IgE on the surface of circulating basophils and markedly superior suppression of skin prick test responses to allergen compared to omalizumab. Another humanized anti-IgE monoclonal antibody, CMAB007, was studied in a phase I, single-arm, open-label study. No anti-CMAB007 antibodies were detected after dosing in any subject. Subcutaneous administration of CMAB007 was well tolerated and seemed to be effective in reducing free IgE in healthy Chinese volunteers. Further trials have yet to be conducted (Zhou et al. 2012).

Omalizumab was approved by the US Food and Drug Administration (FDA) in 2003 for persons 12 years of age and older with moderate-to-severe persistent allergic asthma whose symptoms are inadequately controlled with ICS (Hussar 2004). In the European Union, omalizumab is approved for the treatment of patients 6 years and older with severe persistent allergic asthma inadequately controlled with ICS and LABA.

3 Anti-IL-4/IL-13 Biologics

IL-4, a multifunctional pleiotropic cytokine, was discovered in the mid-1980s (REF). This mediator is produced mainly by activated T cells but also by mast cells, basophils, and eosinophils (Nelms et al. 1999). There is significant functional homogeneity between IL-4 and IL-13 as they play their biological roles by activating a heterodimeric receptor complex consisting of the IL-4 receptor α-subunit (IL-4Rα) and the IL-13 receptor α1-subunit (IL-13Rα1) (Oh et al. 2010).

Functionally, IL-4 is best known for defining the Th2 phenotype of lymphocytes and for regulating cell proliferation, apoptosis, and expression of numerous genes in various cell types, including lymphocytes, macrophages, and fibroblasts, as well as epithelial and endothelial cells (Kelly-Welch et al. 2003). Dupilumab is a fully human monoclonal antibody to the IL-4Rα that inhibits both IL-4 and IL-13 signaling. In a proof of principal study, patients with persistent, moderate-to-severe asthma and an elevated blood or sputum eosinophilia, who used medium-dose to high-dose ICS and LABAs, dupilumab was associated with fewer episodes of loss of asthma control when LABAs and inhaled glucocorticoids were withdrawn, with

improved lung function and reduced levels of Th2-associated inflammatory markers (Wenzel et al. 2013).

Several monoclonal antibodies that have targeted IL-4, or its receptor, have not demonstrated clinical benefit in asthma. AMG 317 is a fully human monoclonal IgG2 antibody to IL-4R. In a phase II, randomized, double-blind, placebo-controlled study, patients received weekly subcutaneous injections of placebo or AMG 317. The primary endpoint was changed from baseline at week 12 in Asthma Control Questionnaire (ACQ) symptom score. AMG 317 did not demonstrate clinical efficacy across the overall group of patients. Clinically, significant improvements were observed in several outcome measures in patients with higher baseline ACQ scores (Corren et al. 2010). Pitrakinra is a recombinant human IL-4 variant that is a potent inhibitor of both the IL-4 and IL-13 receptors (Antoniu 2010). A large clinical trial of 534 patients with moderate-to-severe asthma, who were inadequately controlled on the combination of ICS and LABA, found that the addition of inhaled pitrakinra failed to show clinical benefit compared with placebo (Wenzel et al. 2007). Altrakincept is a soluble recombinant human IL-4R, which inactivates IL-4 without mediating cellular activation. In a small double-blind, placebo-controlled trial, patients with moderate asthma requiring inhaled corticosteroids were randomly assigned to receive a single nebulized dose of altrakincept. Patients in the highest dosing arm required significantly less LABA rescue use. The anti-inflammatory effects were further demonstrated by significantly reduced exhaled nitric oxide (Borish et al. 1999). Finally, pascolizumab is a humanized anti-IL-4 monoclonal antibody that also failed to demonstrate clinical benefit (Hart et al. 2002).

IL-13 increases goblet cell differentiation, promotes eosinophil migration to the airway, activates fibroblasts enabling their transformation into myofibroblasts, and switches B-cell antibody production from IgM to IgE (Corren 2013). IL-13R is a heterodimer complex consisting of IL-13Rα1 and IL-4Rα, expressed on B cells, monocytes and macrophages, dendritic cells, eosinophils, basophils, fibroblasts, endothelial cells, airway epithelial cells, and airway smooth muscle cells (Skowronzwarg et al. 2007). Several biologics have been developed targeting either IL-13 or its receptor IL-13R. Lebrikizumab is an IgG4-humanized monoclonal antibody that binds IL-13 with high affinity at an epitope that strongly overlaps with the binding site of IL-4α and inhibits its activity. In a randomized, double-blind study, poorly controlled asthmatics on high-dose ICS received lebrikizumab or placebo. The primary endpoint of FEV_1 significantly increased by 9.8% from baseline compared with 4.3% for placebo (Corren et al. 2010). Secondary endpoints, including ACQ5 and rates of asthma exacerbations, were not significantly reduced by active treatment, although the study was not powered to detect differences in asthma exacerbations. The effect of lebrikizumab on lung function was greatest in patients with high serum periostin levels. In another study, asthmatic subjects, not on ICS, were treated with either lebrikizumab or placebo. Both primary (post-bronchodilator FEV1 at 12 weeks) and secondary endpoints were not meeting (Noonan et al. 2013).

Tralokinumab is another human anti-IL-13 monoclonal antibody that was studied in a trial involving subjects with uncontrolled asthma. The primary endpoint in this study, ACQ6, was not affected by treatment with tralokinumab compared with placebo. However, changes in FEV1 at week 13 in patients with detectable sputum IL-13 demonstrated a trend toward improvement with tralokinumab (Piper et al. 2013). IMA-638 and IMA-026 are fully humanized IgG1 antibodies that bind to different epitopes on IL-13 and neutralize its bioactivity. These antibodies were studied in subjects with mild, atopic asthma challenged with inhaled allergen. The antibody that prevented binding of IL-13 to IL4Rα, but not that which prevented binding to IL-13Rα1, significantly attenuated allergen-induced early and late asthmatic responses (Gauvreau et al. 2014). There was no effect of either antibody on allergen-induced airway hyperresponsiveness or sputum eosinophils.

4 Anti-IL-5 Biologics

Interleukin-5 (IL-5) has a central role in the maturation, recruitment, activation, and enhanced survival of eosinophils. Many studies of allergen-induced airway responses in mice suggested IL-5 plays an important role in allergen-induced airway eosinophilia and airway hyperresponsiveness. Several clinical trials aimed at neutralizing circulating IL-5 or targeting the IL-5Rα (Lim and Nair 2015; Mukherjee et al. 2014; Nowak et al. 2015).

The flagship of this class of biologics is the anti-IL-5 humanized IgG1 monoclonal antibody mepolizumab (Pavord et al. 2012). Mepolizumab has a high affinity for binding free IL-5. An early clinical trial in nonselected patients with moderate-to-severe asthma did not demonstrate mepolizumab to be effective in improving asthma symptoms or lung function (Flood-Page et al. 2003). However, two studies with carefully phenotyped patients, with persistently elevated eosinophil counts in blood and sputum and who also had history of frequent exacerbations, demonstrated a significant decrease in the exacerbation frequencies and improvement in asthma control. One of these studies also showed an oral steroid-sparing effect in the mepolizumab arm (Nair et al. 2009). Lower doses of mepolizumab (75 and 100 mg) were chosen in the MENSA study where significant reductions in exacerbation rates (47–53%) compared to placebo were seen (Ortega et al. 2014) (Fig. 1). In the SIRIUS study, mepolizumab 100 mg was shown to have a corticosteroid-sparing effect in the order of 50% (Bel et al. 2014). Mepolizumab has been shown to be clinically beneficial in asthma patients with moderate-to-severe asthma with high sputum and/or blood eosinophil counts and has recently been approved for use in severe eosinophilic asthma patients by the European Medicines Agency.

Reslizumab is another humanized monoclonal anti-IL-5 antibody (IgG4/k) that exhibits an extremely long duration of action in mice, monkeys, and guinea pigs (Egan et al. 1999). In an initial clinical trial, reslizumab caused a reduction in sputum eosinophils and a significant improvement in lung function in patients diagnosed with severe refractory eosinophilic asthma (Castro et al. 2011). Two

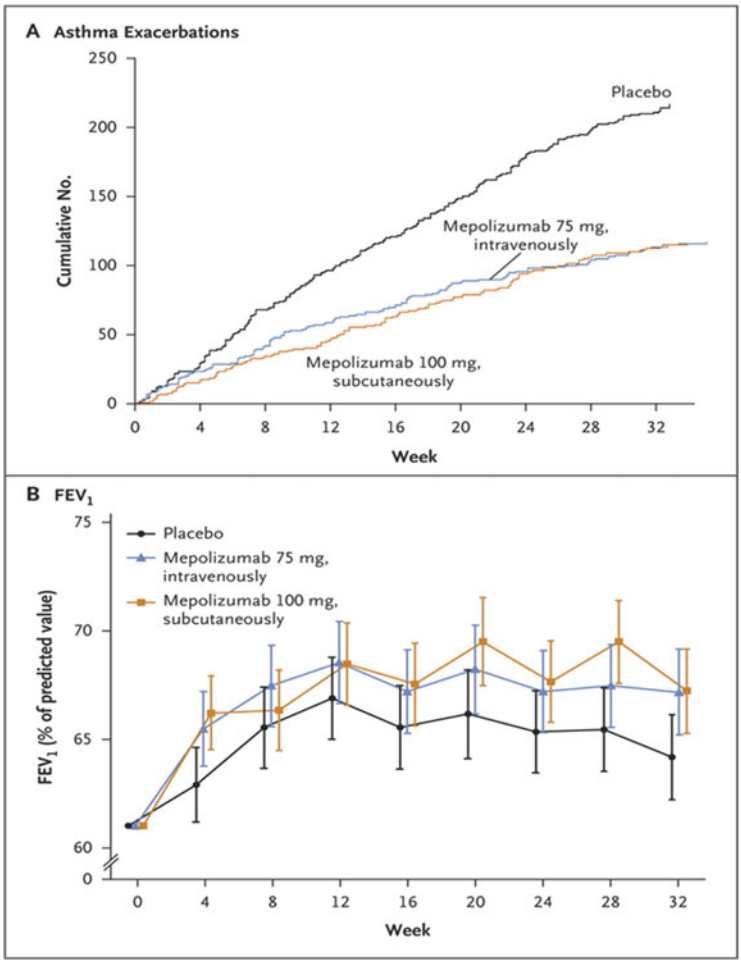

Fig. 1 Effect of mepolizumab treatment or placebo on asthma exacerbations and the forced expiratory volume in 1 second (FEV1) at 32 weeks of treatment (Reproduced with permission from Ortega et al. 2014)

duplicate, multicenter, double-blind, parallel-group, randomized, placebo-controlled trials have compared reslizumab to placebo (Castro et al. 2015). The inclusion criteria were patients with asthma inadequately controlled by medium-to-high doses of inhaled corticosteroid and with a blood eosinophil count of >400/μL and one or more asthma exacerbations in the previous year. In both studies, patients receiving reslizumab had a significant approximately 50% reduction in the frequency of asthma exacerbations compared with those receiving placebo. Adverse events were similar in both the treatment and placebo groups. In the reslizumab arms, 0.4% of subjects suffered anaphylaxis that responded to conventional treatment (Castro et al. 2015).

IL5Rα is expressed by both mature eosinophils and eosinophil-lineage progenitor cells (Rothenberg and Hogan 2006). Benralizumab is a humanized, afucosylated monoclonal antibody. Being afucosylated, benralizumab induces apoptosis in its target cells by enhanced antibody-mediated cellular toxicity. This feature may confer benralizumab with increased efficiency to reduce eosinophil numbers in comparison to the other anti-IL-5 biologics (Ghazi et al. 2012). In a small initial, double-blind, placebo-controlled study, a single-dose intravenous and multiple-dose subcutaneous benralizumab reduced eosinophil counts in airway mucosa and sputum. Benralizumab also suppressed eosinophil counts in the bone marrow and peripheral blood (Laviolette et al. 2013). In another trial, 20–100 mg of subcutaneous benralizumab exhibited significant improvement in annual exacerbation rates, lung function, and asthma scores, with greater benefits seen in patients with blood eosinophil levels >400 cells/μl (Castro et al. 2014).

5 Anti-IL-9 Biologics

IL-9 was first described in the mouse as a T cell and mast cell growth factor (Hultner et al. 1990). The function of IL-9 in asthma and allergy has been investigated by research into its pleiotropic activities on cell types associated with allergic diseases including Th2 lymphocytes, mast cells, B cells, eosinophils, and airway epithelial cells (Xing et al. 2011). In the largest human clinical study of anti-IL-9 to date, 327 subjects were randomized to subcutaneous placebo or MEDI-528 in addition to their usual asthma medications. The authors concluded that the addition of MEDI-528 to existing asthma controller medications was not associated with any improvement in asthma control, asthma exacerbation rates, or FEV_1 (Oh et al. 2010).

6 Anti-IL-17 and Anti-IL-23 Biologics

IL-17A, IL-17F, and IL-23 (IL-23 is a growth factor and inducer of pro-inflammatory Th17 cells, which secrete IL-17 (Aggarwal et al. 2003) can upregulate the antigen-induced recruitment of both neutrophils and eosinophils into the airways (Nakajima and Hirose 2010). In vitro, IL-17A and IL-17F have been shown to augment glucocorticoid receptor-beta expression in bronchial epithelial cells that correlates with decreased cellular response to dexamethasone (Vazquez-Tello et al. 2010). IL-17 cytokines may have a role in steroid-resistant asthma (Vazquez-Tello et al. 2013). They have been shown to be upregulated in bronchial biopsy samples obtained from patients with severe asthma (Al-Ramli et al. 2009). In a randomized placebo-controlled trial of patients with severe asthma that was not adequately controlled by ICS and LABAs, a human IL-17RA-specific monoclonal antibody, brodalumab, failed to show clinical benefit for the overall study population (Busse et al. 2013). Secukinumab, an anti-IL-17 monoclonal antibody that selectively neutralizes IL-17A, is currently being studied in patients

with asthma. Another treatment approach is the use of biologics directed against the IL-17-regulating cytokine IL-23 targeting inhibition of IL-17-dependent recruitment of neutrophils, eosinophils, and lymphocytes into the airways of sensitized mice (Wakashin et al. 2008).

7 Anti-IL-25 Biologics

IL-25, an epithelial-derived cytokine, also known as IL-17E, is a member of the IL-17 cytokine family and is expressed by Th2 cells and activated mast cells, basophils, and eosinophils (Corrigan et al. 2011). The systemic administration of IL-25 in mice causes eosinophilia via the production of IL-5 and enhances the production of other Th2 cytokines such as IL-4, IL-13, and eotaxin (Fort et al. 2001; Tamachi et al. 2006). IL-25 acts principally on its own receptor called IL-25R, a 56-kd single transmembrane protein composed of two subunits, IL-17RA and IL-17RB. IL-25R expression is found on T cells, eosinophils, mast cells, and endothelial cells and is upregulated in response to allergen challenge (Corrigan et al. 2011). Increased plasma levels of IL-25 are seen in allergic asthmatic subjects, and the expression of IL-17RB is higher on eosinophils compared to non-asthmatic controls (Tang et al. 2014). Blocking IL-25 in a mouse model of allergic asthma caused a significant reduction in BAL fluid levels of IL-5 and IL-13, serum IgE, and eosinophils and prevented AHR (Ballantyne et al. 2007). There are no studies yet reported of the effects of blocking IL-25 in asthmatic subjects.

8 Anti-IL-33 Biologics

IL-33 is an IL-1-like pro-inflammatory epithelial-derived cytokine (Haraldsen et al. 2009). In its full form, it is biologically active; however, its bioactivity can be increased tenfold following processing by inflammatory proteases such as neutrophil elastase and cathepsin G, whereas processing by caspases inactivates IL-33 (Lefrancais et al. 2012). IL-33 drives production of Th2-associated cytokines, including IL-4, IL-5, and IL-13, by various hematopoietic cells (Yagami et al. 2010). The receptor for IL-33, ST2L, is an IL-1 receptor-related protein expressed on mast cells and, to a lesser extent, on macrophages, hematopoietic stem cells, NKT cells, eosinophils, basophils, innate lymphoid cells (ILC2), and fibroblasts (Neill et al. 2010; Pecaric-Petkovic et al. 2009). Another form of ST2 exists as a soluble receptor, sST2 that acts a decoy ligand for IL-33 (Ho et al. 2013). IL-33 is expressed at higher levels in asthmatic subjects particularly those with severe asthma (Prefontaine et al. 2009). The IL-33/ST2 axis is involved in Th2/IL-31 and Th17 immune response during the progression of allergic airway disease (Jacob et al. 2016). IL-33 also seems to modulate hematopoietic progenitor cells in patients with allergic asthma (Smith et al. 2015). These features make the inhibiting or blocking of the IL-33/ST2 axis an attractive option in pharmacological management of asthma. Anti-IL-33 monoclonal antibody treatments have been reported to

inhibit allergen-induced eosinophilic airway inflammation, mucus hypersecretion, and Th2 cytokine production in mice (Mizutani et al. 2013). Both the membrane-bound ST2 receptor and the soluble ST2 receptor could also both be feasible targets for biologic asthma therapy (Nabe 2014). A study found that in a murine model of allergic asthma, sST2 exerts a negative regulation on OVA-mediated allergic airway inflammation (Lee et al. 2014). While neutralizing the IL-33/ST2R axis in allergic asthma has significant potential clinical benefits in asthma subject, caution needs to be paid to other biological roles IL-33 has and where its inhibition may be harmful (Schiering et al. 2014).

9 Anti-TSLP Biologics

Thymic stromal lymphopoietin (TSLP), an epithelial cell-derived cytokine, is generated in response to pro-inflammatory stimuli and drives allergic inflammatory diseases such as asthma (Ying et al. 2005, 2008). TSLP is thought to cause airway and blood eosinophilia among patients with allergic asthma by activating airway dendritic cells and mast cells and by increasing the numbers of Th2 cells, resulting in the production of pro-inflammatory cytokines, including interleukin-5 and interleukin-13 (Zhou et al. 2005). Given the plethora of cytokines and cellular dynamics, TSLP is an attractive target to inhibit to blunt the allergen-induced airway response in asthma (Ziegler 2012). AMG 157 is a fully human anti-TSLP monoclonal immunoglobulin G2λ that specifically binds human TSLP and prevents interaction with its receptor (Gauvreau et al. 2014). In a double-blind, placebo-controlled study, 31 patients with mild allergic asthma were randomly assigned to receive three monthly doses of AMG 157 or placebo intravenously. AMG 157 reduced allergen-induced bronchoconstriction and indexes of airway inflammation before and after allergen challenge. AMG 157 attenuated allergen-induced early and late asthmatic responses. In addition, in patients receiving AMG 157, there was a significant decrease in levels of blood and sputum eosinophils before and after the allergen challenge (Fig. 2) (Gauvreau et al. 2014).

10 Anti-TNF Biologics

Tumor necrosis factor alpha (TNFα) is the most widely studied pleiotropic cytokine of the TNF superfamily. In pathophysiological conditions, generation of TNFα at high levels leads to the development of inflammatory responses that are hallmarks of many diseases (Mukhopadhyay et al. 2006). TNFα is stimulated by multiple factors including IL-1, bacterial endotoxins, TNFα itself, platelet-derived growth factor (PDGF), and oncostatin M. In epithelial, endothelial, and fibroblastic cells, secretion of TNFα is induced by IL-17 (Mukhopadhyay et al. 2006).

TNFα has been implicated in the pathogenesis of asthma (Berry et al. 2006; Cembrzynska-Nowak et al. 1993; Thomas and Heywood 2002). Several anti-TNFα biologics have been studied in asthma. In a double-blind, placebo-controlled,

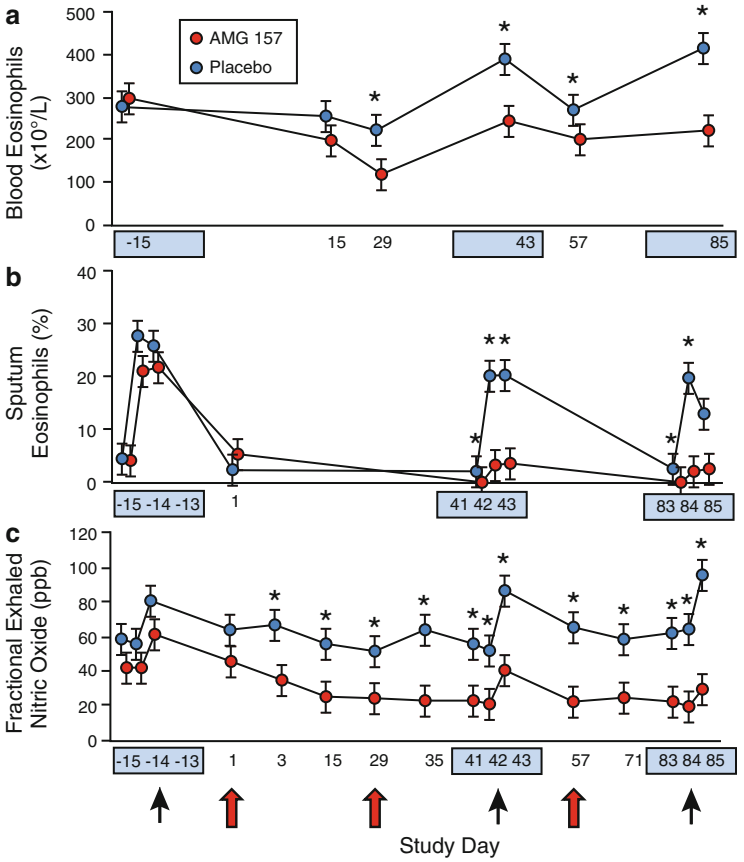

Fig. 2 Effect of treatment with an anti-TSLP hMab or placebo on blood and sputum eosinophils and FeNO before and after allergen inhalation challenge in mild allergic asthmatic subjects. *Red arrows* indicate treatment administered monthly for 3 months. *Black arrows* indicate allergen inhalation challenge before the treatment started (day 14) and after 6 weeks (day 42) and 12 weeks of treatment (day 84). (Drawn from data from Gauvreau et al. 2014)

parallel-group design study in 38 patients with moderate asthma treated with inhaled corticosteroids, but symptomatic during a run-in phase, the recombinant human–murine chimeric anti-TNFα monoclonal antibody infliximab caused a decrease in the number of patients with exacerbations (Erin et al. 2006). Two small trials using etanercept, a soluble TNFα receptor fusion protein administered to patients with severe asthma, demonstrated clinical benefits (Berry et al. 2006; Howarth et al. 2005). However, in a larger randomized, double-blind, placebo-controlled study involving 132 asthmatic subjects with moderate-to-severe persistent asthma over 12 weeks, etanercept was not shown to provide clinical benefit (Holgate et al. 2011). Golimumab (Simponi; Centocor Ortho Biotech), a fully human TNFα-blocking antibody, was assessed in a large multicenter, placebo-

controlled study in patients with uncontrolled severe asthma (Wenzel et al. 2009). No significant improvements in lung function and disease exacerbations were detected. Worryingly serious infectious and neoplastic events were reported, including active tuberculosis, pneumonia, sepsis, and several different malignancies (such as breast cancer, B-cell lymphoma, metastatic melanoma, cervical carcinoma, renal cell carcinoma, basal cell carcinoma, and colon cancer). These findings caused premature termination of the trial. A subgroup analysis of the patients enrolled in the trial identified that the drug possibly benefited older patients with late-onset asthma and a history of hospitalizations or emergency hospital visits during the year before screening and who also had lower baseline FEV1 levels and a post-bronchodilator FEV1 increase of greater than 12% (Wenzel et al. 2009). Overall, conflicting results have been obtained and serious concerns rose with regard to the safety of TNFα blockade in asthma.

11 Anti-granulocyte Monocyte Colony-Stimulating Factor (Anti-GM-CSF)

GM-CSF is a stimulatory growth factor overexpressed in the airway of asthmatic patients and plays a pivotal role in eosinophil recruitment and survival (Park et al. 1998). A human anti-GM-CSF monoclonal IgG1 antibody namilumab has been developed that decreased the survival and activation of peripheral human eosinophils (Krinner et al. 2007). A phase II, double-blind, placebo-controlled, and randomized clinical trial evaluated the safety, tolerability, and efficacy of a single dose (400 mg) of the GM-CSF-targeted monoclonal antibody KB003 in patients with moderate-to-severe asthma inadequately controlled by corticosteroids. It did not achieve significance and further clinical trials have been abandoned.

12 Conclusions

Asthma is a chronic respiratory disorder associated with type 2 airway inflammation as characterized by elevated levels of eosinophils, immunoglobulin E, and cytokines including interleukin (IL)-4, IL-5, IL-9, and IL-13 and tumor necrosis factor (TNF) alpha. However, mounting evidence has shown that considerable heterogeneity exists in human asthma in terms of the nature and intensity of airway inflammation. Novel biologic therapeutics are now emerging for severe asthma, some of the most promising of which are those monoclonal antibodies directed at selective targets. Those monoclonal antibodies act on very specific pathways. Some have shown to be effective, safe, and selective for the established asthma phenotypes, especially in patients with uncontrolled severe asthma, whereas other has proven to be unsafe and/or unsuccessful. The treatment of severe allergic asthma with anti-IgE monoclonal antibody (omalizumab) has been shown to be effective in a large number of patients, and new anti-IgE antibodies with improved pharmacodynamic properties are being investigated. Among developing therapies,

biologics designed to block certain pro-inflammatory cytokines, such as IL-5 (mepolizumab) and IL-13 (lebrikizumab), have a greater chance of being used. Perhaps, blocking more than one cytokine pathway (such as IL-4 and IL-13 with dupilumab) might confer increased efficacy of treatment, along with acceptable safety. It is becoming evident that significant clinical effects with anti-cytokine-based therapies are more likely in carefully selected patients that take asthma heterogeneity into account. It might also be more clinically effective if more than one mediator were to be targeted rather than a single mediator.

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Leukotriene Receptor Antagonists and Antiallergy Drugs

Tsutomu Tamada and Masakazu Ichinose

Contents

Abstract

As one of the candidates of the therapeutic strategy for asthma in addition to inhaled corticosteroids (ICS), leukotriene receptor antagonists (LTRAs) are known to be useful for long-term management of asthma patients complicated by allergic rhinitis (AR) or exercise-induced asthma (EIA). Currently available LTRAs are pranlukast hydrate, zafirlukast, and montelukast. These LTRAs have a bronchodilator action and inhibit airway inflammation, resulting in a significant improvement of asthma symptoms, respiratory function, inhalation frequency of as-needed inhaled β2-agonist, airway inflammation, airway hyperresponsiveness, dosage of ICSs, asthma exacerbations, and patients' QOL. Although cys-LTs are deeply associated with the pathogenesis of asthma, LTRAs alone are less effective compared with ICS. However, the effects of LTRAs in combination with ICS are the same as those of LABAs in combination

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with ICS in steroid-naïve asthmatic patients. Concerning antiallergy drugs other than LTRAs, some mediator-release suppressants, H1 histamine receptor antagonists (H1RAs), thromboxane A2 (TXA2) inhibitors/antagonists, and Th2 cytokine inhibitor had been used mainly in Japan until the late 1990s. However, the use of these agents rapidly decreased after ICS/long acting beta agonist (LABA) combination was introduced and recommended for the management of asthma in the early 2000s. The effectiveness of other antiallergic agents on asthma management seems to be quite limited, and the safety of oral antiallergic agents has not been demonstrated in fetuses during pregnancy. Further effectiveness studies are needed to determine the true value of these orally administered agents in combination with ICS as an anti-asthma treatment.

Keywords
H1RA • LTRA • Mediator-release suppressant • Th2 cytokine inhibitor • TXA2 inhibitor/antagonist

1 Leukotriene Receptor Antagonists (LTRAs)

1.1 Pharmacology of Leukotrienes and Its Receptors

Cysteinyl leukotrienes (cys-LTs) are produced in eosinophils, basophils, mast cells, macrophages, and myeloid dendritic cells, involving several steps including the oxidation of arachidonic acid by 5-lipoxygenase (5-LO) (Laidlaw and Boyce 2012; Kanaoka and Boyce 2004, 2014; Clark et al. 1990). After the unstable epoxide LTA4 is synthesized, LTC4 synthase (LTC4S) conjugates LTA4 to reduced glutathione, forming LTC4, the parent of the cys-LTs (Reid et al. 1990; Lam et al. 1994). Once formed, LTC4 is transported to the extracellular space via the ATP-binding cassette (ABC) transporters 1 and 4 and then metabolized to LTD4 and LTE4 by γ-glutamyl transpeptidases and dipeptidases, respectively. The rapid extracellular metabolism of LTC4 and LTD4 results in short biologic half-lives relative to the stable mediator LTE4, which is abundant and readily detected in biologic fluids. Thus, three different ligands (LTC4, LTD4, and LTE4) arise from a single intracellular synthetic event by successive enzymatic conversions (Laidlaw and Boyce 2012; Kanaoka and Boyce 2014). In addition to this intracellular pathway, there is also a transcellular mechanism for cys-LTs generation that can be carried out by cells that express LTC4S but not the proximal enzyme 5-LO in the pathway (e.g., platelets, endothelial cells). In the latter mechanism, the LTC4S-expressing cells can convert extracellular LTA4 (released by neutrophils or other cells with an active 5-LO enzyme) (Maclouf et al. 1994) and may serve as an additional source of cys-LTs in certain inflammatory states (Laidlaw and Boyce 2012).

The bioactivities of the cys-LTs in the preclinical setting, particularly their potency as smooth muscle constrictors, spurred interest in these mediators as potential therapeutic targets in asthma (Laidlaw and Boyce 2012). The ability to

monitor urinary levels of LTE4 as a reflection of systemic cys-LT generation in vivo provided the proof that cys-LTs are generated by subjects with acute asthma exacerbations (Drazen et al. 1992). Individuals with aspirin-induced asthma (AIA) have especially high baseline levels of urinary LTE4 and a marked further increment in these levels in response to oral challenge with aspirin (Christie et al. 1991). The role of cys-LTs in asthma has been well validated by clinical trials using the available drugs. The 5-LO inhibitor and selective antagonists of CysLT1 receptor both improve lung function, reduce the frequency of asthma exacerbations (Laidlaw and Boyce 2012; Liu et al. 1996; Israel et al. 1996), and reduce the severity of reactions to aspirin challenge in individuals with AIA (Berges-Gimeno et al. 2002).

While the three cys-LTs (C4, D4, E4) share certain functions in vivo, including smooth muscle contraction and vascular leak (Weiss et al. 1982a; Griffin et al. 1983), important differences were identified in early studies that suggested additional and distinct functions for each. Pharmacological profiling of guinea pig lung demonstrated that LTC4 and LTD4 were equipotent as constrictors, whereas LTE4 was inactive (Laidlaw and Boyce 2012). Remarkably, however, LTE4 was ten times more potent for inducing guinea pig tracheal ring contractions in vitro than LTC4 or LTD4 (Lee et al. 1984; Drazen et al. 1982). Together, these in vitro and in vivo functional findings predicted the existence of at least three receptors for cys-LTs: a high-affinity receptor for LTD4, a lower-affinity receptor for LTC4, and a separate receptor for LTE4, with the latter potentially capable of eliciting the secondary production of a prostanoid (Laidlaw and Boyce 2012).

Studies on human subjects also provided compelling evidence for the existence of at least three receptors for cys-LTs (Weiss et al. 1982a, b, 1983). Unlike non-asthmatic subjects, asthmatic subjects were much more sensitive to LTE4 in terms of bronchoconstriction (Davidson et al. 1987). However, asthmatic and non-asthmatic subjects had equivalent dose responses to LTC4 and LTD4 (Griffin et al. 1983). Moreover, subjects with AIA demonstrated even greater sensitivity to LTE4-induced bronchoconstriction than did aspirin-tolerant asthmatic controls (Christie et al. 1993). In another study, inhalation of LTE4, but not of LTD4, provoked the accumulation of eosinophils and basophils into the bronchial mucosa and sputum of asthmatic subjects when the two cys-LTs were administered at doses titrated to produce an equivalent degree of bronchoconstriction (Gauvreau et al. 2001). Inhalation of LTE4 also enhanced the sensitivity of asthmatic subjects to histamine-induced bronchoconstriction, an effect that was blocked by pretreatment with oral indomethacin (Christie et al. 1992). These studies support the concept that LTE4 acts through a receptor(s) that is distinct from those responsible for the actions of LTC4 and LTD4 and suggest that the expression and/or function of the LTE4 receptor may be selectively upregulated in asthma, specifically enhancing the pulmonary responsiveness to it (Laidlaw and Boyce 2012).

The CysLT1 and CysLT2 receptors are both G-protein-coupled receptors (GPCRs) and were cloned and characterized several years after the original descriptive pharmacology predicted their properties (Laidlaw and Boyce 2012). Human and mouse CysLT1 receptors are 87 % identical (Mollerup et al. 2001), and the

CysLT2 receptors are 74 % identical (Hui et al. 2001), suggesting a high level of functional conservation through evolution. Both receptors are structural homologues of the purinergic (P2Y) receptors, which are specialized to recognize extracellular nucleotides, with 25–34 % identity at the amino acid level (Mellor et al. 2001). The CysLT1 receptor binds LTD4 with high affinity (10^{-9} M) and LTC4 with lesser affinity (10^{-8} M), whereas the CysLT2 receptor binds both LTC4 and LTD4 with equal affinity (10^{-8} M) (Laidlaw and Boyce 2012). Neither receptor exhibits substantial affinity for LTE4 in radioligand binding assays, nor does LTE4 elicit strong signaling responses in cells expressing CysLT1 receptor or CysLT2 receptor in isolation (Lynch et al. 1999; Heise et al. 2000). It is therefore unlikely that the pharmacology of LTE4 in vivo is attributable to the CysLT1 receptor and CysLT2 receptor alone (Laidlaw and Boyce 2012). The CysLT1 and CysLT2 receptors are broadly expressed by structural and hematopoietic cells. Some cell types (vascular smooth muscle) express mostly CysLT1 receptors (Heise et al. 2000), whereas others (endothelial cells) dominantly express CysLT2 receptors (Hui et al. 2001). Both receptors are expressed by cells of the innate (macrophages, monocytes, eosinophils, basophils, mast cells, dendritic cells) and adaptive (T cells, B cells) immune system, implying potentially cooperative functions in immunity and inflammation (Kanaoka and Boyce 2004, 2014). Although the CysLT1 and CysLT2 receptors both mediate calcium flux and activate signaling cascades, the blockade or knockdown of CysLT1 receptors in mast cells eliminated most LTD4-mediated signaling despite the presence of CysLT2 receptors on these cells (Laidlaw and Boyce 2012; Mellor et al. 2001, 2003; Jiang et al. 2007). CysLT1 receptors and CysLT2 receptors were found to heterodimerize in primary mast cells, a relatively common feature of GPCRs, which recognize similar ligands (Franco et al. 2007). Thus CysLT2 receptors, by interacting with CysLT1 receptors, limit the surface expression levels and signaling ability of the latter receptors, at least on mast cells. The absence of CysLT2 receptors may also facilitate the formation of CysLT1 receptor homodimers (Lynch et al. 1999) that are strong signaling units for LTD4 (Laidlaw and Boyce 2012).

1.2 Clinical Use of LTRAs

A currently available LTRA is a CysLT1 receptor antagonist. Three types of LTRAs are available: pranlukast hydrate, zafirlukast, and montelukast. LTRAs have a bronchodilator action and inhibit airway inflammation (Minoguchi et al. 2002; Hui and Barnes 1991), resulting in a significant improvement of asthma symptoms, respiratory function, inhalation frequency of as-needed inhaled β2-agonist, airway inflammation, airway hyperresponsiveness, dosage of inhaled corticosteroids (ICSs), asthma exacerbations, and patients' QOL (Tohda et al. 2002; Tamaoki et al. 1997; Drazen et al. 1999).

In several multicenter, randomized, double-blind trials, the usefulness of LTRAs has been investigated as candidate medications to control chronic asthma.

When estimated by FEV1 and symptoms, montelukast significantly improved asthma control during a 12-week treatment period (Reiss et al. 1998). Note that 90 % of the participants had histories of allergic rhinitis (AR) and exercise-induced asthma (EIA) in this study. Concerning EIA, unlike salmeterol, montelukast showed a sustained bronchoprotective effect throughout 8 weeks of the study (Edelman et al. 2000). Additionally, as compared with placebo, montelukast provided significant protection against EIA over a 12-week period and with neither tolerance to the medication nor a rebound worsening of lung function after discontinuation of the treatment (Leff et al. 1998). Although montelukast provides a rapid improvement in FEV1 to patients with chronic asthma, montelukast alone is less effective compared with low doses of ICSs (Peters et al. 2007; Malmstrom et al. 1999) (Fig. 1). On the other hand, LTRAs are known to be useful as agents used concomitantly with an ICS in patients with asthma that cannot be completely controlled even with a medium dose of an ICS, because the additional administration of LTRAs is as effective as a double dose of an ICS (Wada et al. 2000; Price et al. 2003; Laviolette et al. 1999). Additionally, the effects of LTRAs plus ICSs are reported to be the same as those of LABAs plus ICSs in steroid-naïve asthmatic patients (Peters et al. 2007; Price et al. 2011). There are some reports providing evidence for the advantages of LTRAs plus ICSs. Not only inhaled fluticasone (1000 μg/day) for 2 weeks but also oral prednisolone (60 mg/day) for 1 week did

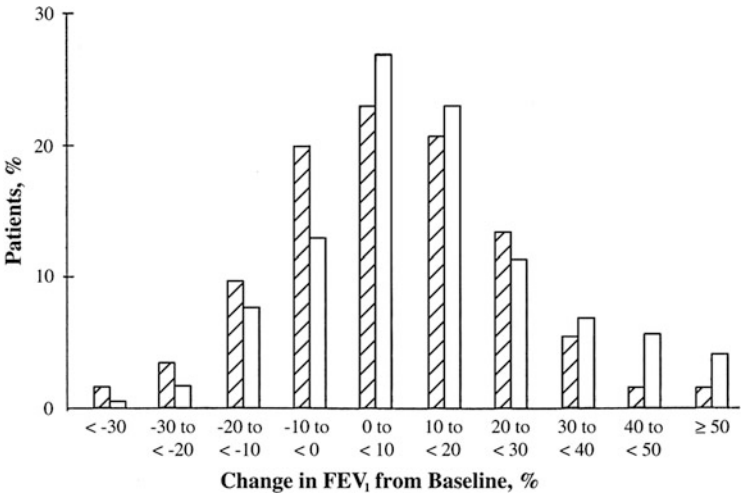

Fig. 1 Distribution of treatment responses for FEV1. The response distributions are shown as histograms for predefined intervals of percentage change in FEV1. Of the montelukast recipients, 42 % had an improvement in FEV1 of at least 11 % from baseline (this was the median response of beclomethasone recipients; that is, 50 % of the beclomethasone recipients had an improvement in FEV1 of at least 11 % from baseline). The proportions of patients who did not show an improvement in FEV1 were 22 % with beclomethasone and 34 % with montelukast. Striped bars represent patients receiving montelukast, 10 mg once daily; white bars represent patients receiving inhaled beclomethasone, 200 μg twice daily. [From reference Malmstrom et al. 1999]

not decrease the LTE4 levels in bronchoalveolar lavage fluids (BALF) (Dworski et al. 1994) and those in urine (O'Shaughnessy et al. 1993). Concerning the effect of LTRA versus LABA added to ICS on asthma controls in patients whose symptoms are inadequately controlled with ICS alone, montelukast used in combination with fluticasone is less effective in improving symptoms and respiratory function and is almost equivalent in preventing exacerbations, when compared with salmeterol in combination with fluticasone (Bjermer et al. 2003; Ilowite et al. 2004). In some patients, respiratory function improves early after the oral administration of an LTRA (in several hours at the earliest, on the following day at the latest) (Hui and Barnes 1991); however, anti-inflammatory effects develop later. Thus, efficacy is generally judged 2–4 weeks after administration.

The potential usefulness of LTRAs for relief from acute asthma has been also investigated. In a study that compared the effect of a single dose of intravenous montelukast (7 mg), oral montelukast (10 mg), and placebo on FEV1 in patients with chronic asthma, the onset of action for intravenous montelukast was faster than that for oral montelukast, and the improvement in airway function lasted over the 24 h observation period for both treatments (Dockhorn et al. 2000). It was also reported that intravenous montelukast added to the standard care produced significant and sustained relief of airway obstruction throughout the 2 h after drug administration, with an onset of action as early as 10 min (Camargo et al. 2010).

As a whole, LTRAs are generally useful for long-term management of patients with asthma complicated by AR (Price et al. 2006), EIA (Leff et al. 1998), and AIA (Dahlen et al. 2002).

While more reports have been published about EGPA patients who have received an LTRA than about those who have received other antiasthmatic drugs, no conclusion has been reached as to whether an LTRA can directly cause the onset of EGPA (Beasley et al. 2008; Nathani et al. 2008). LTRAs are generally safe drugs, although zafirlukast should be used with caution because it may cause severe liver dysfunction and interact with other agents, such as warfarin, since it is metabolized by CYP2C9. It was recently reported that CYP2C8, but neither CYP2C9 nor CYP3A4, contributes to the metabolism of montelukast at clinically relevant concentrations (Filppula et al. 2011; Karonen et al. 2012). LTRAs seem to be relatively safe for pregnant women. In 2008, the US Food and Drug Administration (FDA) first issued a safety alert concerning a potential association between montelukast and increased risk of suicide. However, more recent studies found that the use of LTRAs was not associated with an increased risk of suicide attempts in children, adolescents, and young adults with asthma. Further research needs to be conducted to more fully understand the association between LTRAs and suicide (Manalai et al. 2009; Schumock et al. 2012; Philip et al. 2009).

2 Antiallergic Agents Other Than LTRAs

Antiallergic agents include either mediator-release suppressants or mediator inhibitors and are effective in 30–40 % of the patients with mild-to-moderate atopic asthma, although an administration period of 4–6 weeks or longer is needed to determine their efficacy (Furukawa et al. 1999). The antiallergic agents presented here had been used mainly in Japan, but the use of these agents rapidly decreased after inhaled corticosteroids (ICSs) were introduced in the late 1990s. The safety of oral antiallergic agents has not been demonstrated in fetuses during pregnancy.

2.1 Mediator-Release Suppressants

2.1.1 Pharmacology of Mediator-Release Agents

The anti-inflammatory and antiallergic effect of disodium cromoglycates (DSCG) was shown to work in a dose-dependent fashion through the inhibition of IgE-stimulated mediator release from human mast cells (Netzer et al. 2012; Leung et al. 1988). Recent research has specifically demonstrated that the cromoglycates are potent GPCR35 agonists and that GPCR35 is expressed in human mast cells, basophils, and eosinophils (Kay et al. 1987). GPCR35 mRNA is upregulated upon challenge with IgE antibodies, and cromoglycates may work by dampening the effects of this interaction. DSCG also demonstrated a cell-selective and mediator-selective suppressive effect on macrophages, eosinophils, and monocytes (Yang et al. 2010). DSCG was demonstrated to have anti-inflammatory effects in a study performed using biopsies of bronchial mucosa in nine patients with asthma before and after treatment with inhaled DSCG by a metered-dose inhaler (MDI) (Hoshino and Nakamura 1997). The numbers of eosinophils, mast cells, T-lymphocytes, and macrophages were significantly reduced as a result of DSCG, and the expressions of intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (ELAM-1) were also significantly reduced (Hoshino and Nakamura 1997). Head-to-head comparison of DSCG with beclomethasone dipropionate (BDP) revealed similar anti-inflammatory effects between DSCG and BDP in terms of reduced mucosal populations of eosinophils, mast cells, and T-lymphocytes (Hoshino et al. 1998). A review article revealed that DSCG has a protective effect in reducing bronchial hyperreactivity if it is used continuously for longer than 12 weeks (Hoag and McFadden 1991).

2.1.2 Clinical Use of DSCG

The main effect of mediator-release suppressants is inhibiting the release of chemical mediators from mast cells. It is reported that the long-term use of inhaled DSCG inhibits airway inflammation in adult patients with atopic asthma (Hoshino and Nakamura 1997; Hoag and McFadden 1991). Because DSCG has been recommended as a maintenance treatment for children with moderate asthma, it has been mainly used in children with asthma (Tasche et al. 2000). DSCG is

supposed to be effective in 60 % of cases (Warner 1989), and no serious side effects have been reported in trials (Tasche et al. 2000). However, the use of DSCG has decreased since the 1990s, while the use of ICS is increasing, even in young children (Price and Weller 1995; Warner 1995).

2.2 Histamine H1 Antagonists

2.2.1 Pharmacology of Histamine and Its Receptors

Histamine was first identified as a mediator of biological functions in the early 1900s, and drugs targeting its receptors have been in clinical use for more than 60 years. It is widely known that histamine is increased in the BALF from patients with allergic asthma, and this increase negatively correlates with airway function (Wenzel et al. 1988; Wardlaw et al. 1988; Liu et al. 1990; Jarjour et al. 1991; Casale et al. 1987; Broide et al. 1991). During inflammation, histamine is released from preformed stores in mast cells and basophils. Histamine acts on vascular smooth muscle cells and endothelial cells, leading to vasodilation and an increase in vascular permeability (Thurmond et al. 2008). All of the receptors for histamine are of the GPCR family. In general, it has been found that many cells involved in inflammatory responses express H1, H2, and H4 receptors. H1 receptors couple to Gq proteins leading to phospholipase C activation, inositol phosphate production, and calcium mobilization (Bakker et al. 2002). H2 receptors activate Gαs and increase cyclic AMP formation (Bakker et al. 2002). Activation of the H4 receptor appears to be mainly coupled to pertussis toxin-sensitive Gαi/o proteins, which signal through increases in the intracellular calcium (Thurmond et al. 2008). H1 receptors are expressed on multiple cell types including endothelial cells and smooth muscle cells, where they mediate vasodilation and bronchoconstriction. H1 histamine receptor antagonists (H1RAs), such as diphenhydramine and loratadine, have been used for many years in the treatment of allergic inflammatory responses. Indeed, airway hyperresponsiveness to histamine is one of the hallmarks of asthma, and the plasma histamine concentrations are elevated during the early and late responses to inhaled allergens and may also increase during spontaneous acute asthma episodes. However, ordinary doses of currently available H1RAs have minimal bronchodilator and bronchoprotective activity, and H1RAs have no significant clinical effect in severe persistent asthma (Simons 1999). To date, it is unlikely that monotherapy with most currently available H1RAs will provide significant clinical benefit in asthma (Lordan and Holgate 2002).

2.2.2 Clinical Use of Histamine H1 Antagonists

Specific H1RA completely inhibited histamine-induced bronchoconstriction but failed to completely inhibit inhaled allergen-induced bronchoconstriction (Rafferty et al. 1987). Additionally, singular treatment with H1RA or LTRA caused significant reductions in the mean maximal fall in FEV1 during the early asthmatic reactions (EAR) and late asthmatic reactions (LAR), but the efficacy of H1RA was inferior to that of LTRA (Roquet et al. 1997). H1RAs are not currently a

frontline treatment for asthma (Thurmond et al. 2008; Ohta et al. 2014; Global Initiative for Asthma (GINA) 2014). H1RAs are beneficial for asthma accompanied by allergic rhinitis or atopic dermatitis. Adverse effects may include sleepiness and malaise.

2.3 Thromboxane A2 (TXA2) and Its Receptors

2.3.1 Pharmacology of TXA2 and Its Receptors

Like cys-LTs (C4, D4, E4), TXA2 is a lipid mediator that powerfully contributes to the airflow limitation by constricting bronchial smooth muscles, increasing mucous secretion and microvascular leakage, and acting as a chemoattractant for inflammatory cells such as T-lymphocytes, eosinophils, and activated mast cells (Rolin et al. 2006). TXA2 is generated by thromboxane synthase, which belongs to the cytochrome P450 superfamily (Tanabe and Ullrich 1995; Nusing et al. 1990). Due to its prothrombotic and vasoconstrictor effects, this prostanoid is the physiological antagonist of prostacyclin (Rolin et al. 2006). The human TXA2 receptor termed TP was the first eicosanoid receptor cloned (Hirata et al. 1991). Two isoforms of this receptor were described: TPα and TPβ. Both isoforms functionally couple to a Gq protein leading to phospholipase C activation, calcium release, and the activation of protein kinase C (Huang et al. 2004; Shenker et al. 1991; Dorn and Becker 1993). Nevertheless, they couple oppositely to adenylate cyclase. TPα activates adenylate cyclase, while TPβ inhibits this enzyme (Hirata et al. 1996). TXA2 is mainly produced by platelets, monocytes, macrophages, neutrophils, and lung parenchyma (Nusing et al. 1990; Widdicombe et al. 1989; Higgs et al. 1983; Hamberg et al. 1975). TXA2 is a potent stimulator of platelet shape change and aggregation as well as a potent stimulator of smooth muscle constriction and proliferation and bronchial hyperresponsiveness (Kurosawa 1995). TXA2 plays a crucial role in the pathogenesis of bronchial asthma since it is a potent constrictor of bronchial smooth muscles and a stimulator of airway smooth muscle cell proliferation (Tamaoki et al. 2000a; Morris et al. 1980; Devillier and Bessard 1997). It has been shown that TXA2 is increased in the airways of patients suffering from asthma after allergen challenge (Wenzel et al. 1991). Several studies also demonstrated increased concentrations of this mediator and metabolites in BALF, urine, and plasma from asthmatic patients (Kumlin et al. 1992; Oosterhoff et al. 1995; Wenzel et al. 1989). Activation of the prostanoid TP receptors present in bronchial smooth muscle cells by TXA2 leads to intracellular calcium mobilization with bronchoconstriction as a consequence (Capra et al. 2003; Hall 2000). Prostanoid TP receptor activation also contributes to bronchial smooth muscle hyperplasia and airway remodeling, which occur in response to chronic airway inflammation of asthma (Vignola et al. 2003). Using a model of allergen-induced cough in guinea pig, it was demonstrated that airway mucous cells are an important source of TXA2 and that this prostanoid facilitates cough (Rolin et al. 2006). Moreover, this team showed the localization of thromboxane synthase by immunohistochemical detection in the airways, mainly in epithelial goblet cells and tracheal glands (Xiang et al. 2002).

2.3.2 Clinical Use of TXA2 Inhibitors/Antagonists

TXA2 synthesis inhibitors and TXA2 receptor antagonists inhibit airway inflammation, improve airway hyperresponsiveness (Hoshino et al. 1999; Fujimura et al. 1986, 1991), and improve the impaired mucociliary transport (Fujimura et al. 1991). However, like other antiallergic agents described in this chapter, the use of TXA2 antagonists has decreased since ICS and ICS/LABA therapies became widely disseminated worldwide, including in Japan. Their adverse effects include a tendency for increased bleeding; thus, we should be cautious about the concomitant use of other agents with inhibitory effects on platelet aggregation.

2.4 Th2 Cytokine Inhibitor

2.4.1 Pharmacology of Th2 Cytokine Inhibitor

Suplatast tosilate (IPD) is a unique compound that inhibits the release of Th2 cytokines, such as IL-4 and IL-5, and inhibits tissue infiltration by eosinophils (Corry and Kheradmand 2006; Horiguchi et al. 2001; Yamaya et al. 1995; Oda et al. 1999). This orally administered agent is effective in reducing the ECP level in induced sputum, improving the peak expiratory flow (Horiguchi et al. 2001) and airway hyperresponsiveness (Yoshida et al. 2002; Sano et al. 2003). These anti-inflammatory features might be responsible for their beneficial effects on airway function (Corry and Kheradmand 2006).

2.4.2 Clinical Use of Th2 Cytokine Inhibitor

In a randomized, double-blind, placebo-controlled, parallel-group study, IPD improved pulmonary function and symptom control and enabled a decrease in the dose of inhaled corticosteroid without significant side effects in steroid-dependent asthma (Tamaoki et al. 2000b). This promising investigational agent is currently available only in Japan. IPD enables a reduction in the dose of ICS (Tamaoki et al. 2000b).

3 Conclusion

Although cys-LTs are deeply associated with the pathogenesis of asthma, LTRAs alone are less effective compared with ICS. However, the effects of LTRAs in combination with ICS are the same as those of LABAs in combination with ICS in steroid-naïve asthmatic patients. Currently, the use of LTRAs is mostly limited to asthma patients with AR or EIA. Antiallergic agents other than LTRAs had been used mainly in Japan, but the use of these agents rapidly decreased after ICS was introduced and recommended for the management of asthma in the late 1990s. Further effectiveness studies are needed to determine the true value of these orally administered agents in combination with ICS as an antiasthma treatment.

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Glucocorticoids

Ian M. Adcock and Sharon Mumby

Contents

Abstract

The most effective anti-inflammatory drugs used to treat patients with airways disease are topical glucocorticosteroids (GCs). These act on virtually all cells within the airway to suppress airway inflammation or prevent the recruitment of inflammatory cells into the airway. They also have profound effects on airway

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structural cells to reverse the effects of disease on their function. Glucocorticosteroids act via specific receptors—the glucocorticosteroid receptor (GR)—which are a member of the nuclear receptor family. As such, many of the important actions of GCs are to modulate gene transcription through a number of distinct and complementary mechanisms. Targets genes include most inflammatory mediators such as chemokines, cytokines, growth factors and their receptors. GCs delivered by the inhaled route are very effective for most patients and have few systemic side effects. However, in some patients, even high doses of topical or even systemic GCs fail to control their disease. A number of mechanisms relating to inflammation have been reported to be responsible for the failure of these patients to respond correctly to GCs and these provide insight into GC actions within the airways. In these patients, the side-effect profile of GCs prevent continued use of high doses and new drugs are needed for these patients. Targeting the defective pathways associated with GC function in these patients may also reactivate GC responsiveness.

Keywords
Airway • Asthma • COPD • Inflammation • Nuclear receptor • Pharmacology

1 Introduction

Glucocorticoids (GC) are endogenous adrenal hormones and the secretion of cortisol is elevated increases in response to stress (Magiakou and Chrousos 2002). Cortisol does not just perform a role as a marker of stress but is a modulator of cellular and tissue function. The immune and inflammatory systems that are activated in the normal response to exogenous stimuli/challenges are potently suppressed by GCs and this characteristic has enabled their use as highly effective therapeutic agents (Barnes and Adcock 2003). Indeed, synthetic GCs are the mainstay of anti-inflammatory therapy for asthma and many other chronic inflammatory diseases (Barnes 2006b). This chapter discusses the general pharmacologic aspects of GCs, their mechanism of anti-inflammatory actions and possible mechanisms for their limited effectiveness in severe treatment refractory asthma and COPD (GINA).

Inflammatory and immune functions in the body display a diurnal variation which is also seen in asthma physiology (Gibbs et al. 2012) and reflects systemic cortisol levels (Magiakou and Chrousos 2002). The diurnal changes in cortisol levels is regulated by local and central circadian “clocks” which therefore control the endogenous anti-inflammatory responses seen in asthma (Farrow et al. 2012). The timing of exogenous GC dosing may, as a result, affect the efficacy of endogenous GCs by enhancing their anti-inflammatory properties if given at maximal trough or peak times (Farrow et al. 2012).

2 Chemical Structures

Modern GCs such as prednisolone, fluticasone, budesonide and dexamethasone are based on the cortisol (hydrocortisone) structure with modifications to enhance the anti-inflammatory effects such as the introduction of 6α-fluoro further substitutions (Johnson 2004; Daley-Yates 2015). Reduced binding to the mineralocorticoid receptor is achieved by insertion of a C=C double bond at C1,C2 and lipophilic substituents such as 21α-esters attached to the D-ring increase glucocorticosteroid receptor (nuclear receptor subfamily 3, group C, member 1; NR3C1; GR) binding, enhance topical deposition and hepatic metabolism. This substitutions are seen with budesonide and fluticasone (Hochhaus et al. 1991; Daley-Yates 2015) (Fig. 1).

The ligand-binding domain (LBD) of GR has a pocket on the floor of the binding cleft that lies beneath the C17 residue of the steroid backbone (Bledsoe et al. 2002). The degree of occupancy of this pocket affects the affinity, duration of action and side-effect profile of ligands and computational chemistry can design drugs with improved attributes including those without a steroid backbone to improve safety (Daley-Yates 2015).

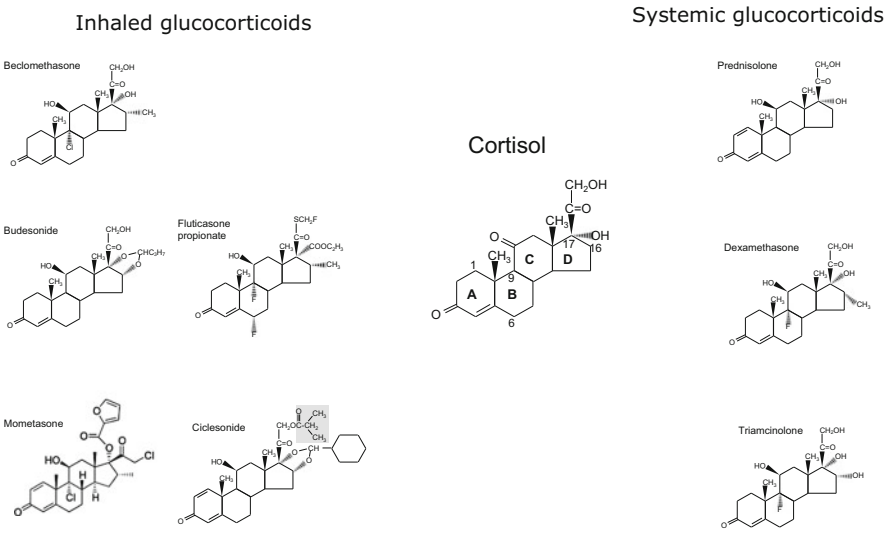

Fig. 1 Structure of clinically used topical inhaled glucocorticosteroids beclomethasone, budesonide, fluticasone propionate, mometasone, and ciclesonide (the cleaved ester is *shaded*) and systemic glucocorticosteroids prednisolone, dexamethasone and triamcinolone which are all based on modifications of the natural cortisol structure

2.1 Pharmacokinetics

The lipophilic nature of synthetic GCs enable their ready absorption after topical administration and helps prolong their retention in the airways (O'Connor et al. 2011). Modern inhaled GCs (ICS) have high receptor affinity, are retained in the airways and are rapidly metabolised after absorption from the GI tract which accounts for their good safety profile even when used in more severe asthmatics and during exacerbations (Barnes 2006a; Daley-Yates 2015). The side effects seen with ICS are dose-dependent and are the same as those seen with oral GCs (Schacke et al. 2002; Daley-Yates 2015). Metered dose inhalers (MDI) and dry powder inhalers (DPI) deliver 10–20 % of the inhaled dose to the lungs but >50 % is deposited in the oropharynx and mouth. The drug may then be swallowed and taken up from the gut and become systemically available.

ICS as a group all have a good therapeutic index resulting from a small particle size enabling low oral bioavailability and rapid metabolism/clearance combined with high plasma protein binding to give a short systemic half-life (O'Connor et al. 2011; Daley-Yates 2015). The plasma half-life of currently used ICS varies from <2 h (budesonide) to >5 h (BDP/BMP, fluticasone and mometasone). This is in contrast to their biological effects which last for 18–36 h (Winkler et al. 2004; Daley-Yates 2015) (Table 1). In general, ICS treatment efficacy and side effects are directly related to tissue dose although there is some evidence that this may vary with the drug and patient profile (O'Connor et al. 2011).

Most patients with asthma are treated with ICS with oral preparations being limited to patients with severe disease on account of the risk of adverse side effects (see below) (Schleimer 2004; Umland et al. 2002). Interestingly, there is a tenfold

Table 1 Relative potencies of common glucocorticoids

Drug	Potency relative (hydrocortisone)	Equiv. dose (μg)[a]	Duration of action[b] (h)
Inhaled drugs			
Budesonide	3,750	400	1.5–2.8
Fluticasone	7,200	200	3.1–14
Mometasone	8,800	200/400	4.5
des-Ciclesonide	4,800	320	0.7–7
Beclomethasone (BDP/BMP)	2,100/5,400	400	0.5/2.7
Oral drugs		*Equiv. dose (mg)*[c]	
Hydrocortisone	1	20	8–12
Cortisone	0.8	25	8–12
Prednisolone	4	5	12–36
Triamcinolone	5	4	12–36
Dexamethasone	25	0.75	36–72

BDP Beclomethasone dipropionate, *BMP* Beclomethasone monopropionate
[a]Equivalence to BDP
[b]Biological half life
[c]Equivalence to hydrocortisone

variability in plasma concentrations of GCs after oral administration in asthmatics and normal volunteers when given the same dose although the reasons for this are unclear (Winkler et al. 2004).

2.2 Glucocorticoid Responsiveness in Asthma

Asthma has long been known as a chronic inflammatory disease of the central airways and the beneficial effect of the potent anti-inflammatory prednisolone in asthmatic patients further emphasised this point (GINA). Interestingly, in relation to later clinical trials using anti-eosinophil directed biologics, blood eosinophil levels were not altered in some patients with more severe asthma who were relatively refractory to oral prednisolone treatment (Grant 1961). Treatment with prednisolone was related to adverse side effects however (Grant 1961). Dramatic improvements in asthma symptoms were also seen with the introduction of ICS which had few systemic side effects (Clark 1982; Brompton Hospital/Medical Research Council Collaborative Trial 1974). In this initial studies only 40 % of asthmatics responded well to ICS with respect to improvements in lung function—it was not investigated whether this related to a lack of compliance, poor inhaler technique or a true relative insensitivity to ICS.

As with other chronic inflammatory diseases, ICS reduce the inflammatory markers seen in the asthmatic airways and this results in the improvement in FEV_1 and the reversal of AHR back to levels seen in healthy non-asthmatic subjects in most subjects with mild-moderate disease (GINA). However, since discontinuation of ICS leads to a return of the symptoms of asthma and of airway inflammation, they are not a cure for asthma (Adcock et al. 2008a, b; Durham et al. 2016). It is now recognised that asthma is not a single disease but is composite of several diseases or a syndrome with many potential phenotypes existing. Future therapies will depend upon understanding the inflammatory drive for each patient/phenotype to enable the most effective therapeutic regimen for each patient to be determined (Chung and Adcock 2013). The results of many single centre groups worldwide but increasingly of large pan-European and pan-USA consortia have defined subgroups of asthma and severe asthma based on clinical features and the addition of minimal inflammatory parameters (Chung and Adcock 2013; Bel et al. 2011; Kupczyk and Wenzel 2012). For example, five asthma clusters were reported by the SARP consortia (Moore et al. 2010) and four clusters by the group from Leicester (Haldar et al. 2008). Severe asthma patients were found amongst several clusters which indicates that clinical variables alone are not helpful in defining the underlying mechanism(s) of asthma in these subjects. When inflammatory characteristics such as sputum or blood eosinophilia and/or genomic signatures into account, the ability to predict the therapeutic efficacy of some drugs was improved. For instance, poor glucocorticoid responses were associated with neutrophilic airway inflammation (Wenzel et al. 1997) and eosinophilic inflammation led to better disease outcome with ICS therapy than standard clinical management (Green et al. 2002; Kupczyk et al. 2013). Sputum and blood eosinophilia also appears to be a better predictor of

the response to anti-IL-5 treatment (Nair et al. 2009; Pavord et al. 2012; Ortega et al. 2014) but not to anti-IL-4R therapy (Wenzel et al. 2013, 2016).

2.3 Effects of Glucocorticoids on Asthmatic Inflammation

The majority of asthma, usually accompanied by atopy, is characterised by an inflammatory response within the airways involving mast cell activation, eosinophil influx and increased numbers of activated type 2 T helper (Th2) cells (Holgate et al. 2010). However, this single mechanistic view has been modified with the realisation that subsets of asthmatic patients exist which may even reflect different diseases (Haldar et al. 2008; Moore et al. 2010) and in particular that inflammatory phenotypes may define the response to GCs (Haldar et al. 2008; Woodruff et al. 2007; Choy et al. 2015). For example, the subgroup of patients with severe asthma who present with high sputum or blood eosinophilia despite high dose ICS or oral steroids use are those most likely to respond to the anti-IL-5 therapy. Interestingly, the effect is seen on exacerbation rate rather than lung function or other asthma outcome measures (Haldar et al. 2009; Nair et al. 2009; Pavord et al. 2012) and may also be steroid sparing (Bel et al. 2014).

GCs are the most successful anti-inflammatory treatment used in asthma as they target all the cells implicated in asthmatic inflammation (GINA) (Fig. 2). The routine use of ICS to prevent airway inflammation in combination with relievers such as β_2 agonists, which help the airway smooth muscle to relax after contraction, is effective in treating symptoms, reducing exacerbations and improving lung function in most asthmatics and has resulted in great improvements in asthma control and the quality of life of most asthmatics (Chung et al. 2014; Chung and Adcock 2013). Unfortunately a minority of asthmatics show refractoriness to GC treatment (Adcock et al. 2008a; Barnes and Adcock 2009). The burden of costs (economic, morbidity and mortality) of these GC-refractory patients is much greater than that of GC-sensitive non-severe asthmatic subjects (Adcock et al. 2008a, b; Chung and Adcock 2013; Durham et al. 2016; Accordini et al. 2013).

The GC refractory nature of the inflammatory response is not confined to a subset of asthmatics but is also seen to a greater or lesser extent in most chronic inflammatory diseases (Barnes and Adcock 2009). The inflammatory patterns found in refractory asthma may also contribute to relative GC insensitivity as drivers of specific disease subphenotypes may, in themselves, be GC refractory. A greater understanding of the mechanisms underlying GC actions in regulating inflammation and an elucidation of the processes that prevent their effectiveness in some patients will result in novel therapeutic agents, or combinations of agents, to treat severe asthmatics (Barnes and Adcock 2009).

GCs have profound effects on infiltrating immune cells as well as on the function of airway structural cells. ICS prevent eosinophil recruitment from the bone marrow as well as their migration into the airways and this probably explains the greater beneficial effect of oral GCs (Giembycz and Lindsay 1999). GCs also suppress the expression of eosinophil survival factors and induce eosinophil apoptosis (Giembycz and Lindsay 1999). In contrast, GCs enhance peripheral blood

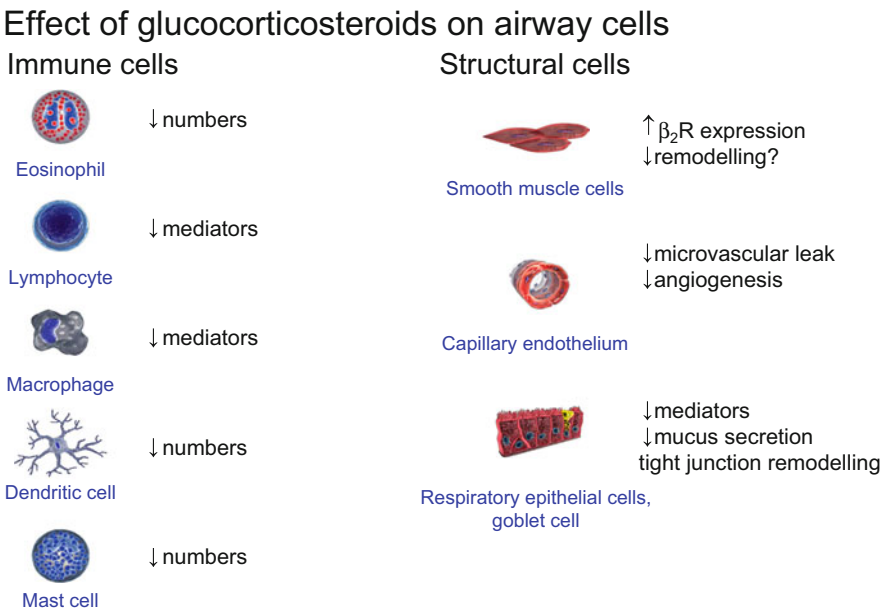

Fig. 2 The function of inflammatory and structural cells is modulated by glucocorticosteroids in asthma. The activity (T-lymphocytes and macrophages) and/or number of infiltrating cells (eosinophils, T-lymphocytes, macrophages, basophils, mast cells and dendritic cells) are decreased by glucocorticoids. Glucocorticoids also have a suppressive effect on resident structural cells and reduce mediator release and expression on epithelial and endothelial cells, microvascular leak from blood vessels, angiogenesis and both the numbers of mucus glands and release of mucus from these glands

neutrophilia (Hallett et al. 2008) and prevent neutrophil apoptosis (Hallett et al. 2008).

Total blood lymphocyte numbers are reduced in asthmatic subjects who receive oral GCs. GCs inhibit lymphocyte activation and inflammatory mediator expression through a variety of mechanisms and induce lymphocyte apoptosis (Rhen and Cidlowski 2005). The effects of ICS on lymphocytes are varied and dependent upon mitochondrial function and downstream effects on apoptosis (Eberhart et al. 2011; Psarra and Sekeris 2011). We have previously shown that $CD4^+$ lymphocytes from severe asthmatics differentially express cofilin-1, a protein which regulates mitochondrial function (Vasavda et al. 2006). GCs can also affect $CD4^+CD25^+$ $Foxp3^+$ regulatory T cells (Tregs) expression and function (Urry et al. 2012; Umland et al. 2002). In comparison to the marked effects on T-cell function, ICS have little effect on B-cell IgE production in vivo in asthma (Umland et al. 2002) although higher doses may be effective in COPD and in vitro (Lee et al. 2016).

ICS have profound effects on the function, terminal differentiation and activation status of macrophages and monocyes in asthma (Donnelly and Barnes 2012). In particular, they reduce the expression of macrophage-derived pro-inflammatory cytokines and chemokines (Donnelly and Barnes 2012). ICS treatment reduces

peripheral blood levels of monocytes and also low affinity IgE receptors expression (Umland et al. 2002). Dendritic cells (DCs) are key players in allergic asthmatic inflammation (Lambrecht and Hammad 2012) and ICS, by regulating DC CCR7 expression, can modulate DC migration to local lymphoid collections (Lambrecht and Hammad 2012). Furthermore, the release of Th1 and Th2 polarising cytokines is suppressed by GCs (Ito et al. 2006a; Umland et al. 2002) whilst that of IL-10 is increased (Lambrecht and Hammad 2012).

Overall, although most inflammatory responses in the airway are suppressed by GCs some innate immune responses including neutrophil production and survival, macrophage phagocytosis and epithelial cell survival are either unaffected or even increased (Schleimer 2004; Zhang et al. 2007). Furthermore, GCs often increase rather than suppress the expression of Toll-like receptors, complement, pentraxins, collectins, SAA and other host defence genes (Schleimer 2004; Zhang et al. 2007).

2.4 Effects of Glucocorticoids on Airway Structural Cells

GCs suppress the expression and release of most inflammatory mediators and growth factors from primary airway epithelial cells (Holgate et al. 2010) probably via targeting NF-κB (Ito et al. 2006a; Heijink et al. 2014). GCs also modulate mucus production and secretion (Chen et al. 2012), epithelial fluid flux (Holgate et al. 2010; Kato and Schleimer 2007; Proud and Leigh 2011) and integrity (Holgate et al. 2010). This may involve a specific effect on modulating cladin eight expression (Kielgast et al. 2016). In contrast, GCs enhance surfactant protein (SP)-A & D which are important in host defence (Schleimer 2004).

GCs are also very effective in suppressing the synthetic and proliferative functions of primary human airway smooth muscle cells (Chung 2005; Perry et al. 2014, 2015) although this may be dependent, on part, upon the matrix on which the cells are grown (Chung 2005; Clifford et al. 2011). The ability of these cells to response to GCs reflects the disease severity and cells from patients with severe asthma are less responsive than those from non-severe asthmatics (Chang et al. 2012, 2015). This may reflect the relative expression of the dual MAPK phosphatase 1 (MKP-1) and of the p38 mitogen activated protein kinase (MAPK) (Bhavsar et al. 2010).

3 GC Effects in COPD

3.1 Reduced Response to the Anti-Inflammatory Action of Corticosteroids in Stable COPD

In contrast to asthma, glucocorticoid treatment of stable COPD is rather ineffective in reducing airway inflammation and the decline of lung function (Barnes 2013). A Cochrane review of the role of regular long-term treatment with ICS alone versus placebo in patients with stable COPD has concluded that it reduces significantly the

mean rate of exacerbations and the rate of decline of quality of life but not the decline in FEV_1 or mortality rates (Yang et al. 2012). Current national and international guidelines for the management of stable COPD patients recommend the use of inhaled long-acting bronchodilators, ICS, and their combination for maintenance treatment of moderate to severe stable COPD (GOLD 2016). ICS treatment is also associated with side effects such as increased risk of oropharyngeal candidiasis, hoarseness, and pneumonia (Yang et al. 2012).

Several large controlled clinical trials of inhaled combination therapy with ICS and LABAs in a single device in stable COPD have shown that this combination therapy is well tolerated and produces a modest but statistically significant reduction in the number of severe exacerbations and improvement in FEV_1, quality of life, and respiratory symptoms in stable COPD patients, with no greater risk of side effects than that with use of either component alone. Increased risk of pneumonia is a concern; however, this did not translate into increased exacerbations, hospitalisations, or deaths (Nannini et al. 2013). In addition, the Towards a Revolution in COPD Health (TORCH) study showed a 17 % relative reduction in mortality over 3 years for patients receiving salmeterol (SAL)/fluticasone propionate (FP), although this just failed to reach significance (Calverley et al. 2007; Scott et al. 2015). Blood eosinophil counts are a promising biomarker of the response to ICS in COPD (Steiling et al. 2014) and could potentially be used to stratify patients for different exacerbation rate reduction strategies (Pascoe et al. 2015). Indeed, in retrospective analysis of COPD patients taking inhaled combination therapy, there was an increasing improvement in steroid response according to the level of blood eosinophilia (Pavord et al. 2016).

3.2 Side Effects of GCs

GCs are powerful anti-inflammatory and immunosuppressive agents and not surprisingly high doses of GCs used over a long time lead to an increased risk for adverse effects. All currently available ICS topical GCs have some systemic effect but this is minimal compared to that seen with oral GCs. Prolonged use is the highest risk factor although dosage, dosing regime and the specific drug used and individual patient variability are also important (Schacke et al. 2002; Mattishent et al. 2014). The most common GC side effects are glaucoma, cataracts, tissue atrophy and reduced wound healing, adrenal suppression and osteoporosis (Schacke et al. 2002; Mattishent et al. 2014). There is an increased risk of infection, particularly in COPD patients, which is dose- and duration-dependent (Scott et al. 2015).

The use of oral steroids is associated with more severe side effects which include skin and muscle atrophy, delayed wound healing, increased risk of infection, osteoporosis and bone necrosis, glaucoma and cataracts, behavioural changes, hypertension, peptic ulcers and GI bleeding and diabetes which are again dose- and duration of use-dependent (Schacke et al. 2002; Mattishent et al. 2014). GCs cause major tissue atrophy which presents as permanent striae (“stretch marks”) in the skin whilst early skin atrophy is reversible (Schacke et al. 2002; Mattishent et al.

2014). These can occur concomitantly as seen with Cushing's Syndrome (Magiakou and Chrousos 2002; Schacke et al. 2002; Mattishent et al. 2014). Acute administration of GCs suppresses the hypothalamic–pituitary–adrenal (HPA) axis resulting in cortisol suppression, a marker of compliance. The benefit/risk ratio is a serious issue in patients with severe asthma taking regular high dose GCs and is a major drive for the lack of compliance in some patients and drives the search for novel anti-inflammatory drugs with reduced side effects compared with GCs (Table 2).

Table 2 Tissue/organ specific side effects of high dose topical and systemic glucocorticosteroids

Cardiovascular system
Hypertension
Dyslipidemia
Thrombosis
Vasculitis
CNS
Disturbances in mood, behaviour, memory and cognition
"steroid psychosis", steroid dependence
Cerebral atrophy
Endocrine system, metabolism, electrolytes
Cushing's syndrome
Diabetes mellitus
Adrenal atrophy
Growth retardation
Hypogonadism, delayed puberty
Increased sodium retention and potassium excretion
Eye
Glaucoma
Cataract
Gastrointestinal
Peptic ulcer
Gastrointestinal bleeding
Pancreatitis
Immune system
Increased risk of infection
Re-activation of latent viruses
Skeleton and muscle
Muscle atrophy/myopathy
Osteoporosis
Bone necrosis
Skin
Atrophy, striae, distension
Delayed wound healing
Steroid acne, perioral dermatitis
Erythema, teleangiectasia, petechia, hypertrichosis

The mechanism(s) that drive GC side effects are varied and not fully resolved although it is likely that diabetes and glaucoma result from GR transactivation whilst HPA suppression is due to transrepression. Osteoporosis probably requires both gene induction and gene repression (Schacke et al. 2002; Mattishent et al. 2014). Some newer ICS such as ciclesonide have reduced side-effect profiles and a lesser effect on cortisol suppression. Des-ciclesonide, the active form of the drug, is produced by cleavage of the precursor by lung-specific esterases (Kanniess et al. 2001).

4 Mechanisms of Glucocorticosteroid Action

4.1 Glucocorticoid Receptors

GCs diffuse rapidly through the cell membrane and bind the ligand binding domain of their cytoplasmic receptor (GR, NR3C1—nuclear receptor subfamily 3, group C, member 1) to induce activation (Beck et al. 2009; Xavier et al. 2016). Once activated, GR translocates into the nucleus where it interacts with transcriptional coactivators or repressors to repress inflammatory genes or enhance the expression of anti-inflammatory genes (Beck et al. 2009; Xavier et al. 2016). GR exists in all cells within the airway as the predominant GRα form (Lu and Cidlowski 2006). Other forms exists such as the GRβ isofiorm that has been implicated in steroid insensitvity in some patients by acting as a dominant negative regulator of GRα (Kino et al. 2009). The presence of GRα in airway cells explains the pronounced effect that GCs have on airway resident and inflammatory cells and their clinical efficacy in most subjects with asthma (Adcock et al. 1996).

The function of GRα is affected by post-translational modifications with the effect of phosphorylation being the most studied and Ser211 phosphorylation has been linked to alterations in ligand binding, nuclear translocation and transactivation and co-factor association (Weigel and Moore 2007; Avenant et al. 2010a). GR Ser226 phosphorylation, in contrast, is associated with greater transcription efficacy (Avenant et al. 2010b). Correct GR phosphorylation is essential for optimal GR function with phosphorylation at both Ser226 and Ser221 being seen with activation by dexamethasone (Verhoog et al. 2011).

GR can also be acetylated on K494 and K495 following activation (Ito et al. 2006b). Acetylation of GR affects the ability of GR to interact with p65 and removal of these tags is important for the suppression of subsets of inflammatory genes (Ito et al. 2006b).

Small Ubiquitin-like Modifier (SUMO) proteins can also modify GR and affect its function. Sumoylation affects GR transactivation potential particularly at promoters with multiple GREs (Davies et al. 2008) whilst K293 GR SUMOylation is essential for GC-induced inverted repeated negative GC response element (IR - nGRE)-mediated direct transrepression and for NF-κB/AP1-mediated GC-induced tethered indirect transrepression (Hua et al. 2016a, b).

Shuttling of GR between the nucleus and cytoplasm is regulated by nuclear import and export receptors in a dynamic manner (Maneechotesuwan et al. 2009). GR possess two nuclear localisation signals (NLS), NLS1 and NLS2 (Savory et al. 1999). GR interacts with several importins including importins 7, 8, 13 and the α/β heterodimer (Freedman and Yamamoto 2004). Defects in nuclear translocation observed in patients with relative steroid refractory asthma may result from abnormal levels of importin 7 or its ability to interact with GR under the influence of oxidative stress (Hakim et al. 2013; Chang et al. 2015).

Nuclear GR can induce gene expression following DNA binding at specific Glucocorticoid Response Elements (GREs) or, acting as a monomer interact with DNA-bound pro-inflammatory factors and thereby enable transcriptional regulator proteins to be positioned such that they repress activated gene expression (Beck et al. 2009; Xavier et al. 2016). Pro-inflammatory transcription factors such as AP-1 and NF-κB are the major targets for this tethering process (Ito et al. 2006a; Xavier et al. 2016) although recent ChIP-seq analysis in airway epithleial cells indicates that tethering between GR and p65 for example is not essential for repression (Kadiyala et al. 2016).

4.2 Gene Induction by Corticosteroids

The GRE is the imperfect palindrome AGAACA*nnn*TGTTCT (Adcock and Caramori 2001; Kadiyala et al. 2016) with GR able to interact with each heaxmer independently (Meijsing et al. 2009). Even small changes in the GRE sequence can have a profound effect on transcriptional activity (Meijsing et al. 2009). Indeed, the GRE may be considered as different type of GR ligand which is able to modify GR function by altering the association with transcriptional co-factors, changing the local chromatin configuration and thereby affecting downstream functional actions of GR (Xavier et al. 2016).

The activated GR only remains associated with the GRE for a few seconds before being replaced by a different GR in a process called assisted loading (Biddie et al. 2011; Biddie et al. 2012). Binding of the first GR to a GRE initiates and ATP-dependent chromatin remodelling process that provides site more ameneable for GR-GRE interaction and highlights the importance of co-ordinated GRE interactions to obtain the full GC response in a cell- and tissue-dependent manner (Biddie et al. 2012). These data also emphasised the importance of an extensive AP-1-GR interaction network in the control of GR-GRE binding and function (Biddie et al. 2011; Biddie et al. 2012).

ChIP-seq analysis in A549 cells reported >10,000 GR binding sites (GBS) of which only 13 % were able to induce transcriptional activation in response to GC exposure (Vockley et al. 2016). The GBS lacking activation potential clustered around the inducible GBS and interactions between these direct and tethered GBS across 10,000 s Kbp were necessary for the full gene activation response to GC (Vockley et al. 2016). In a separate study, it was reported that co-operative binding of GR and NF-κB p65 occurred at GREs to enhance the expression of key anti-inflammatory genes such as A20/TNFAIP3 (Kadiyala et al. 2016). In addition, there

was a large variability in the sites associated with gene repression in response to TNF.

4.3 Gene Repression by Corticosteroids

GR plays a critical role in suppressing inflammatory gene expression. The mechanisms involved generally evoke tethering of activated GR to an activated transcriptional complex driven by DNA-bound NF-κB, for example (Beck et al. 2009; Xavier et al. 2016). The interaction between GR and NF-κB is mutually antagonistic with GR repression seen with NF-κB activation (Ito et al. 2006b; Xavier et al. 2016). Importantly, increased NF-κB activation at the nuclear localisation and expression level is associated with severe asthma (Ito et al. 2006a).

This process is driven in part by HDAC2-mediated alterations in GR acetylation status (Ito et al. 2006b). HDAC2 expression and/or activity linked to enhanced HAT activity is reduced in severe asthma patients, particularly children (Su et al., 2009; Hew and Chung 2010). Interestingly, GRβ has been reported to reduce HDAC2 expression in human BAL macrophages (Li et al. 2010). A lack of HDAC activity may also evoke local changes in histone acetylation at inflammatory gene promoters and thereby modulating gene expression (Beck et al. 2009; Xavier et al. 2016).

In addition to interactions with AP-1 and NF-κB, GR can also associate with, and repress, the function of many other transcription factors including the signal transducer and activator of transcription (STAT) family of transcription factors (Langlais et al. 2012). Many inflammatory and acute phase genes are under STAT regulation induced by mediators such as IFNs, IL-5 and IL-6, for example (O'Shea and Plenge 2012). Interestingly, inflammatory mediated induced by IFNγ-stimulated airway epithelial cells can be inhibited by JAK-STAT inhibitors but not by steroids (Fenwick et al. 2015).

Several other mechanisms of GR function have been reported included effects on mRNA stability. GCs affect the expression of pro-inflammatory gene mRNAs which contain adenylate-uridylate–rich elements (AREs) within their 3′ untranslated regions through targeting tristetrapolin (TTP) and Hu antigen R (HuR) family members which control mRNA decay and stability, respectively (Smoak and Cidlowski 2006). This mechanism is used by dexamethasone, for example, to down-regulate COX-2 and CCL11 expression acting via the p38 MAPK–MKP-1 axis (Smoak and Cidlowski 2006; Ishmael et al. 2008).

It has become increasingly clear that non-coding RNAs (ncRNAs) are intimately involved in modifying GR expression and function and vice versa (Maltby et al. 2016). ncRNAs such as microRNAs (miRNAs) control cellular pathways by impacting upon mRNA degradation and/or translation with the effect depending upon the degree of homology between each specific miRNA and the target mRNA. The expression of certain key miRNAs are regulated by GR and GR is itself the target of other miRNAs (Kabesch and Adcock 2012; Maltby et al. 2016). Induction of GILZ expression by GR is reduced by miR18 and miR124a in human and rat

cells and aberrant expression of these miRNAs may be involved in the relative steroid insensitivity seen in some patients with severe asthma (Kabesch and Adcock 2012; Maltby et al. 2016). Hydrocortisone elicited a threefold increase in miR124 in sepsis patients which caused GRα down-regulation and steroid insensitivity (Ledderose et al. 2012).

MiR145, miR21 and let-7b regulate several features of asthma and inhibition of miR145 prevented eosinophilia, mucous secretion and airway hyperresponsiveness to the same extent as dexamethasone in a mouse model of asthma (Kabesch and Adcock 2012; Maltby et al. 2016). Long ncRNAs (lcRNAs) are defined as being >200 nucleotides in length and two specific lncRNAs have opposite effects on GR function. Steroid receptor RNA activator (SRA) is a constituent of the steroid receptor coactivator (SRC)-1/SRC-2 complex and it increases GR transcriptional (Lanz et al. 1999). In contrast, growth arrest specific 5 (Gas5) is a GRE decoy by binding to the DNA binding site of active GR (Kino et al. 2010).

4.4 Steroid Refractory Asthma

Some patients with severe asthma are unable to suppress asthmatic inflammation with high dose ICS or even oral glucocorticoids (Bel et al. 2011; Chung et al. 2014). These subjects account for a large percentage of the costs for asthma and are a major healthcare problem worldwide (Bel et al. 2011; Chung et al. 2014; Accordini et al. 2013). These patients are distinct from those who are non-compliant with their treatment or subjects without access to the correct therapies (Bel et al. 2011; Chung et al. 2014). The reduced GC function in refractory asthma may be multi-factorial and each stage of GR activation, namely GR expression, ligand binding, nuclear translocation and/or binding to the GRE and other transcription factors has been proposed as a mechanism (Beck et al. 2009; Hew and Chung 2010; Chung et al. 2014).

The transcriptome of airway epithelial cells of mild/moderate asthmatics has identified a gene profile that predicts ICS responsiveness—namely, an IL-13-induced gene signature (Woodruff et al. 2009). The expression of this signature is variable in asthma (Choy et al. 2011) and is inversely correlated with Th17 cells which are linked with steroid insensitivity (Peters et al. 2014) and with IL-6 a marker of neutrophilic asthma which is also associated with more severe disease (Peters et al. 2016).

High levels of IL-2, Il-4 and Il-13 reduce steroid responses in T-cells by reducing the affinity of GR for its ligand (Ito et al. 2006a; Kino et al. 2009). This may reflect differences in GR phosphorylation status under the control of the p38 MAPK pathway (Bhavsar et al. 2010; Irusen et al. 2002). Increased p38 MAPK activity is also seen in peripheral blood monocytes and BAL macrophages from patients with severe asthma and p38 MAPK inhibitors restored GC responsiveness in these cells (Bhavsar et al. 2010; Goleva et al. 2009). Similar results were seen in cells from COPD patients (Armstrong et al. 2011). There is some evidence that this may be linked to changes in HDAC and HAT activities (Hew and Chung 2010). p38

MAPK may also modulate GR responses by changing GR phosphorylation status (Galliher-Beckley et al. 2011). Phosphorylation of GR on Ser134 is p38 MAPK-dependent manner and significantly down-regulates dexamethasone-dependent genome-wide transcriptional responses and cell functions (Galliher-Beckley et al. 2011). MKP-1/DUSP1 is a GC-inducible gene which dephosphorylates and inactivates p38 MAP kinases and its expression and induction is impaired in severe asthma (Hew and Chung 2010).

The exact stimulus given to a cell modifies the intracellular pathway(s) activated and other signalling pathways such as the MEK/ERK pathway have been implicated in controlling relative GC-refractoriness (Ito et al. 2006a; Goleva et al. 2008). Furthermore, cyclin-dependent kinases (CDK), glycogen synthase kinase-3 and JNKs can also target GR phosphorylation or phosphorylation of GR-associated co-factors (Adcock and Barnes 2008; Barnes and Adcock 2009; Ngkelo et al. 2015).

Neutrophillic asthma is associated with GC refractoriness and increased IL-17 expression and Th17 cells (Zijlstra et al. 2012; Peters et al. 2014). An animal model of asthma demonstrated that Th17 cell transfer causes a dexamethasone-insensitive neutrophilic inflammatory response and BHR to metacholine (McKinley et al. 2008). More importantly, IL-17 inhibits budesonide sensitivity in primary human bronchial epithelial cells through modulating PI3K and HDAC2 expression (Zijlstra et al. 2012).

4.5 Asthma Exacerbations and Steroid Sensitivity

Viral and bacterial infections cause asthma exacerbations in children and adults which is not readily resolved by GCs (Jackson et al. 2011; Hewitt et al. 2016). In experimental models of asthmatic exacerbations neither ICS (Grunberg et al. 2001) nor oral prednisolone (Gustafson et al. 1996) prevents the worsening of airway inflammation or improve clinical symptoms. Principal component analysis indicated that IFNγ was one of the dominant variables for chronic persistent obstruction in severe asthma (Kaminska et al. 2009) and primary bronchial epithelail cells stimulated with IFNγ do not respond to dexamethasone (Fenwick et al. 2015). These inflammatory responses are, however, completely ablated by treatment of cells with a JAK-STAT inhibitor. Exposure of primary human airway epithelial cells to RV-16 causes a relative GC resistance by preventing GR nuclear import (Papi et al. 2013). This is reversed by suppression of the RV-16-induced JNK and NF-κB pathways.

Although some patients with severe asthma have low serum-specific IgE and negative skin prick tests, they have IgE present within the airways (Barnes 2009a). Epithelial cell colonisation by microbial superantigens such as Staphylococcal enterotoxins may drive this local IgE production (Barnes 2009a). They may also induce relative GC insensitivity through ERK MAPK pathway activation resulting in increased GRβ expression or by modulating GRα phosphorylation (Li et al. 2004). In addition, TLR7 and TLR9 activation of blood-derived DCs can reduce dexamethasone sensitivity (Guiducci et al. 2010).

4.6 Oxidative Stress and GC Refractoriness

Patients with COPD and also some patients with severe asthma have raised levels of oxidative stress (Rahman and Adcock 2006) and hydrogen peroxide (H_2O_2) has been shown to reduce GR translocation in primary human airway fibroblasts (Rahman and Adcock 2006) and attenuates suppression of inflkammation by budesonide and epithelial cell integrity (Heijink et al. 2014). It is possible that redox-sensitive activation of the AP-pathway may drive relative steroid refractoriness in PBMCs from severe steroid refractory asthmatics (Adcock et al. 2008b). Indeed, the expression of AP-1 components and its upstream activators is greater in PBMCs and bronchial biopsies from patients with corticosteroid-resistant asthma (Ito et al. 2006a) and their expression is not altered by high doses of oral glucocorticoids (Adcock et al. 2008a, b). Nitrosative stress may also impact upon steroid responsiveness and peroxynitrite formation causes nitration of specific tyrosine residues results in the loss of enzymic activity (Y146) and degradation (Y253) of HDAC2 (Osoata et al. 2009). However, reduced HDAC2 expression and/or activity is not seen in all patients with therapy refractory asthma possibly reflecting the heterogeneity of severe asthma phenotype (Moore et al. 2010; Chung and Adcock 2013).

In contrast, HDAC2 expression and activity is reduced in COPD (Ito et al. 2005). Overexpression of HDAC2, but not HDAC1, improved steroid sensitivity in primary macrophages from COPD patients (Ito et al. 2006b) through a mechanism that involves the phosphoinositide-3-kinase (PI3K)-δ pathway (Marwick et al. 2009; To et al. 2010). Sub-bronchodilator doses of theophylline can enhance HDAC2 activity in vitro and combined theophylline and ICS treatment improved lung function and sputum neutrophilia in COPD patients (Ford et al. 2010) and lung function in smoking asthmatics (Barnes 2009b; Spears et al. 2009). However, use of PI3K-selective inhibitors may prove more efficacious in improving patient responses to ICS.

5 Clinical Implications

Unbiased cluster analysis of clinical features incorporating inflammatory indices will lead to better phenotyping of patients with asthma and COPD than currently achieved. It is likely, however, that unbiased, or semi-biased, analysis of omic and multi-omic analysis will be required to define the key pathways that drive the various clinical traits observed involved in the inflammatory profile observed in these patients that may indicate novel therapeutic targets (Moore et al. 2007; Chung and Adcock 2013). Current evidence indicates that sub-phenotyping patients will aid targeting of novel therapeutic agents to the correct patient at the correct time, e.g., anti-IL-5 (Haldar et al. 2009; Nair et al. 2009; Bel et al. 2014), anti-IL-13 (Corren et al. 2011) and JAK/STAT inhibitors (Fleischmann et al. 2012). However, not all patients respond to these therapies despite the use of biomarker selection and other biologics have lacked efficacy or even been detrimental such as anti-TNFα (Adcock et al. 2008a, b; Chung and Adcock 2013; Wenzel et al. 2009).

Understanding the mechanisms by which GCs have reduced efficacy in some patients may provide an alternative approach to treatment of these patients. In this manner, drugs will be used as add-on therapies to enhance clinical efficacy. The results of in vitro, ex vivo and in vivo experiments have given some insights into the signalling pathways that may provide potential steroid-enhancing options but these will need to be tested in clinical trials. These may include combinations of ICS with p38 MAPK or PI3K inhibitors where neither drug provides adequate effects on their own (Chung 2011).

The development of non-invasive biomarkers for possible responders-non-responders is critical if the drive towards personalised or stratified medicine in airways disease is to be achieved. Failure to obtain a good reliable biomarker of pathway activation or possible clinical respond may prevent the right subjects being treated. It is also imperative that patients are aware of the importance of taking their drugs (Heaney et al. 2016; McNicholl et al. 2012) as no drug, however potent, will have clinical benefit if the patient does not take it.

Bacterial and viral infection drive exacerbations of severe asthma and COPD should be treated where possible but this is more complicated as it also affects GC responsiveness (Papi et al. 2013). Although anti-infective agents may be beneficial in many of these patients with severe disease, it is unclear which patients are the most likely to benefit and what would be the best dosing regimen.

6 Conclusions

The optimal anti-inflammatory treatment for patients with asthma will remain ICS but these are not adequate for a significant sub-population of patients. The causes of this lack of response are heterogeneous with possible roles for genetic, behavioural (compliance) and environmental risk factors. Greater understanding of the precise pathways that underpin GC refractory asthma and many patients with COPD may identify new targets that either restore GC responsiveness or prevent inflammation on their own. Integrated multi-omic analysis of samples from the airways of large cohorts of these patients along with suitable predictive biomarkers will be necessary in the future to help us understand mechanisms of GC actions and inactions in these patients.

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Bifunctional Drugs for the Treatment of Respiratory Diseases

Clive Page and Mario Cazzola

Contents

Abstract

Over the last decade, there has been a steady increase in the use of fixed dose combinations for the treatment of a range of diseases, including cancer, AIDS, tuberculosis and other infectious diseases. It is now evident that patients with asthma or chronic obstructive pulmonary disease (COPD) can also benefit from the use of fixed dose combinations, including combinations of a long-acting β_2-agonist (LABA) and an inhaled corticosteroid (ICS), and combinations of LABAs and long-acting muscarinic receptor antagonists (LAMAs). There are now also "triple inhaler" fixed dose combinations (containing a LABA, LAMA and ICS) under development and already being made available in clinical practice, with the first such triple combination having been approved in India. The use of combinations containing drugs with complementary pharmacological actions in the treatment of patients with asthma or COPD has led to the discovery

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and development of drugs having two different primary pharmacological actions in the same molecule that we have called "bifunctional drugs". In this review we have discussed the state of the art of bifunctional drugs that can be categorized as bifunctional bronchodilators, bifunctional bronchodilator/anti-inflammatory drugs, bifunctional anti-inflammatory drugs and bifunctional mucolytic and anti-inflammatory drugs.

Keywords
Anti-inflammatory drugs • Asthma • Bifunctional drugs • Bronchodilators • COPD

1 Background

Asthma and COPD are common complex inflammatory diseases of the respiratory tract that usually require treatment with multiple drug classes (Boulet et al. 2012; Vestbo et al. 2013). Over the last decade, there has been a steady increase in the use of fixed dose combinations of two or more drugs for the treatment of a range of diseases including cancer (van Meir et al. 2014), AIDS (Flexner and Saag 2013), tuberculosis (Dawson and Diacon 2013) and other infectious diseases (Huang et al. 2013).

It is now recognized that the treatment of asthma and COPD can benefit from the use of fixed dose combinations of two or more drug classes (Cazzola et al. 2012a). Today the treatment of asthma and COPD globally is dominated by the use of inhaled fixed dose combinations of a LABA and an ICS, although the wide usage of ICS in the treatment of patients with COPD is being questioned because of a potential increased risk of pneumonia (Kew and Seniukovich 2014) and because of recent observations questioning their efficacy (Magnussen et al. 2014). Nonetheless many fixed dose combinations of ICS/LABA and ICS/LAMA and indeed triple inhalers containing fixed doses of LABA/LAMA and ICS are in development.

However, the use of fixed dose combinations for the treatment of asthma and COPD is not a new concept with inhaled fixed dose combinations of both short-acting β_2-agonists (SABAs) and short-acting muscarinic receptor antagonists (SAMAs) having been used for many years in the treatment of patients with asthma or COPD (Goodman & Gilman 2005), and such combinations are particularly used in the treatment of acute exacerbations of these diseases. The success of these medicines has stimulated the development of longer-acting β_2-agonists (LABAs) and ultra-LABAs and longer-acting muscarinic receptor antagonists (LAMAs). These drugs are now widely used as bronchodilators for the treatment of patients with COPD and more severe asthma (Cazzola et al. 2012a).

These developments in treatment using fixed dose combinations reflect our growing understanding that there is a need to treat both the underlying inflammation and the symptoms of airway obstruction that characterize asthma and COPD. Moreover, the use of multiple drugs in a single inhaler is thought to improve the

adherence to treatment, as it is well recognized that patients prescribed a bronchodilator and an anti-inflammatory drug as monoinhalers will often stop the anti-inflammatory drug when symptoms improve (Stempel et al. 2005), despite current understanding suggesting that regular use of ICS (at least in patients with asthma) may be necessary to optimize lung function and reduce exacerbations of the disease in the long term (Bateman et al. 2008).

However, the development of formulations to allow the use of more than one drug class in a single inhaler is sometimes challenging, as there are often differences in duration of action of the mono-components and issues concerning chemical compatibility and stability as well as gallenic challenges relating to the different physiochemical properties of the different drug classes (Cazzola et al. 2012a). This is especially so with the development of triple inhalers, and to date only one such medicine containing tiotropium bromide, ciclesonide and formoterol fumarate has been approved in India, although others are in late-stage clinical development (Cazzola and Matera 2014).

However, an alternative approach to delivering complementary pharmacological activities for the treatment of patients with asthma or COPD is to develop molecules designed to have two distinct primary pharmacological actions, which we will term bifunctional drugs. In this article we review the current status of bifunctional drugs in development or currently in use for the treatment of respiratory diseases.

2 Bifunctional Bronchodilator Drugs

It has long been recognized that β_2-agonists and muscarinic receptor antagonists improve lung function by distinct pharmacological mechanisms, β_2-agonists acting to relax airway smooth muscle irrespective of the cause of the bronchoconstriction and muscarinic receptor antagonists by blocking M_3 receptors on airway smooth muscle to limit the actions of the neurotransmitter acetylcholine (ACh) released from parasympathetic nerves innervating the lung (Cazzola et al. 2012a; Cazzola and Molimard 2010).

Moreover, β_2-agonists can amplify the bronchial smooth muscle relaxation caused by a muscarinic receptor antagonist by decreasing the release of ACh via a modulation of cholinergic neurotransmission that involves calcium-activated potassium (K_{Ca}) channels rather than adenylyl cyclase and subsequent increases in intracellular levels of cyclic adenosine monophosphate (c-AMP). Activation of K_{Ca} channels is thought to hyperpolarize the cell membrane, thus causing reductions in the concentration of intracellular Ca^{2+} and ACh release in prejunctional parasympathetic nerves (Cazzola and Molimard 2010; Cazzola et al. 2013a) and thus potentially providing additional bronchodilation above the effects seen with antagonism of muscarinic receptors alone on the airway smooth muscle. However, this mechanism seems unlikely to contribute in a significant way in clinical practice as there is evidence clearly indicating that β_2-agonists facilitate, rather than inhibit release of Ach from airway parasympathetic nerves (Meurs and Dekkers 2013; Meurs et al. 2013). Therefore, it has been suggested that crosstalk

between the signalling mechanisms arising from antagonism of muscarinic receptors and activation of β_2 adrenoceptors within airway smooth muscle provides a more plausible explanation of the additional bronchodilation seen when both classes of drug are used together rather than individually (Meurs and Dekkers 2013; Meurs et al. 2013). Indeed, crosstalk between G_q-coupled M_3 receptors and G_s-coupled β_2 adrenoceptors may have a major influence on β_2-agonist-induced relaxation, presumably by activation of protein kinase C (PKC) and subsequent phosphorylation of the β_2 adrenoceptor and/or G_s protein (Meurs and Dekkers 2013; Meurs et al. 2013). Moreover, at postsynaptic level β_2AR signalling limits M_3 receptor-mediated inositol triphosphate (IP_3) production by several distinct mechanisms, most presumed to involve protein kinase A (PKA) (Pera and Penn 2014).

Recent findings have also demonstrated that β_2 adrenoceptors and muscarinic receptors mediate opposing effects on endothelin-1 expression in human lung fibroblasts (Ahmedat et al. 2012). Since muscarinic receptor-mediated upregulation of endothelin-1 contributes to profibrotic effects induced by muscarinic agonists, inhibition of endothelin-1 expression by a muscarinic receptor antagonist could contribute to long-term beneficial effects of these drugs. Moreover, β_2-agonists and muscarinic receptor antagonists have been demonstrated to provide additive control of transforming growth factor (TGF)-β1-mediated neutrophilic inflammation in patients with COPD (Profita et al. 2012) that has implications also for the use of such drugs in the treatment of patients with neutrophilic asthma. LABAs and anticholinergic drugs might also contribute to control the ACh-induced increased levels of Th17 cells in systemic inflammation of COPD (Profita et al. 2014).

These complementary pharmacological actions have led to LABAs and LAMAs often being used together, particularly in the treatment of patients with COPD (van der Molen and Cazzola 2012), but they are also now being investigated for the treatment of asthma where combinations of SABAs and SAMAs have been widely used for several decades (Goodman & Gilman 2005). These observations have led to the development of a number of new drugs referred to as MABAs, which have both β_2-agonist activity and muscarinic receptor antagonism in the same molecule, some of which have now reached early clinical development (Cazzola et al. 2012a). These bifunctional (or dual pharmacophore) muscarinic antagonist β_2-agonist (MABA) agents are exemplified by the drugs GSK 961081 (batefenterol) and THR 200495 which have recently been shown to induce bronchodilation in patients with COPD that lasts for up to 24 h and that is comparable to a combination of salmeterol and tiotropium (Bateman et al. 2013; Norris and Ambery 2013). Other examples include AZD 2115, LAS 190792, TEI3252, PF-3429281 and PF-4348235 (Hughes and Jones 2011; McNamara et al. 2012).

The MABA approach circumvents the potential problems associated with formulating different drugs in one inhaler yet still providing a fixed ratio of muscarinic antagonism and β_2 agonism compared with combination therapy (Cazzola et al. 2013b). However, what is not yet clear is the relative contribution of the two different pharmacological activities to the overall improvement in lung function and indeed on which pharmacological action such drugs should be

optimally dosed, as some examples of MABAs have different pharmacodynamic half-lives for their β_2-agonist activity and the muscarinic receptor antagonist activity within the same molecule (Bateman et al. 2013; Norris and Ambery 2013). A recent clinical study has demonstrated that treatment with 3 separate doses of GSK 961081 showed superior improvements in lung function in patients with COPD compared with a standard dose of salmeterol suggesting that the MABA provides a better treatment than monotherapy with a β_2-agonist (Wielders et al. 2013), although a similar trial has not yet been performed comparing a MABA with monotherapy with a muscarinic receptor antagonist. Thus, whilst MABAs show promise, there is a lot that still needs to be understood as to how to best use this class of drug and how they will compare to existing fixed dose combination inhalers (Cazzola et al. 2013b).

3 Bifunctional Bronchodilator/Anti-inflammatory Drugs

Xanthines such as theophylline have been widely used as treatments for both asthma and COPD for more than 100 years, and whilst early clinical studies with such drugs have stressed their bronchodilator activity, they were originally introduced into clinical practice to treat an inflammatory renal disease, glomerular nephritis (Persson 1985). It has been recognized for some time that xanthines could exhibit anti-inflammatory activity experimentally in the lung, additional to their bronchodilator activity (Persson 1985; Spina et al. 1998), an observation that has been confirmed clinically in patients with asthma (Crescioli et al. 1991; Sullivan et al. 1994; Jaffar et al. 1996; Evans et al. 1997; Lim et al. 2001) or COPD (Culpitt et al. 2002; Ford et al. 2010). Such observations provided some of the earliest evidence that it was possible to have both bronchodilator and anti-inflammatory activity in a single molecule. The problem with xanthines is their very narrow therapeutic window (Boswell-Smith et al. 2006a) that has limited their wider use. Furthermore, the advent of newer inhaled bronchodilator and anti-inflammatory drugs, particularly inhaled β_2-agonists and ICS, has seen a progressive decline in the use of xanthines (that are usually administered systemically), even though there is clear evidence that withdrawal of theophylline from patients with asthma or COPD leads to worsening of airways inflammation and symptoms, even in patients taking glucocorticosteroids and other classes of bronchodilator drug (Baba et al. 2001; Minoguchi et al. 1998; Kidney et al. 1995). Such observations suggest that xanthines possess other useful pharmacological properties not shared with glucocorticosteroids and other classes of bronchodilator.

This has led at least one pharmaceutical company to develop a combination inhaler using theophylline and an ICS (Barnes et al. 2010). Other pharmaceutical companies have tried to find safer xanthines and a number of been investigated in the clinic, including bamiphylline (Spinelli et al. 1991), enprophylline (Pauwels et al. 1985), isbuphylline (Manzini et al. 1993), acebrophylline (Tapadar et al. 2014) and doxophylline (Page 2010; van Mastbergen et al. 2012), some of which have been approved for the treatment of asthma and COPD. Like

theophylline, each of these drugs has been shown to possess anti-inflammatory and bronchodilator actions to varying degrees and, in the case of doxophylline, a wider therapeutic window than theophylline (Page 2010).

Theophylline and the related xanthine isobutyl methylxanthine (IBMX), in particular, have often been described as the archetypal non-selective phosphodiesterase (PDE) inhibitors as a possible mechanism of action of these drugs (Nicholson et al. 1991), although of course it is now recognized that this is probably not the only molecular mechanism contributing to their therapeutic benefit in the treatment of patients with asthma or COPD (Boswell-Smith et al. 2006a). Thus, another approach to try and improve the therapeutic window of xanthines has been to develop more selective inhibitors of the growing family of PDEs, as it is now recognized that PDE 3 and 4 are found in airway smooth muscle, whilst PDE 3, 4 and 7 are found in the majority of inflammatory cells thought to be involved in the pathogenesis of asthma and COPD (Spina et al. 1998; Page 2014). Specifically, the PDE3 isoenzyme is considered to predominate in airway smooth muscle, and inhibition of this enzyme, rather than PDE4 inhibition, leads to airway smooth muscle relaxation, whereas the PDE4 isoenzyme is the predominant isoenzyme in the majority of inflammatory cells, including neutrophils, which are implicated in the pathogenesis of COPD and severe asthma, and in eosinophils, which characterize inflammation in patients with asthma (Page 2014).

Recently the selective PDE4 inhibitor roflumilast-n-oxide has been approved for the treatment of severe COPD, although the side effect profile of this drug still limits the wider use of this agent (Calverley et al. 2009; Fabbri et al. 2009). This clinical benefit of roflumilast is thought to arise from the anti-inflammatory action of this selective PDE4 inhibitor (Grootendorst et al. 2007), as whilst PDE4 is found in human airway smooth muscle, it is now clear from a number of clinical studies with a variety of PDE4 inhibitors administered either orally (Grootendorst et al. 2003; Harbinson et al. 1997) or by inhalation (Singh et al. 2010), that this drug class is not able to induce acute bronchodilation.

In contrast, a number of selective PDE3 inhibitors have been shown to elicit acute bronchodilation in man (Bardin et al. 1998; Myou et al. 1999), and indeed recently PDE3 has been documented to be upregulated in airway smooth muscle obtained from patients with asthma (Yick et al. 2013). These observations have led to the development of drugs having dual inhibitory activity for both PDE3 and PDE4 in order to obtain both bronchodilator and anti-inflammatory activity in the same molecule. The first such drug was zardaverine, which clearly exhibited bronchodilation in patients with asthma but unfortunately was halted during clinical development because of gastrointestinal side effects (Brunnée et al. 1992). Another example was benzafentrine (AH 21-132) (Foster et al. 1992), which clinically elicited bronchodilation but was later discontinued from clinical development, as was another dual PDE 3/4 inhibitor, pumafentrine (Rieder et al. 2013).

Other compounds having both PDE3 and 4 inhibitory activities have been described by Kyorin Pharmaceuticals in Japan, but these have also been stopped at the preclinical stage because of unwanted gastrointestinal side effects (Ochiai et al. 2013). However, another inhaled dual PDE3/4 inhibitor, RPL 554 (Boswell-

Smith et al. 2006b), has been shown in early clinical studies to have both bronchodilator and anti-inflammatory actions at the same dose, without having significant side effects (Franciosi et al. 2013), representing potentially a new class of drug for the treatment of patients with asthma or COPD (Wedzicha 2013). Furthermore, recent data investigating this drug in combination with either a muscarinic receptor antagonist or a β_2-agonist has shown synergistic interactions on the relaxation of human bronchi (Calzetta et al. 2013) or human small airways (Calzetta et al. 2015). If such findings translate into the clinical setting, such observations raise the distinct possibility of combination of dual PDE 3/4 inhibitors with other drug classes. Furthermore, PDE5 inhibitors such as sildenafil are also able to induce bronchodilation in addition to their well-documented effects on pulmonary vascular smooth muscle (Charan 2001) as well as suppress the pulmonary inflammation and airway hyperreactivity that follow allergen and lipopolysaccharide (LPS) challenge (Toward et al. 2004). Thus, it has been suggested that a new molecule that inhibits both PDE4 and PDE5 could act at multiple levels in patients with COPD, reducing lung inflammation and, possibly, remodelling, as well as decreasing arterial pulmonary hypertension, and improving lung function (Giembycz 2005). One such example is LASSBio596, designed as a hybrid of thalidomide and aryl sulfonamide, which is a drug that exhibits potent inhibitory effects on both PDE4 and PDE5 (Rocco et al. 2003). In a murine model of elastase-induced emphysema, LASSBio596 reduced lung inflammation and remodelling as well as being able to improve lung mechanics (Guimaraes et al. 2014). Interestingly, it has also been documented that LASSBio596 has the potential to block proliferation of fibroblasts (Campos et al. 2006).

Another approach to combining anti-inflammatory and bronchodilator actions in a single molecule has been to combine the bronchodilator actions of nitric oxide (NO) with the anti-inflammatory actions of an ICS. NO-budesonide (TPI 1020) (Boulet et al. 2009; Turner et al. 2010) was the first example of such a drug, although this drug has been dropped from further development. Others have attempted to combine NO and salbutamol into a single molecule (NCX 950) to obtain both bronchodilator and anti-inflammatory actions, and this approach has shown some promise preclinically (Lagente et al. 2004). Another innovative approach is the use of a mutual prodrug designed to allow local metabolism to the active forms of the parent constituents at the site of action, thus reducing unwanted systemic side effects. GS424020, a novel mutual prodrug of salmeterol and desisobutrylciclesonide, exhibited intriguing pharmacological activity in preclinical studies (Barrett et al. 2010).

GS-5759 is a novel bifunctional PDE4 inhibitor/LABA drug that displays PDE4 inhibition and β_2 agonism comparable to roflumilast and indacaterol, respectively (McDonald et al. 2012). More recently a series of molecules that combine the anti-inflammatory drug roflumilast with the LABA salmeterol (Barrett et al. 2010; Huang et al. 2014; Liu et al. 2013) or formoterol (Tannheimer et al. 2012) have been described that are other potential examples of a new drug class that combine anti-inflammatory and bronchodilator actions into a single molecule, although to date there are very limited biological data with these molecules. A potential

advantage of these compounds is that both β_2-agonists and PDE4 inhibitors rely on modulation of the secondary messenger cyclic AMP to elicit their effects, and it is possible that such a bifunctional drug could provide additive or even potentially synergistic anti-inflammatory activity in the lungs (Tannheimer et al. 2012). Also bifunctional compounds in which a PDE4 inhibitor is connected to a muscarinic receptor antagonist have been described (Phillips and Salmon 2012) that utilize a pyrazolopyridine as the PDE4 inhibitor and a biaryl-containing muscarinic antagonist but differ in the linker used to combine these two activities into the same molecule. Another example of such an approach is UCB-101333-3, a 4,6-diaminopyrimidine (Provins et al. 2007).

RO 50-24118, a stable analogue of vasoactive intestinal peptide (VIP) that is highly selective for the $VPAC_2$ receptor, has been shown to have dual bronchodilatory and anti-inflammatory effects, in that it relaxes airway smooth muscle cells, inhibits bronchoconstriction and attenuates the influx of neutrophils and $CD8^+$ T cells in inflammatory lung disease (Tannu et al. 2010) suggesting another potential approach to combining anti-inflammatory and bronchodilator actions into a single molecule.

4 Bifunctional Anti-inflammatory Drugs

Glucocorticosteroids are currently recognized as the gold standard anti-inflammatory drugs for the treatment of inflammatory airway diseases such as asthma and COPD, in part because they exhibit polypharmacy via a range of actions, such as reducing the activation and recruitment of inflammatory cells into the lung (Adcock et al. 2008). However, this drug class can also be associated with significant side effects when they enter the systemic circulation as well as having local side effects when applied topically. Nonetheless, given the success of glucocorticosteroids as anti-inflammatory drugs, it is perhaps not surprising that the "holy grail" of the pharmaceutical industry for many years in the respiratory field has been to find an alternative anti-inflammatory drug to glucocorticosteroids that retain the efficacy but that have a better safety profile (Adcock et al. 2008). There have been many new classes of anti-inflammatory drug developed (Adcock et al. 2008; Cazzola et al. 2012b), most of which have failed, except in the treatment of a subset of more severe patients with asthma (Nair et al. 2009) or COPD (Calverley et al. 2009; Fabbri et al. 2009). Many of these have been drugs or biologics directed against a single inflammatory mediator, and these failures (Adcock et al. 2008; Cazzola et al. 2012b; Bryan et al. 2000; Leckie et al. 2000; Nair et al. 2012; Gauvreau et al. 2014) perhaps suggest that the complexity of the inflammatory response in both asthma and COPD requires drugs that have actions at more than one biological target.

Thus, a number of drugs have been developed having bifunctional anti-inflammatory activity, including drugs exhibiting antagonism for the receptors for platelet-activating factor (PAF) and histamine, and also mast cell secretion-blocking effects in the same molecule such as rupatadine (Saint-Martin

et al. 2004; Church 2010), as well as drugs behaving as thromboxane receptor antagonists and cys-LT antagonists in the same molecule (Arakida et al. 1998). However, both of these classes of drug have to date only shown limited efficacy, at least in the treatment of allergic airways disease.

Dual targeting of IL-13 and IL-4 is another approach that holds promise for achieving great efficacy. Persistence of asthma symptoms despite high-dose corticosteroids has been linked to increased IL-13 levels in the lungs, suggesting that IL-13 expression might contribute to corticosteroid resistance in some patients. IL-4 shares functional redundancy with IL-13 because of a shared receptor, the type 2 IL-4R; however, IL-4 can also signal exclusively through the type 1 IL-4R (composed of IL-4Rls in the lungs, suggest) (Kasaian et al. 2013; Kau and Korenblat 2014 Dec). A biotherapeutic agent targeting both murine IL-4 and IL-13 was generated by combining well-characterized binding domains in an optimal configuration, using appropriate linker regions (Kasaian et al. 2013). This bifunctional IL-4 and IL-13 antagonist demonstrated high affinity for both cytokines and reduced the IL-4-dependent rise in serum IgE and reduced IL-13-dependent BHR, lung inflammation, mucin gene expression and serum chitinase responses in mice. Effective dual blockage of IL-13 and IL-4 resulted in greater therapeutic benefit than could be achieved by targeting either cytokine alone raising the possibility of bifunctional biologics. Other multiple agents that target both IL-4 and IL-13 have entered into clinical development, including AMG-317, dupilumab and pitrakinra. Pitrakinra, a recombinant IL-4 mutein that blocks the effects of both IL-4 and IL-13, improved the exacerbation incidence in response to medication withdrawal in asthmatic patients with high peripheral blood eosinophil counts ($>$350 cells/mm^3) (Wenzel et al. 2010). In patients with persistent, moderate-to-severe asthma and elevated eosinophil levels who used ICS and LABAs, therapy with dupilumab, a fully humanized mAb to the IL-4Rα receptor that inhibits both IL-4 and IL-13, as compared with placebo, was associated with fewer asthma exacerbations when LABAs and inhaled glucocorticoids were withdrawn, with improved lung function and reduced levels of Th2-associated inflammatory markers (Wenzel et al. 2013). Bispecific antibodies, antibodies that are able to inhibit the action of two targets simultaneously, have been designed for IL-4 and anti IL-13 and could be an additional therapeutic option in individuals with an unfavourable IL-4Rα polymorphism (Spiess et al. 2013).

The co-expression of PDE4 and PDE7 in most immunoinflammatory cells and the synergistic effects of PDE7- and PDE4-selective drugs in the suppression of inflammation in cell-based studies have fuelled speculation that dual inhibition of PDE7 and PDE4 could be an effective strategy to treat COPD (Page and Spina 2012). IR-284 is a dual PDE4–PDE7 inhibitor, but there is no published study that has documented its effects in patients with COPD (Matera et al. 2014). Nonetheless, TPI 1100, which comprises two antisense oligonucleotides targeting the messenger RNA (mRNA) for the PDE4B/4D and PDE7A isoforms, has been shown to reduce neutrophil influx and key cytokines in an established smoking mouse model (Seguin and Ferrari 2009).

Since pathological airway remodelling is mediated by PDE1 and PDE1 inhibitors that block smooth muscle mitogenesis (Chan and Yan 2011), combined PDE1 and PDE4 inhibitors could have utility in the treatment of patients with COPD (Giembycz and Maurice 2014). The dual PDE1/4 inhibitor KF19514 is also reported to be able to suppress inflammation and arrest airway remodelling, at least in a murine model of chronic asthma (Kita et al. 2009).

5 Bifunctional Anti-inflammatory/Mucolytic/Antioxidant Drugs

Carbocysteine (Rahman and MacNee 2012), N-acetyl cysteine (NAC) (Rahman and MacNee 2012) and erdosteine (Cazzola et al. 2010) are all drugs that have been used for many years in the treatment of COPD. They have been variously described as antioxidants or mucolytic drugs and continue to be prescribed in many countries. However, recent data with NAC (Zheng et al. 2014) has reported significant effects on exacerbations in patients with COPD which suggest that this class of drug may need to be used more widely. It is of interest that both NAC (Zheng et al. 2014) and erdosteine (Cazzola et al. 2010) have both been shown to be anti-inflammatory, as well as mucolytic, activities which would both be expected to contribute to the clinical efficacy observed with these drugs in reducing exacerbations. As such they provide templates for the development of novel bifunctional drugs exhibiting both anti-inflammatory and mucolytic activities and have the distinct advantage over many existing drugs in being orally active and well tolerated.

6 Conclusions

It is clear that there is a growing trend to develop drugs with bifunctional activity for the treatment of respiratory diseases that have the potential benefit of being easier to formulate than combinations of multiple drugs in a single inhaler, thus improving adherence and potentially being able to offer additive or even synergistic benefit, as such drugs may target different cellular compartments than when individual drugs are presented to cells individually. It is also likely that the development of bifunctional drugs may serve as a basis for improved "triple-therapy" combinations through coformulation that could deliver three complementary therapeutic effects for patients with asthma or COPD using only two drugs; an example is provided by the recent evidence that the use of the dual PDE3/4 inhibitor, RPL554 in combination with an M_3 muscarinic antagonist, may provide synergistic activity on the relaxation of human airway smooth muscle which suggests that if this drug was combined with an anticholinergic drug this could translate into further clinical benefit (Franciosi et al. 2013). Furthermore, the MABA, GSK961081, has recently been evaluated as twice daily fixed combination with fluticasone propionate (Cazzola et al. 2012a).

It is evident therefore that bifunctional drugs offer an exciting new approach to the treatment of respiratory diseases where there remains significant unmet need, and we anticipate that bifunctional drugs will have a significant role to play in the future treatment of patients with asthma or COPD.

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Drugs Affecting TRP Channels

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Contents

Abstract

Chronic obstructive pulmonary disease (COPD) and asthma are both common respiratory diseases that are associated with airflow reduction/obstruction and pulmonary inflammation. Whilst drug therapies offer adequate symptom control for many mild to moderate asthmatic patients, severe asthmatics and COPD patients symptoms are often not controlled, and in these cases, irreversible structural damage occurs with disease progression over time. Transient receptor

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potential (TRP) channels, in particular TRPV1, TRPA1, TRPV4 and TRPM8, have been implicated with roles in the regulation of inflammation and autonomic nervous control of the lungs. Evidence suggests that inflammation elevates levels of activators and sensitisers of TRP channels and additionally that TRP channel expression may be increased, resulting in excessive channel activation. The enhanced activity of these channels is thought to then play a key role in the propagation and maintenance of the inflammatory disease state and neuronal symptoms such as bronchoconstriction and cough. For TRPM8 the evidence is less clear, but as with TRPV1, TRPA1 and TRPV4, antagonists are being developed by multiple companies for indications including asthma and COPD, which will help in elucidating their role in respiratory disease.

Keywords
'Chronic obstructive pulmonary disease' (COPD) • 'Transient receptor potential' (TRP) • Asthma • Cough • TRPA1 • TRPM8 • TRPV1 • TRPV4

1 Transient Receptor Potential Channels

Transient receptor potential channels are a superfamily of 28 transmembrane cation permeable channels that can be subdivided into seven families – namely, TRP ankyrin (TRPA), canonical (TRPC), melastatin (TRPM), mucolipin (TRPML), NOMPC (TRPN), polycystin (TRPP) and vanilloid (TRPV) – on the basis of sequence homology. With the exception of TRPN channels, which have only been detected in fish, 27 of these ion channels spanning the other six TRP families are expressed in mammals.

In general, TRP channels share certain properties, namely, they are generally Ca^{2+}-preferring cation channels (albeit with varying selectivities), and possess six transmembrane domains with a pore region between the fifth and sixth transmembrane regions (Clapham 2005; Szallasi et al. 2007). Collectively, the TRP ion channel family form an array of cellular sensors for a huge range of endogenous and exogenous chemical and physical stimuli, and their ability to coordinate and integrate a spectrum of physiological stimuli has implicated them with key roles in the pathogenesis of many mammalian diseases. For the purposes of this chapter, we will focus on those TRP channels that have been heavily implicated with roles in asthma and chronic obstructive pulmonary disease (COPD), namely, TRPA1, TRPV1, TRPV4 and TRPM8. We will first briefly summarise the characteristics of COPD and asthma. The structural and functional properties of each TRP channel will then be briefly discussed, along with the evidence for the channels involvement in asthma and/or COPD. Then the drugs affecting that channel will be highlighted, with a particular emphasis on those drugs currently in, or aimed for, clinical trials. Finally the fortunes of current TRP channel drug development will be summarised, and the future merits and prospects of TRP channel drug development will be considered.

1.1 COPD

Chronic obstructive pulmonary disease (COPD) is a prevalent and debilitating respiratory disease with associated systemic comorbidities. It is a leading cause of death and disability worldwide, and disease incidence is predicted to continue increasing, such that COPD is predicted to be the third leading cause of death by 2020 (Vestbo et al. 2013). COPD is characterised by irreversible and progressive reduction of airflow, measured as a decline in lung function through spirometry (Barnes and Stockley 2005; Rabe et al. 2007; Paredi et al. 2010). Current treatments provide essentially only moderate symptomatic relief and do not halt progression of the disease (Barnes 2013). The airflow limitation in COPD is accompanied by an abnormal inflammatory response to noxious inhaled particulates or gases, for example, tobacco smoke, which is thought to be one of the primary causative agents for the initiation of COPD (Barnes and Stockley 2005; Rabe et al. 2007; Salvi and Barnes 2009). Typically such exposures must take place over a long duration before the symptoms of COPD appear – hence most diagnoses of COPD are made when patients are in middle age.

Chronic cough is often one of the first complaints that patients present with prior to a diagnosis of COPD: such that the recent GOLD strategy for diagnosis, management and treatment of COPD notes that patients presenting with chronic cough accompanied by a decline in actual compared to predicted spirometry values should be considered for a diagnosis of COPD (Vestbo et al. 2013). Indeed, cough was found to be the most commonly experienced symptom, as reported by 70% of 3,265 COPD sufferers interviewed, and occurring daily in 46% of the same population (Rennard et al. 2002).

One of the primary characteristics of COPD is an abnormal inflammation of the airways that is unresponsive to standard anti-inflammatories, including the gold-standard treatment of corticosteroids (Barnes 2013). It is thought that chronic exposure to noxious gases/particles drives inflammation in the lungs (Decramer et al. 2012). Cigarette smoke (CS), for example, stimulates multiple pulmonary immune cells (in particular macrophages, but also neutrophils, and $CD4^+$ Th1 and $CD8^+$ Tc lymphocytes) to release many inflammatory mediators, which recruits further inflammatory cells (in particular neutrophils, but also macrophages and $CD4^+$ Th1 and $CD8^+$ Tc lymphocytes) to the airways (Barnes 2008). It is thought that this positive feedback loop, along with repeated stimulation provided by chronic CS exposures, drives a persistent and progressive inflammation. This inflammation, along with exposure to the damaging components of CS, causes destruction of the alveolar structure and enhances mucus production (via goblet cell hyperplasia and hypertrophy) and fibrosis, resulting in reduction in the surface area for gas exchange, reduced elastic recoil and increased airflow obstruction (Hogg et al. 2004; Barnes 2008; Lai and Rogers 2010). The exact proportion of these structural changes may vary between patients, as COPD is an umbrella term that covers several interrelated lung diseases, including chronic bronchitis, small airway disease (SAD) and emphysema (Barnes 2004; Sturton et al. 2008). Whatever the

proportion in an individual patient, these inflammatory-driven structural changes contribute to the reduction in airflow, as assessed by spirometry.

1.2 Asthma

Defining asthma is somewhat difficult, as there is no single definitive genetic or environmental cause or trigger for development of this disease (Hargreave and Nair 2009). However, broadly speaking, asthma is a chronic inflammatory disease of the airways, characterised by sudden but transient decreases in airflow associated with dyspnea, cough and wheeze, which are generally fully reversible by bronchodilator treatment (Morosco and Kiley 2007). It has been estimated that 300 million people worldwide may suffer from asthma, with the highest incidences in the Americas, Europe and Australia of 5–10% of the population (Masoli et al. 2004).

Sudden bronchospasm of airway smooth muscle in asthmatics ('asthma attack') causes breathlessness and wheeze. Bronchospasm may be triggered by many stimuli, which in normal subjects would be innocuous, including dust and other allergens, pollen, air pollution, exercise and cold air (Eder et al. 2006). This airway hyperresponsiveness (AHR) is driven either by chronic airway inflammation and/or structural changes to the airways induced by this inflammation (Lommatzsch 2012). Whilst the chronic inflammation in asthma also involves an abnormal activation of multiple immune cells, it is different from the inflammation observed in COPD in that in the majority of asthmatics it is suppressed by anti-inflammatory therapy and involves different cell types, including $CD4^+$ Th2 cells, mast cells and eosinophils (Barnes 2008).

2 TRPV1

TRPV1 is well known as the receptor responsible for the perception of heat, particularly so for mediating the 'spicy hot' effects of capsaicin, the active constituent of chilli peppers from piquant *Capsicum* spp. plants, which activates TRPV1 on sensory nerves (Caterina et al. 1997). However, TRPV1 is a polymodal receptor, responding to capsaicin, its ultra-potent structural analog resiniferatoxin, and also xenobiotics, noxious heat (>42°C), acidic conditions/protons (low pH), as well as endogenous agents such as anandamide and inflammatory eicosanoids such as bradykinin and PGE_2 (Fig. 1) (Caterina et al. 1997; Vriens et al. 2009; Grace et al. 2012). Some of these agents, such as capsaicin, heat, acid and anandamide, activate TRPV1 via a direct interaction with the channel to cause a lowering of its voltage dependency, leading to opening of the pore domain (Caterina et al. 1997; Zygmunt et al. 1999; Jordt et al. 2000; Vriens et al. 2004). By contrast, other activators of the channel, such as PGE_2 and bradykinin, act indirectly by second messengers, in these cases released subsequent to activation of G-protein-coupled receptors (respectively the B_2 and EP_3 receptors) (Maher et al. 2009; Grace et al. 2012).

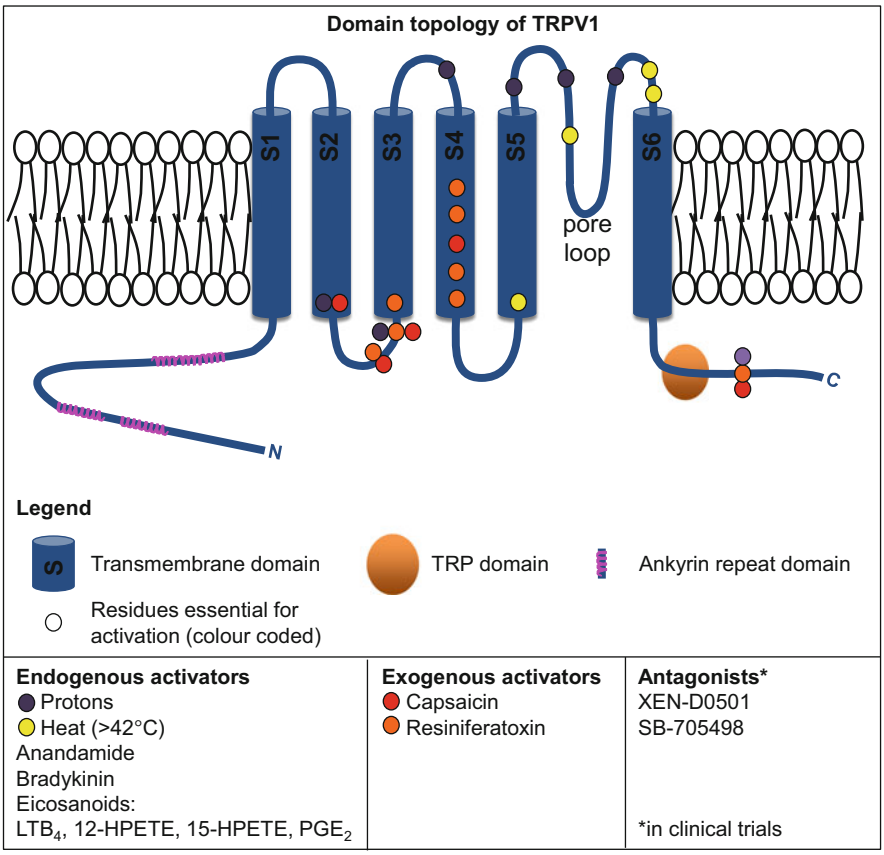

Fig. 1 Diagram showing domain topology and residues important in activation of TRPV1, along with selected endogenous/exogenous TRPV1 activators and TRPV1 antagonists in clinical trials for respiratory indications (Szolcsányi and Sándor 2012)

2.1 TRPV1 Roles in Airway Disease

TRPV1 receptors are predominantly expressed in the peripheral nervous system and, relevant to the control of airway functions, in a subset of vagal (both jugular and nodose origin) ganglia sensory neurons, as well as pulmonary innervating dorsal root and nasal trigeminal ganglia neurons (Banner et al. 2011). TRPV1-positive fibres innervate multiple tissue types, including the nose, trachea, parenchyma, alveoli and vessels, throughout the respiratory tract (Grace et al. 2014). Classically, TRPV1 is thought to be expressed on a capsaicin-sensitive subset of slow-conducting unmyelinated C-fibres (Coleridge and Coleridge 1984); however it is now acknowledged that there is a wider population of TRPV1-expressing neurons which includes the fast-conducting myelinated Aδ-fibres (Adcock et al. 2014). Due to their expression in these nerve fibres innervating the lung,

TRPV1 receptors in the airway have received particular attention for their ability to provoke cough in both animal species and human subjects. Indeed, the threshold for cough provocation by capsaicin has been found to have been lowered in various populations of asthmatics and COPD patients who have chronic cough compared to healthy control subjects (Choudry and Fuller 1992; Wong and Morice 1999; Doherty et al. 2000; Weinfeld et al. 2002; Blanc et al. 2009; Belvisi et al. 2016). Furthermore, a lowered capsaicin cough threshold, or rather increased sensitivity to capsaicin, is one of the key clinical measures which define a population of patients proposed to have cough hypersensitivity syndrome (Chung 2011; Millqvist 2011).

As well as playing a role in eliciting cough, TRPV1-expressing C-fibres have been demonstrated to release pro-inflammatory neuropeptides such as substance P (SP) and calcitonin gene-related peptide (CGRP) which mediate neurogenic inflammation via their retrograde release from peripheral terminals in rodent airways, although it is unclear if this phenomenon occurs in humans (Belvisi 2003). Indeed, capsaicin inhalation is also associated with parasympathetic bronchoconstriction, mucus hypersecretion, vasodilatation and the sensation of dyspnea (Couto et al. 2013), further implicating the TRPV1 receptor a role in symptoms other than cough in asthma and COPD. Furthermore, administration of the TRPV1 antagonist SB-704498 was found to reduce subsequent airway hyperresponsiveness to histamine in an ovalbumin-sensitised guinea pig model of allergic asthma (Delescluse et al. 2012).

In respiratory disease settings, TRPV1 function in the lung is thought to be modulated by some or all of three main mechanisms: elevated levels of direct TRPV1 agonists or channel openers; sensitisation of the channel by, for example, phosphorylation to induce activation to otherwise innocuous stimuli; and the increase in expression or de novo expression of TRPV1 in individual cells.

In the case of the first two mechanisms, the progressive and persistent pulmonary inflammation observed in asthmatics and COPD patients elevates the levels of multiple TRPV1 activators and sensitisers, including, e.g. inflammatory prostanoids and eicosanoids, neurotrophins, lowered pH and proteases (PAR2 agonists) (Adcock 2009; Grace et al. 2014; Veldhuis et al. 2015).

It should be noted that it is difficult in some cases to make a distinction between a ‘sensitiser’ and an ‘activator’ of TRPV1, with some GPCR agonists, having been shown to both sensitise TRPV1 to subsequent stimuli and to cause activation of TRPV1 (Fischer and Reeh 2007; Maher et al. 2009). Especially in the context of the inflammatory milieu, it is difficult to determine the exact role a single constituent plays. It is clear however that modulation of TRPV1 by GPCRs is an important factor in inflammatory diseases given that TRPV1 contains domains for binding of proteins, such as A-kinase-anchoring protein (AKAP), that can influence its activity, by, for instance, facilitating interactions with the signalling cascade kinases PKA and PKC (Veldhuis et al. 2015). Interestingly it is thought that the subcellular localisation of TRPV1 along with accessory proteins such as AKAP facilitates the regulation of TRPV1 by a multitude of signalling pathways, allowing TRPV1 to integrate signals to a wide range of stimuli – recently reviewed in Nilius and

Szallasi (2014). Sensitisers are generally thought to enhance the activity of TRPV1 via phosphorylation of specific residues, enabling the channel to respond to normally innocuous stimuli, or even to become activated spontaneously (Nilius and Szallasi 2014).

In addition to the increase in TRPV1 activation currents via elevated levels of activators/sensitisers, expression of TRPV1 may be increased under inflammatory conditions. This may occur in cells that had previously expressed TRPV1, in which case the increased surface expression would be expected to contribute, along with increased levels of activators/sensitisers to increased TRPV1 activation. However, intriguingly Lieu et al. have recently shown in guinea pigs that ovalbumin sensitisation/challenge or neurotrophin administration increases the number of TRPV1-expressing neurons, particularly of Aδ-type fibres, suggesting that the neural pathways innervating the airways are plastic and may be moulded by the inflammatory environment seen in asthma and COPD (Lieu et al. 2012). This data is corroborated by two other studies demonstrating increases in the proportion of TRPV1-expressing neurons in nodose ganglia in both a rat ovalbumin sensitisation/challenge model of allergic asthma and a guinea pig cigarette smoke exposure model of COPD (Zhang et al. 2008; Wortley et al. 2014a). It seems likely therefore that TRPV1 expression can be induced in neurons that previously did not express TRPV1, increasing the number of peripheral sensory inputs which may cause cough or airway hyperresponsiveness. In human subjects with chronic cough and severe asthma, capsaicin cough hypersensitivity has been reported (Doherty et al. 2000; Belvisi et al. 2016), and increased expression of TRPV1-like immunoreactivity has been detected in bronchial biopsies (Groneberg 2004; Mitchell et al. 2005; McGarvey et al. 2013).

By contrast, less evidence has been published regarding the expression of non-neuronal TRPV1. However, preliminary data has suggested that TRPV1 mRNA expression in the whole lung homogenate of emphysema patients is increased compared with healthy nonsmokers and nonsmokers, suggesting that TRPV1 expression may be increased in other tissues as well as neuronal cells (Grace et al. 2014). In addition, the TRPV1 inhibitor JNJ17203212 reduced the release of ATP from human bronchial epithelial cells (HBEC), and in vivo, *TRPV1*$^{-/-}$ mice exhibited less cigarette smoke-induced ATP release and subsequent neutrophilic inflammation in bronchoalveolar lavage fluid (Baxter et al. 2014). Despite these intriguing data, the role of TRPV1 in other lung tissues and cells in respiratory health and diseases such as asthma and COPD is relatively poorly understood (Gharat 2007).

There are no current firm descriptions of TRPV1 variant channelopathies per se (Banner et al. 2011). However Smit et al. more recently described associations between several TRPV1 SNPs and increased risk of usual and nocturnal cough in non-asthmatics, which was also correlated with cigarette smoking and occupational irritant exposures (Smit et al. 2012). Whilst the functional effects of SNPs associated with increased risk of cough are unknown, another SNP variant TRPV1-I585V, which – by contrast – is associated with a reduced risk of current cough or wheeze in asthmatics, is reported to decrease channel activity by 20–30%

(Cantero-Recasens et al. 2010). The associations of these SNPs with either increased risk or protection from cough and wheeze may suggest potential benefit could be derived from the antagonism of TRPV1 in disease, particularly in asthmatics and COPD patients who suffer from these symptoms.

2.2 TRPV1 Channel Antagonists

Of all the TRP channels, antagonists for TRPV1 are the most advanced in terms of drug development and clinical trials. However, development of these compounds has not been as straightforward as was initially hoped. The state of development of TRPV1 antagonists has been well documented in recent reviews (Preti et al. 2012; Nilius and Szallasi 2014), and here we will focus on selected compounds to describe the difficulties in development of TRPV1 antagonists, as well as updating with the latest reports of the most promising compounds, especially those targeted for respiratory conditions.

The main difficulties with TRPV1 antagonist development have been related to its function as a thermosensor for hot temperatures, and in particular this has meant that adverse effects such as increased body temperature and latent withdrawal to noxious hot stimuli have dogged development. For instance, the TRPV1 antagonist AMG 517 was entered into phase 1 and 1b clinical trials, where administration in patients following molar extraction caused significant and long-lasting increases in body temperature to above 40°C (Gavva et al. 2008). Due to safety concerns, these trials were terminated before the analgesic effect could be determined.

Another compound, MK-2295 (or NGD-8243), a Merck/Neurogen compound, entered a phase 2A POC trial also against dental pain, with 182 subjects receiving either drug or placebo (clinicaltrials.gov identifier NCT00387140). In this study the compound was similarly reported to have undesired effects, including causing an increase in body temperature and altering noxious heat sensation threshold (Xia et al. 2011). These adverse effects were correlated with target engagement, and reportedly meant a dose regimen could not be established that would allow efficacy whilst avoiding risk for burn injury, with some subjects reportedly unable to detect potentially harmful hot temperatures (Xia et al. 2011; Moran et al. 2011).

By contrast, the compound PHE377 has completed a phase 1b PoP trial, with the developing company PharmEste reporting that the compound was ‘well tolerated’, has ‘on-target activity’ and ‘does not increase body temperature’ (PharmEste website 2012). Unfortunately we could not find a peer-reviewed publication presenting this data; however the company is reportedly seeking partners for further development in a ‘phase 2 clinical study in chronic pain with different aetiologies’. It seems, therefore, that the development of TRPV1 antagonists which avoid the problem of hyperthermia may be possible, an idea substantiated by the preclinical development of BCTP, which is highly efficacious at inhibiting responses to capsaicin, RTX and heat, yet exhibits only mild effects on body temperature in rats (0.6°C increase) (Nash et al. 2012; Ferrer-Montiel et al. 2012).

Indeed, the GSK candidate SB-705498 recently completed phase 2 clinical trials for chronic refractory cough and was reported to be well tolerated, with no significant increases in tympanic temperature. This was the first TRPV1 antagonist to be examined clinically as an antitussive, but disappointingly, SB-705498 lacked efficacy in improving 24 h cough counts (Belvisi et al. 2014). However, the shift in objectively measured capsaicin-evoked cough, whilst statistically significant, appeared to be slight, and TRPV1 occupancy was estimated to be approximately only 40% (±20% confidence intervals). The targeting of chronic idiopathic cough was based on the observation by Belvisi et al. that cough responses to capsaicin were differential across different disease groups, with COPD and chronic cough patients in particular exhibiting increased cough responses to capsaicin inhalation and additional studies showing that chronic cough patients show high spontaneous cough frequency over 24 h of ambulatory recording compared to other patient groups (Decalmer et al. 2007; Belvisi et al. 2016; Sumner et al. 2013). However, it remains to be seen if higher potency compounds that have greater efficacy at inhibiting capsaicin-evoked cough can be effective at reducing increased cough frequency in chronic idiopathic cough patients or chronic cough of other aetiologies.

Of note, another TRPV1 antagonist, XEN-D0501, has recently been reported to cause increases of only 0.74°C in a phase 1 clinical trial after a single dose, and following twice-daily repeated dosing, this increase above placebo was reduced to 0.3°C (Round et al. 2011). What is more, Wortley et al. have recently shown that XEN-D0501 was approximately 1,000 times more potent than SB-705498 at inhibiting capsaicin depolarisation of human vagus nerve in vitro, and 100 times more potent at inhibiting capsaicin-evoked cough in conscious guinea pigs (Wortley et al. 2014b). XEN-D0501 is currently in ongoing phase 2 clinical trials for both chronic idiopathic cough (clinicaltrials.gov identifier NCT02233699) and cough in COPD (clinicaltrials.gov identifier NCT02233686), which are expected to conclude in 2015.

2.2.1 Other Notable Clinical Candidates Now Discontinued

The sensory neural pathways underlying pain disorders share many similarities with those that cause abnormal cough, and other TRPV1 antagonists which have been in development as analgesics include AZD1386 and GRC-6211 which both were trialled in patients with dental pain following molar extraction and appeared efficacious. It appears however that subsequently both compounds have been discontinued, in the former case due to liver enzyme elevations (Bonney and Carr 2013) and the latter for unspecified reasons following the sale of the compound by Glenmark to Lilly (Lilly press release 2007; Kym et al. 2009; Xia et al. 2011).

3 TRPA1

TRPA1, formerly known as ANKTM1, is currently the only mammalian-expressed member of the TRP ankyrin family and is named for the large number of ankyrin repeat motifs (14–19) on its N-terminus (Nilius et al. 2012). Near this ankyrin repeat domain are residues that enable TRPA1 to be activated by electrophilic compounds, which include a wide range of endogenous and exogenous reactive chemicals and irritants, so implicating TRPA1 with a key role as a noxious chemosensor. This list of TRPA1 activators includes environmental irritants (constituents of cigarette smoke, air pollution and vehicle exhaust fumes), pungent components of foods, hypochlorite (produced endogenously by immune cells and exogenous constituent of warfare agents) and endogenous agents, commonly produced in inflammatory processes (Nilius and Szallasi 2014). The newest addition to the list of TRPA1 activators are components of bacterial cell walls, an intriguing finding that suggests that the peripheral sensory nervous system (which expresses TRPA1 and responds to LPS) can detect and respond to bacterial infections independently of the immune system (Meseguer et al. 2014). A selected list of some of the wide range of the compounds activating TRPA1 is illustrated in Fig. 2. However as well as chemical ligands, TRPA1 was also initially discovered to be a sensor of physical stimuli, responding to noxious cold temperatures (below 17°C) (Story et al. 2003) and contributing to cellular responses to mechanical stresses (Brierley et al. 2011). However there is some controversy surrounding its supposed cold sensitivity, with one group suggesting that rat and mouse TRPA1 expressed in heterologous expression systems *are* activated at cold temperatures (Chen et al. 2013), but that in vivo a TRPA1 antagonist *does not* affect paw withdrawal time to noxious cold (Chen et al. 2011). In support of this latter finding, Zhou et al. report that TRPA1 does not play a role in cold sensation in afferent bronchopulmonary C-fibres (Zhou et al. 2011). This confusion was furthered by two recent contradictory reports, with Chen et al. suggesting that human TRPA1 (albeit in heterologous expression systems) is unresponsive to cold temperatures, whereas Moparthi et al. suggest that human TRPA1 is intrinsically cold sensitive (Chen et al. 2013; Moparthi et al. 2014). It seems therefore that the thermoTRP status of TRPA1 as a cold sensor is far from definitively proven.

3.1 TRPA1 in Asthma and COPD

Although TRPA1 was first identified in human cultured fibroblasts (Jaquemar et al. 1999), expression studies have shown that TRPA1 is predominantly expressed in sensory nociceptive neurons, including, in the respiratory tract, those of vagal and dorsal root ganglia, as well as those of nasal trigeminal ganglia origin (Story et al. 2003; Bandell et al. 2004; Bautista 2005; Nassenstein et al. 2008; Jang et al. 2012).

Recently, however, TRPA1 expression has also been demonstrated in immune cells involved in the inflammatory response in asthma and COPD – such as B cells,

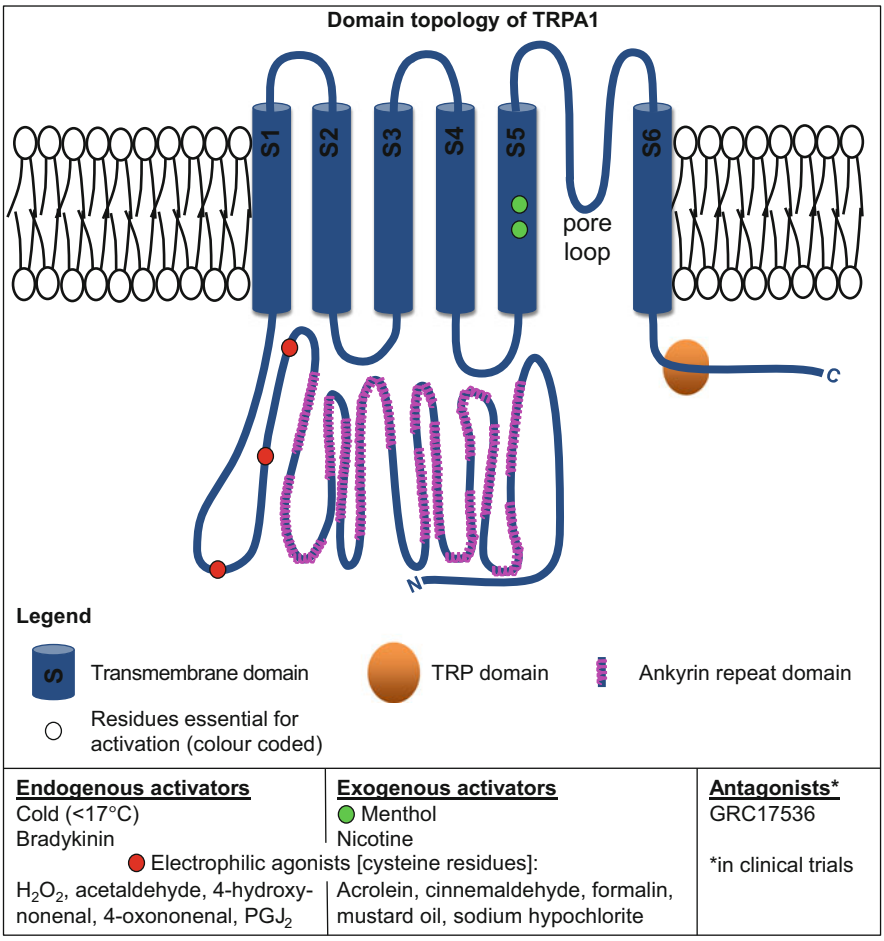

Fig. 2 Diagram showing domain topology and residues important in activation of TRPA1, along with selected endogenous/exogenous TRPA1 activators and TRPA1 antagonists in clinical trials for respiratory indications (Hinman et al. 2006; Macpherson et al. 2007; Bang and Hwang 2009)

$CD4^+$ and $CD8^+$ T cells and mast cells (Prasad et al. 2008; Banner et al. 2011). Additionally, TRPA1 expression has been observed in human lung bronchial epithelial cell lines, and these observations have recently been extended to native pulmonary epithelial cells (Mukhopadhyay et al. 2011; Büch et al. 2013).

Activation of TRPA1 channels has been shown to depolarise vagal pulmonary C-fibres and Aδ-nociceptors in rodent species (Bessac et al. 2008; Taylor-Clark et al. 2008; Nassenstein et al. 2008; Birrell et al. 2009; Andrè et al. 2009; Grace et al. 2012; Adcock et al. 2014), and by extension TRPA1 receptors on these fibre types are thought to mediate cough provoked by TRPA1 agonists in human subjects (Birrell et al. 2009). Indeed, the wide range of exogenous irritants and noxious agents that activate TRPA1, along with its ability to provoke cough, has indicated

TRPA1 with a key role as a key defensive noxious sensor in the lungs (Geppetti et al. 2009; Grace and Belvisi 2011).

Interestingly, in pulmonary vagal ganglia neurons, TRPA1 is expressed almost exclusively in a subpopulation of TRPV1-expressing cells, with up to 98% of TRPA1-expressing cells also expressing TRPV1 (Hondoh et al. 2010). TRPA1 subunits have been demonstrated to form functional TRPA1/V1 heterodimers with TRPV1, although there is no dependency between the two for the formation of functional channels (Akopian 2011). What is more, a recent screen of compounds acting on TRPV1 and TRPA1 suggests that the responsiveness to various agonists may be modulated depending on whether these channels are either expressed individually or together (Sadofsky et al. 2014). It will be particularly interesting and relevant to future clinical development to discover whether native cells that co-express TRPV1/A1 differ in their functional responses to agonists compared to cell populations expressing TRPV1 or TRPA1 alone.

As well as evoking cough, many TRPA1-activating irritants cause asthma-like symptoms, including cough, wheeze and dyspnea, hinting at a role for TRPA1 in asthma (Grace et al. 2014). Indeed, TRPA1 has been linked with a key role in the airway hyperresponsiveness (AHR) and bronchoconstriction characteristic of asthma, with a TRPA1 antagonist (HC030031) reversing the AHR to acetylcholine in an ovalbumin-sensitised mouse model (Caceres et al. 2009) and abolishing the late asthmatic response observed following ovalbumin sensitisation/challenge in rat and murine models of asthma (Raemdonck et al. 2012). Additionally, Trankner et al. recently demonstrated that TRPV1-expressing nerves are essential for the development of allergic AHR in a murine model, with selective ablation of TRPV1-expressing nerve fibres abolishing AHR, and direct optogenetic stimulation of these same fibres induces AHR in non-challenged but sensitised control mice (Tränkner et al. 2014). Given that a TRPV1 antagonist did not block this effect and that TRPA1 is almost only expressed in TRPV1-expressing nerve fibres, this supports the concept that TRPA1 plays a key role in the development of AHR in asthma. Furthermore, recent work by Hox et al. has demonstrated that nonallergic AHR can be induced by a single exposure of hypochlorite (TRPA1 agonist) + ovalbumin in wild-type, but not *TRPA1*$^{-/-}$ mice (Hox et al. 2013).

It is thought that the excessive activation of TRPA1 on sensory nerves in asthma and COPD is due to the increased levels of both endogenous pro-inflammatory signalling molecules (the 'inflammatory soup') and the exogenous stimuli which are implicated in the development of COPD – and to some extent asthma – such as cigarette smoke (Andrè and Campi 2008; Simon and Liedtke 2008; Lin et al. 2010; Kanezaki et al. 2012). However, as well as being excessively activated by pro-inflammatory stimuli, it is also thought that TRPA1 activation itself causes the release of pro-inflammatory agents that help to sustain the persistent inflammation observed in asthma and COPD. COPD-relevant TRPA1 agonist sources include cigarette smoke constituents (including acrolein, crotonaldehyde and nicotine), other potential COPD-causative agents such as wood smoke and ozone as well as endogenous aldehydes produced following oxidative stress exposure, such as 4-hydroxynonenal (Taylor-Clark et al. 2007; Trevisani et al. 2007; Shapiro

et al. 2013). Recent studies have suggested the expression of TRPA1 in non-neuronal pulmonary cell types, including fibroblasts, epithelial cells and smooth muscle cells, and that expression and release of pro-inflammatory cytokines can be induced from these cell types (Nassini et al. 2012).

Whilst there is some evidence that TRPV1 expression is increased under disease conditions and specifically in asthma and COPD (as discussed previously), there is very limited evidence that TRPA1 expression is similarly modulated in inflammatory states. What little information is available indicates that mechanisms of enhanced TRPA1 expression are likely to be tissue-type specific, and to date TRPA1 up-regulation has not been investigated in tissue types or disease models of relevance to COPD (Malin et al. 2011; Bautista et al. 2013).

Again, by comparison to TRPV1, where Smit et al. found an association between TRPV1 SNPs and increased risk of cough and wheeze, the same authors could find no association between 29 TRPA1 SNPs and cough or wheeze in the same population (Smit et al. 2012).

The only known TRPA1 channelopathy is familial episodic pain syndrome, which is associated with functional activation of the channel at normal resting potentials, causing debilitating upper body pain; however there is no data available concerning how this syndrome impacts on the respiratory tract (Kremeyer et al. 2010; Nilius and Szallasi 2014). There are currently no reported associations between SNPs in TRPA1 and susceptibility to airway diseases (Nilius and Szallasi 2014).

3.2 TRPA1 Channel Antagonists

In contrast to TRPV1, TRPA1 antagonists have had less development time (due to the more recent identification of the TRPA1 receptor), and therefore with far fewer candidate compounds, only two candidate compounds have reached clinical trial stages. A recent (and thorough) review of the patent literature however suggests that several companies possess patented TRPA1 antagonists in various states of preclinical/biological testing, including Abbott (two compounds), Merck Sharp & Dohme, Scripps Research Institute, Janssen (two compounds), Glenmark Pharmaceuticals (seven compounds) and Hydra Biosciences (four compounds), with some indicated for asthma and respiratory conditions (Preti et al. 2012).

One of those compounds that has reached clinical trials however is CB-189,625, which was developed by Cubist Pharmaceuticals and Hydra Biosciences. Indicated for acute (perioperative) pain and certain inflammatory conditions, CB-189,625 completed phase 1 clinical trials, with no adverse effects reported apart from those attributed to vehicle (Bokesch et al. 2012; Preti et al. 2012). However, according to a publicly available quarterly financial report in 2013, the compound was discontinued due to ‘solubility concerns’ (Cubist Pharmaceuticals 2013).

By contrast, the Glenmark Pharmaceuticals candidate GRC-17536 appears to have fared better, having completed phase 1 and, recently, phase 2 trials in subjects with painful diabetic peripheral neuropathy (clinicaltrials.gov identifier

NCT01726413). There are to the best of our knowledge no published works on the results of these trials; however a press release indicates that the compound was well tolerated in phase 2, with no adverse effects reported and a statistically significant and clinically relevant positive response observed (Evaluate Group press release 2014).

In addition, and following preclinical data with this compound showing an antitussive effect against CA-provoked cough (Mukhopadhyay et al. 2014), GRC-17536 is currently in another phase 2 clinical trial for chronic refractory cough (clinicaltrialsregister.eu identifier 2013-002728-17). The trial is described as a double-blind crossover placebo-controlled trial of the effect of GRC-17536 on 24 h cough counts in chronic cough patients refractory to treatment, with target engagement judged by GRC-17536 inhibition of CA-induced cough. Interestingly, the compound has been formulated as a dry powder for inhalation, although the reason for this change of formulation from previous clinical trials is unknown. Whilst it is unclear when it is due to conclude, the results of this first clinical trial of a TRPA1 antagonist in a respiratory condition are highly anticipated.

4 TRPV4

TRPV4 was initially discovered to be expressed in rat kidney and was originally identified as a putative osmosensor with sequence similarities to TRPV1 and TRPV2; hence its original designation of VR-OAC, or vanilloid receptor-related osmotically activated channel (Liedtke et al. 2000). TRPV4 is a Ca^{2+}- and Mg^{2+}-permeable nonselective cation channel composed of 871 amino acids with three ankyrin repeats near the NH_2-terminus (Fig. 3). Like TRPA1 and TRPV1, TRPV4 is a thermosensor, although unlike the former two, TRPV4 is thought to be involved in the sensation of tepid to warm temperatures, imbuing TRPV4 with constitutive activity in basal conditions as its activation range of 24–42°C overlaps with normal body temperatures (Nilius et al. 2005; Belmonte and Viana 2008). Again in similarity to TRPV1 and TRPA1, TRPV4 is a polymodal sensor which responds to a range of endogenous and exogenous chemical and physical stimuli including arachidonic acid and derivatives, endocannabinoids, synthetic α-phorbols and mechanical stimuli, such as changes in osmolarity (Watanabe et al. 2003; Vriens et al. 2004; Willette et al. 2008).

4.1 TRPV4 Role in Airway Disease

Its wide tissue expression in tissues all over the body (including heart, lung, kidney, CNS, skin and sweat glands) and variously in multiple neuronal and non-neuronal cell types aligns with the many different cellular functions in which TRPV4 is involved (Vincent and Duncton 2011; Grace et al. 2014).

Unlike that of the TRPA1 and TRPV1 channels discussed previously, the role of neuronally expressed TRPV4 in the lungs is not well characterised (Grace

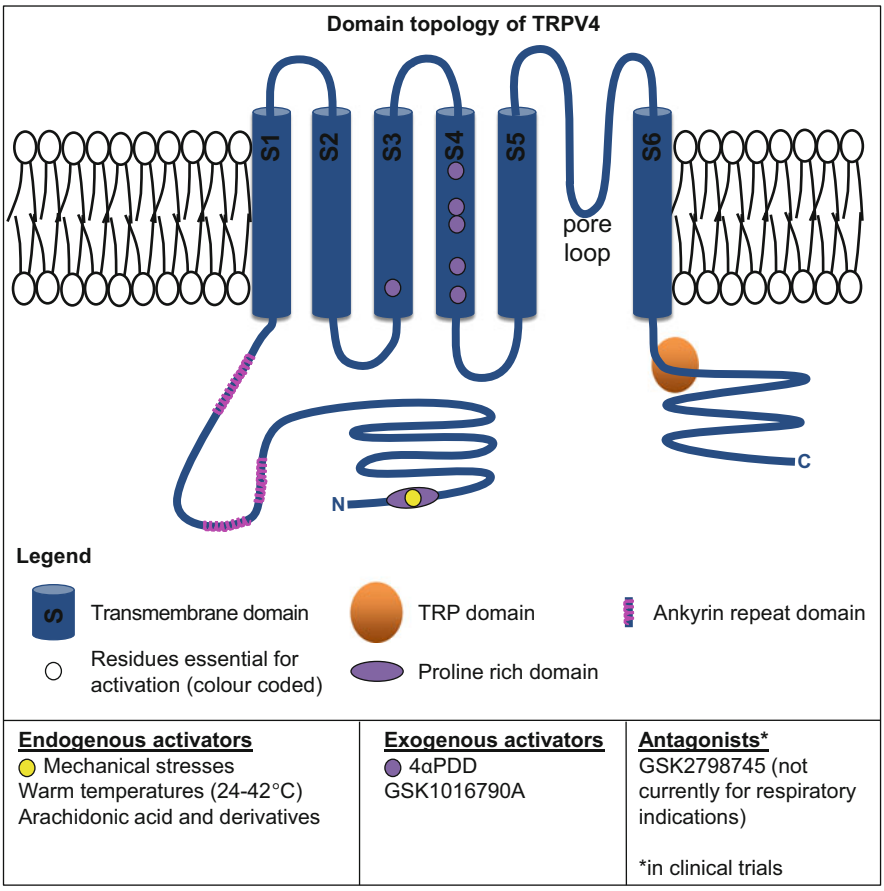

Fig. 3 Diagram showing domain topology and residues important in activation of TRPV4, along with selected endogenous/exogenous TRPV4 activators and TRPV1 antagonists in clinical trials (Vriens et al. 2004, 2007; Vennekens et al. 2008; D'hoedt et al. 2008; Everaerts et al. 2010)

et al. 2014). However, recently it has been demonstrated that airway sensory nerves can be activated by TRPV4 agonists (Bonvini et al. 2016). In this study, TRPV4 agonists evoked cough in guinea pigs and also induced depolarisation or calcium entry into vagal nerves and neurons – specifically nodose but not jugular neurons. This finding means it is possible that TRPV4 could play a role in the abnormal cough response in COPD and asthmatic patients, where the osmolarity of the pulmonary surfaces is altered, and levels of endogenous activators (such as arachidonic acid) are increased, although this idea requires validation with further work (Grace et al. 2014).

Whilst neuronal pulmonary TRPV4 has not been widely investigated, more is known about TRPV4 for its expression and functional role in a wide range of non-neuronal lung tissues, including structural cells such as airway smooth muscle,

epithelial cells, pulmonary vessels as well as in inflammatory cells such as alveolar macrophages and neutrophils (Liedtke et al. 2000; Jia et al. 2004; Yang 2006; Alvarez et al. 2006; Dietrich et al. 2006; Banner et al. 2011). Due to the postulated increased levels of TRPV4 activators present, it is thought that excessive activation of TRPV4 could play multiple roles in the pathology of asthma and COPD, dependent on the specific tissues in which it is expressed.

For example, it has been demonstrated that TRPV4 is expressed in human airway smooth muscle cells (Jia et al. 2004), and a specific TRPV4 agonist elicits significant contraction of both guinea pig and human smooth muscle (via the release of cysteinyl leukotrienes), which is blocked using a selective TRPV4 antagonist, implicating TRPV4 with a role in the variable airflow obstruction observed in asthma (Bonvini et al. 2013; McAlexander et al. 2014). Furthermore, TRPV4 activation in both human airway smooth muscle and human airway epithelial cells has been shown to cause the enhanced release of ATP (Seminario-Vidal et al. 2011; Takahara et al. 2014; Baxter et al. 2014), *TRPV4*$^{-/-}$ mice had reduced pulmonary ATP release and neutrophilic inflammation following CS exposure and TRPV4 expression was upregulated in whole lung tissues from COPD patients (Baxter et al. 2014). What is more, TRPV4 activation has also been suggested to contribute to both neurogenic inflammation via the release of neuropeptides and the stimulation of alveolar macrophages to release reactive oxygen and nitrogen species (Vergnolle et al. 2010; Hamanaka et al. 2010). These data collectively suggest TRPV4 plays a role in the inflammatory processes underlying asthma and COPD pathologies (Esther et al. 2008; Willart and Lambrecht 2009; Mortaz et al. 2010; Riteau et al. 2010; Eltom et al. 2014). What is more, in rodent and murine models TRPV4 activation has been implicated with a role in the formation of pulmonary oedema (Thorneloe et al. 2012), which is thought to be due to its role as a regulator of endothelial permeability (Jian et al. 2008).

Similarly to TRPV1 and TRPA1, TRPV4 can be activated by multiple stimuli including endogenous inflammatory lipids, changes in airway surface osmolarity and mucus production (Vincent and Duncton 2011; Grace et al. 2014). In addition to the evidence that levels of TRPV4 activators are upregulated in asthma and COPD, there is also the suggestion that TRPV4 may be sensitised to subsequent stimulation via phosphorylation of the channel, for example via PKA and PKC activity (Grant et al. 2007; Poole et al. 2013).

Two known diseases are caused by genetic changes resulting in abnormal TRPV4 function: Charcot-Marie-Tooth disease type 2C and scapuloperoneal spinal muscular atrophy (Wu et al. 2010). Whilst there are serious systemic repercussions of both channelopathies (bone dysplasia and peripheral nervous degeneration), there is no evidence linking these channelopathies of TRPV4 with asthma, COPD or other respiratory diseases, although it is unclear whether a gain or loss of function is encoded by the TRPV4 mutant protein (Nilius and Owsianik 2010). As Nilius and Owsianik point out when discussing TRPV4 channelopathies, there appears to be a disconnect between the anticipated problems (osmoregulation, pulmonary hypertension or endothelial dysfunction) and those observed in TRPV4-related channelopathies (Nilius and Owsianik 2010).

By contrast, however, several SNPs of TRPV4 have been found to confer increased susceptibility to COPD pathologies, indicating that the increased activation of TRPV4 may contribute to disease progression in COPD patients (Zhu et al. 2009). Interestingly, the study by Cantero-Recasens et al., which found an association between TRPV1 SNPs and cough/wheeze in asthma, found no similar associations with known TRPV4 SNPs and asthma symptom risk (Cantero-Recasens et al. 2010). It may be, however, that there are other asthma risks (e.g. for exacerbation) that are associated with TRPV4 SNPs that were not considered in the design of this study.

Whilst much work remains to be done to highlight directly the role of TRPV4 in asthma and COPD in the specific context of these diseases, there is evidence that activators of TRPV4 are elevated in disease and that activation of TRPV4 leads to the development of asthma and COPD pathologies in animal models. When taken together with the associations between TRPV4 SNPs and risk for COPD, there seems to be a role for TRPV4 activation in the development of these diseases, providing a rationale for the clinical development of specific antagonists of TRPV4.

4.2 TRPV4 Antagonists

There are few TRPV4 antagonist clinical candidates in advanced stages of development. Currently, the GlaxoSmithKline compound GSK2798745 is in a phase 1 trial to assess safety and tolerance in healthy subjects and stable heart failure patients (clinicaltrials.gov identifier NCT02119260). Otherwise it is unclear whether existing compounds in preclinical testing for respiratory indications will be advanced to clinical trials. Given the channelopathies related to systemic change of function of this channel, and its wide expression profile, it may be that the lungs are ideal target tissues for antagonists of TRPV4, with the potential for compounds to be targeted relatively selectively to the lung using inhaled formulations.

5 TRPM8

TRPM8 is another ‘thermoTRP’, in that, like TRPA1, it is thought to respond to cold – or more accurately cool – temperatures in the range 15–28°C (Peier et al. 2002; McKemy 2013). In this way, TRPM8 is thought to be more a physiological sensor of innocuous cool temperatures, rather than a sensor for the detection of painful noxious cold temperatures (McKemy 2013).

Whilst it is a cold thermosensor, TRPM8 shares little sequence homology with TRPA1 and in particular lacks the N-terminus ankyrin repeat sequence structure of TRPA1, as well its responsiveness to electrophilic noxious compounds (Story et al. 2003; Latorre et al. 2007). Instead, chemical TRPM8 activators, such as menthol, icilin and eucalyptol, in general seem to have the shared characteristic of eliciting a cooling sensation (Peier et al. 2002; Zhou et al. 2011). Menthol itself has been variously reported to be analgesic, to have antitussive properties, to be a

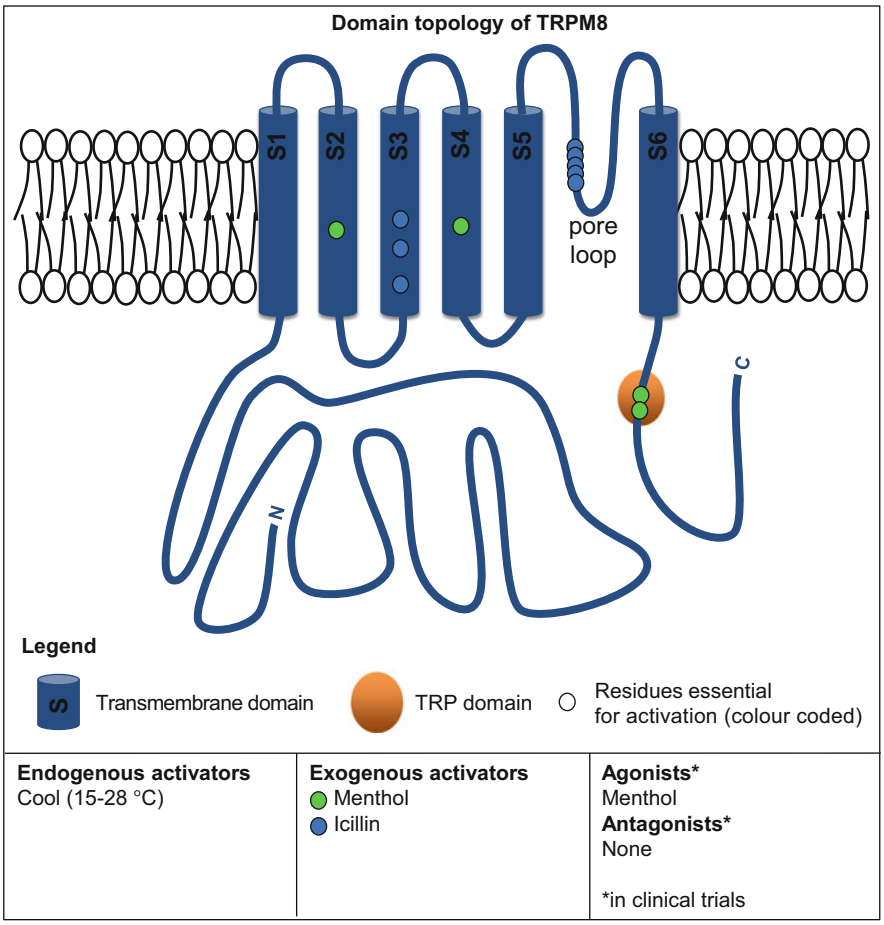

Fig. 4 Diagram showing domain topology and residues important in activation of TRPM8, along with selected endogenous/exogenous TRPM8 activators and TRPM8 ligands in clinical trials for respiratory indications (Latorre et al. 2011)

bronchodilator and to reduce airway irritation and inflammation caused by cigarette smoke irritants (Morice et al. 1994; Wright et al. 1997; Galeotti et al. 2002; Willis et al. 2011; Millqvist et al. 2012). However, whilst menthol is commonly thought of as a TRPM8 agonist, it acts as a TRPA1 agonist at higher concentrations, and may well also activate a host of other targets, including possibly opioid receptors (Galeotti et al. 2002; Karashima et al. 2007). Indeed, data presented recently indicates that the beneficial (bronchodilator/antitussive) effects of menthol in the airways are not due to TRPM8 agonism (Maher et al. 2014). This highlights the particular difficulty in interpreting the literature on TRPM8 due to the use of nonselective ligands (Fig. 4).

5.1 TRPM8 in Asthma and COPD

TRPM8 is primarily expressed in neurons, particularly in subpopulations of neurons originating in the dorsal root and trigeminal ganglia (Clapham et al. 2001; Peier et al. 2002; Story et al. 2003). Interestingly, the subpopulation of TRPM8-expressing neurons appears to be mostly distinct from those subpopulations that express TRPA1, which could imply a distinction in the physiological responses to 'cool' versus 'noxious cold' sensing (Clapham et al. 2001; Peier et al. 2002; Story et al. 2003; Hondoh et al. 2010). It may therefore be relevant to note that whilst TRPA1 expression almost completely overlaps with the well-known pain/cough receptor TRPV1, only about 30% of TRPM8 also express TRPV1 in DRG neurons (Okazawa et al. 2004). Limited TRPM8 expression in vagal ganglia neurons has also been observed in some studies (Xing et al. 2008; Nassenstein et al. 2008), and whilst TRPM8 mRNA has been detected in retrograde-stained airway jugular neurons, it is thought that the proportion of TRPM8-expressing vagal ganglia neurons is much lower than the reported 60% of TRPM8-expressing nasal trigeminal neurons (Hondoh et al. 2010; Plevkova et al. 2013).

Inhalation of cold air can cause bronchoconstriction and cough and induce plasma protein extravasation and mucus production (Yoshihara et al. 1996; Peier et al. 2002; Carlsen and Carlsen 2002; Xing et al. 2008). However, it is unclear whether this is due to the activation of TRPM8, or the noxious cold sensor TRPA1, or some relative proportion of the two channels. Furthermore, contradictory data exists from multiple studies indicating that activation of TRPM8 (mostly via menthol inhalation/application) inhibits cough and bronchoconstriction (Morice et al. 1994; Kenia et al. 2008; Ito et al. 2008; Millqvist et al. 2012; Wise et al. 2012).

There is also the suggestion that activation of TRPM8 by menthol in nasal trigeminal neurons can inhibit the cough reflex, although this may tell us more about the neurological integration of peripheral nervous signals in the CNS than it does about the role of TRPM8 in disease conditions (Buday et al. 2012).

A truncated TRPM8 splice variant has been detected in human bronchial epithelial cells, and this activation of this variant has been shown to induce pro-inflammatory cytokine transcription (Sabnis et al. 2008a, b). Furthermore, TRPM8 activation has been shown to play a key role in mucus production and mast cell activation (Cho et al. 2010; Li et al. 2011; Grace et al. 2014).

It is unknown whether the expression of TRPM8 is altered in disease states. What is more, whilst it is thought that modulators (usually activators) of TRPV1, TRPA1 and TRPV4 are increased in disease states or inflammatory conditions, it is unclear if in a similar manner TRPM8 activity is modulated by the inflammatory milieu, with no known endogenous activators of TRPM8 having been described (Grace et al. 2014).

To the best of our knowledge there are no SNPs of TRPM8 with relevance to asthma or COPD, and likewise there are no known channelopathies that can offer insight into the function of TRPM8 in the airways.

The various apparently conflicting findings of the consequences of TRPM8 activation make it hard to predict whether TRPM8 agonism or antagonism would be of more benefit in asthma or COPD. Perhaps future work with more specific agonists would help to elucidate the role of this channel in various disease states and tissues. Therefore the next section of this chapter will discuss the development and use of both TRPM8 agonists and antagonists.

5.2 Drugs Affecting TRPM8 Channels

Menthol is already currently available and widely used in OTC lozenges, nasal sprays and cough syrups; usually indicated as cough and cold remedies (Morice et al. 1994; Laude et al. 1994; Kenia et al. 2008; Preti et al. 2012). In these OTC remedies, menthol is often ascribed with antitussive properties and increasing airflow (reducing dyspnea). However, whilst clinical studies have found that menthol does inhibit the cough response to inhaled capsaicin and citric acid (Morice et al. 1994; Laude et al. 1994; Wise et al. 2012), appropriate controls for inhalation of menthol have not been established. This makes these data hard to interpret, given the conscious control of the cough reflex, and the demonstrated effect of mindfulness on the cough reflex (Young et al. 2009). What is more, recently menthol has been shown to increase the perception of nasal patency, but no effect on nasal airflow (Eccles 2003; Kenia et al. 2008).

Interestingly, menthol can attenuate respiratory irritation induced by TRPA1 and TRPV1 agonist constituents of cigarette smoke (Willis et al. 2011). However, as menthol is known to activate several receptors besides TRPM8 (as discussed previously), it is hard to know whether to attribute any clinical benefits to TRPM8 agonism.

To the best of our knowledge, currently no TRPM8 antagonists have reached clinical development stages for asthma and COPD. However, the Pfizer candidate PF-05105679 has completed a phase 1 trial (clinicaltrials.gov identifier NCT01393652) and was reported to be generally well tolerated whilst reducing inhibition of pain induced by the cold pressor test (Winchester et al. 2014). It was also reported that around a third of participants receiving PF-05105679 experienced a sensation of ‘feeling hot’; however no effect on core temperature was observed – although the reason for this observation was not established.

Furthermore, antagonists are in development and biological testing by Bayer HealthCare, Glenmark Pharmaceuticals, RaQualia Pharma Inc., Amgen, and Janssen (for more details, see the excellent patent review by Preti et al. 2012). Of note, Janssen and Amgen have specifically mentioned COPD and asthma (respectively) in their patent applications, although Janssen has multiple compounds that are also indicated for ‘respiratory conditions’, although to date there are no published validations of these compounds in preclinical models of asthma or COPD (Preti et al. 2012).

It seems likely that future preclinical biological work to understand the mechanisms of how TRPM8 modulation affects the course of disease is required to 'pathfind' for the preclinical and clinical development of TRPM8 drugs.

6 Discussion

Targeting TRP channels with antagonist drugs may be an effective strategy for reducing the elevated activity of these channels and consequent adverse effects/symptoms in asthma and COPD. Whilst it is apparent from the first generation of antagonists that TRP channels play a role in homeostasis as well as as gatekeepers of these adverse effects, it may be possible to design compounds that can avoid these adverse effects. For example, hot temperature gating of TRPV1 is structurally separate to ligand-gating site(s), and this has allowed the development of specific antagonists that block activation of the ligand-gating site to prevent chemical activators, but allow activation by hot temperatures (it is thought). Such antagonists hold promise that they may allow the desired blockade of adverse effects caused by TRP activation whilst still allowing the activation of the TRP channel by normal endogenous stimuli such as hot temperatures. This offers an optimal clinical profile for patients, especially in the case of TRPV1, for example, allowing the reflexive sensing of dangerous heat for the avoidance of burning.

TRPV4 and TRPM8 channel antagonists are in various stages of preclinical development, and their use in animal models has the potential to tell us much more about the role of the these channels in respiratory disease. By contrast, the clinical trials of TRPV1 and TRPA1 antagonists for the indication of chronic cough in various patient populations are currently ongoing, and the results of these trials may inform us much more about the nature of these channels in human respiratory disease.

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Evaluation of New Drugs for Asthma and COPD: Endpoints, Biomarkers and Clinical Trial Design

Dave Singh

Contents

Abstract

There remains a considerable need to develop novel therapies for patients with asthma and chronic obstructive pulmonary disease (COPD). The greatest challenge at the moment is measuring the effects of novel anti-inflammatory drugs, as these drugs often cause only small effects on lung function. Measurements that demonstrate the pharmacological and clinical effects of these drugs are

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needed. Furthermore, we now recognise that only subgroups of patients are likely to respond to these novel drugs, so using biomarkers to determine the clinical phenotype most suitable for such therapies is important. An endotype is a subtype of a (clinical) condition defined by a distinct pathophysiological mechanism. An endotype-driven approach may be more helpful in drug development, enabling drugs to be targeted specifically towards specific biological mechanisms rather than clinical characteristics. This requires the development of biomarkers to define endotypes and/or to measure drug effects. This newer approach should continue alongside efforts to optimise the measurement of clinical endpoints, including patient-reported outcome measurements, required by drug regulatory authorities.

Keywords
Asthma • Biomarkers • COPD • Induced sputum • Minimal clinical important differences • Patient reported outcomes

1 Introduction

The development of new medicines for the treatment of asthma and chronic obstructive pulmonary disease (COPD) involves a series of clinical trials designed to assess pharmacokinetics, clinical efficacy and safety. Initial studies are usually conducted in healthy volunteers (phase 1 studies) with a focus on safety assessments and pharmacokinetics. Pharmacokinetic analysis is used to help determine the most appropriate dosing regimen (e.g. once or twice per day). It is usual to conduct a single ascending dose (SAD) and multiple ascending dose (MAD) study in healthy volunteers before moving into phase 2 studies that involve patients. A summary of the different phases of respiratory clinical trials is shown in Fig. 1.

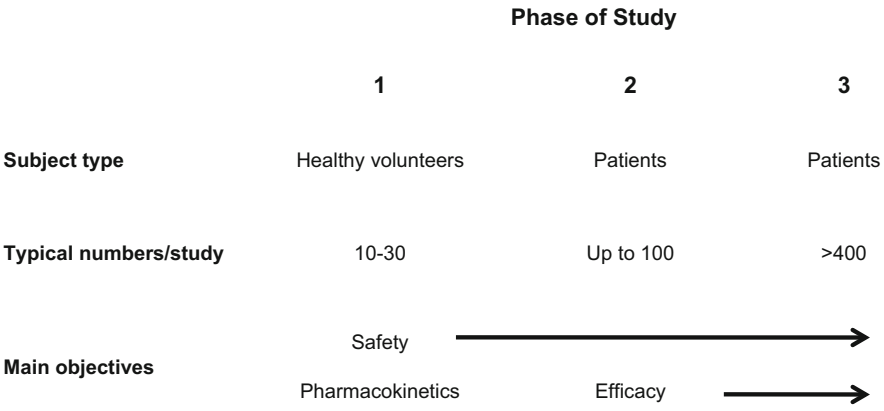

Fig. 1 Different phases of a clinical development programme

Phase 1 healthy volunteer studies usually do not include assessments of the pharmacological actions of the drug; this is called pharmacodynamics and is easier to study in patients with disease. However, the mode of action of the drug may allow some pharmacodynamic measurements to be made in healthy volunteers. For respiratory drugs, measurements from blood such as cells or proteins may be informative, and models of airway inflammation in healthy volunteers have been used (which will be covered in depth later in the chapter). These pharmacodynamic measurements can enable dose-response relationships to be better understood, thus aiding the selection of doses for phase 2 studies.

Phase 2a studies are often called proof of concept trials, as they investigate for the first time whether the drug has an impact on disease processes. Phase 2b studies are usually larger and involve a range of doses, so that the optimum doses can be identified. Phase 3 studies are of longer duration and are required by the regulatory authorities to prove the long-term clinical effectiveness of the drug in a robust manner using a large sample size. The costs and timelines of clinical development programmes need to be closely managed with early decisions being made to identify candidate drugs that are most likely to show clinical benefit in later phase studies that are larger and more expensive.

It is important for clinical development programmes to evaluate pharmacodynamics in the lungs as early as possible. Consequently, there is an increasing focus on innovative ways of conducting early clinical development programmes including novel trial designs, early patient exposure to evaluate pharmacodynamics and studies being done in healthy volunteer and patients in parallel. These studies often require novel pharmacodynamic measurements to be developed and applied. The measurement of forced expiratory volume in 1 s (FEV_1) is commonly used to assess drug effects in asthma and COPD clinical trials. This is a well-established and reproducible measurement that is recognised by drug regulatory authorities (Cazzola et al. 2008). However, this lung function measurement can be relatively insensitive for detecting beneficial drug effects, particularly in COPD studies of anti-inflammatory drugs where the improvements in lung function are small, but there are important benefits on other clinically important parameters such as the prevention of exacerbations. Alternative ways of measuring the effects of novel anti-inflammatory drugs being developed for asthma or COPD are often needed. A biomarker is defined as a measurement that is related to a clinical characteristic, such as the presence of disease, severity of disease, prognosis or response to therapy. Biomarkers are being increasingly used in asthma and COPD clinical trials to measure pharmacodynamics.

This chapter reviews the commonly used methods for assessing the effects of drugs in asthma and COPD clinical trials. This includes challenge methodologies, which are often used at earlier phases, and biomarkers. The clinical endpoints commonly used in later phase studies are also reviewed.

2 Challenge Models Used in Early Phase Studies

2.1 Allergen Challenge

The inhaled allergen challenge model has been frequently used in patients with asthma to investigate the potential efficacy of novel anti-inflammatory drugs designed to suppress the allergic response (Diamant et al. 2014; Kent et al. 2013; Singh et al. 2013; Singh et al. 2010; Wenzel et al. 2007). The inhalation of allergen in sensitised individuals can result in an early asthmatic response (EAR) due to bronchoconstriction. A proportion of patients subsequently experience a late asthmatic response (LAR) due to airway inflammation; the time profile of the EAR and LAR is shown in Fig. 2. This model is mainly used in drug development to identify drugs that attenuate the LAR. The EAR is generally measured at 0–2 h after challenge, while the LAR is measured at later time points, for example, from 4 to 10 h after challenge. The maximum fall in FEV_1 and the area under the curve (AUC) for the fall in FEV_1 during the EAR and LAR are the endpoints used to evaluate drug effects.

Inhaled corticosteroids (ICS) have a profound inhibitory effect on the LAR (Duong et al. 2007; Palmqvist et al. 2005). Long-acting beta-agonists (LABAs) also attenuate the LAR, and the allergen challenge model has been used to demonstrate the additive effects of using ICS with LABAs on the prevention of allergic inflammation (Palmqvist et al. 2005). Montelukast also inhibits the LAR, although the magnitude of inhibition caused by this drug is less than ICS and varies greatly between studies (Singh et al. 2007; Palmqvist et al. 2005). This highlights the difficulty of comparing drug effects between studies, as differences between patient characteristics and experimental protocols contribute to variability in drug effects.

Novel drugs with different mechanisms of action have demonstrated attenuation of the LAR in phase 2a clinical trials, including phosphodiesterase (PDE)

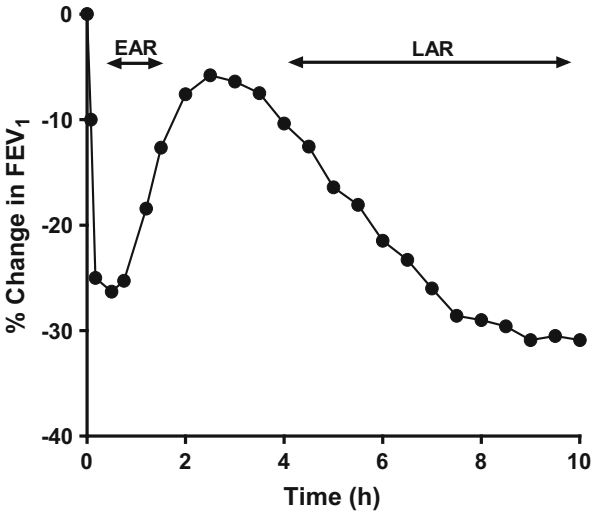

Fig. 2 Time course of EAR (early asthmatic reaction) and LAR (late asthmatic reaction)

4 inhibitors (Singh et al. 2010), CRTH2 antagonists (Diamant et al. 2014; Singh et al. 2013) and monoclonal antibodies directed against cytokines involved in allergic inflammation (Wenzel et al. 2007). Such positive results serve as a proof that the drug interferes with allergic airway inflammation and therefore is a suitable candidate to be advanced into phase 2b studies that focus on clinically relevant endpoints such as lung function and symptoms. In contrast, failure to attenuate the LAR is usually treated as a signal to stop further development of the drug. An example of the allergen challenge model being used to make such decisions at phase 2a is the evaluation of the iNOS inhibitor GW274150 which was designed to reduce nitric oxide (NO) production in the airways, based on the hypothesis that NO plays a role in the pathogenesis of allergic airway inflammation (Singh et al. 2007). GW274150 inhibited exhaled NO levels but had no effect on the LAR, thereby disproving the hypothesis being tested. An important element of the study design was the evaluation of exhaled NO levels, as this enabled confirmation that the dose of the drug administered had biological activity in the lungs. In general, a negative result on a bronchial challenge or clinical endpoint is easier to interpret if one has information on whether the drug had sufficient pharmacological activity in the lungs. Terms such as "proof of pharmacology" or "target engagement" are often used for this concept. Another important aspect of the study design was the use of the positive control drug montelukast in a three-way placebo-controlled crossover design. Montelukast inhibited the LAR, thus providing confidence that the study protocol was able to demonstrate anti-inflammatory effects.

Monoclonal antibodies that inhibit the activity of IL-5 are in clinical development (Corren 2012). These drugs suppress eosinophil recruitment and activation. An allergen challenge study showed that anti-IL-5 treatment with SB-240563 reduced eosinophil numbers in the lung, but there was no effect on the LAR (Leckie et al. 2000). However, subsequent studies have shown that this drug reduces exacerbations in moderate-to-severe asthma patients with evidence of eosinophilia (Haldar et al. 2009; Ortega et al. 2014). This serves as a note of caution when considering negative results in allergen challenge studies.

Although allergen challenge studies are usually performed in ICS-naïve subjects, it is possible to provoke an LAR in patients taking ICS (Lee et al. 2014). These subjects may be a more relevant population to use when studying novel drugs that will eventually be prescribed in addition to ICS.

Allergen challenges are usually performed on a single occasion using a relatively high allergen dose that exceeds real-life exposure levels. Consequently, these high-dose allergen challenges are somewhat unrepresentative of the real-life response to allergen. Low-dose allergen protocols using repeated daily exposure have been developed to overcome this issue (Lee et al. 2014). While the rationale for using such a protocol is attractive, there are practical difficulties in implementing such a protocol that requires subjects to attend the clinical trial site on repeated occasions for allergen challenge. Furthermore, the effects of drugs on lung function are less easy to measure with the low-dose challenge, as the decrease in lung function is relatively small.

One of the attractions of the single-dose allergen challenge model in drug development is the relatively small sample size required. Reproducibility studies of allergen challenges provide data that can be used to perform power calculations for these early phase studies (Aul et al. 2013). Many phase 2 allergen challenge studies have been conducted with < 40 patients (Diamant et al. 2014; Kent et al. 2013; Singh et al. 2013; Singh et al. 2010), enabling relatively rapid recruitment and study completion, thus keeping drug development timelines as short as possible.

2.2 Models of Neutrophilic Airway Inflammation

Increased airway neutrophil numbers are a characteristic feature of COPD and severe asthma. Models of acute neutrophilia in the lungs have been used in healthy volunteer studies to test the effects of novel drugs designed to suppress neutrophilic inflammation (Michel et al. 2007; Franciosi et al. 2013). One drawback of these models is that they induce acute neutrophilia in subjects with healthy lungs, which is different to chronic neutrophilia in diseased lungs. Nevertheless, these models can be used in healthy subjects at an early stage of clinical development to assess whether the drug has any pharmacological impact on neutrophil influx into the airways.

The inhalation of bacterial lipopolysaccharide (LPS) causes acute airway neutrophilia through activation of Toll-like receptor 4 (TLR4) signalling (Michel et al. 1997). This is a safe procedure in healthy subjects, although it does cause a transient systemic illness involving a mild fever that resolves within 24 h. Induced sputum is a relatively non-invasive way to measure neutrophil numbers in the airway lumen after LPS challenge, although the more invasive procedure of bronchoscopy has also been performed (Maris et al. 2005), as this allows sampling of bronchial mucosal tissue as well as the airway lumen. LPS can also be administered by endobronchial challenge to a segment of the lung (Hohlfeld et al. 2008); this has the advantage of not exposing the entire lung to LPS. Of note, treatment with oral prednisolone for 6 days had no effect on sputum neutrophilia (Michel et al. 2007), suggesting that this is a corticosteroid-resistant model in healthy subjects.

Recent work has shown that the inhaled LPS model can be safely performed in smokers with normal lung function (Aul et al. 2012) and mild COPD patients (Gupta et al. 2014). The advantage of using these subjects is that the effect of LPS is studied in the context of pre-existing smoking-related neutrophilic inflammation in the lungs. This may more closely resemble bacterial exposure leading to COPD exacerbations. On a practical note, the magnitude of increase in sputum neutrophils in smokers and COPD patients is less than that observed in healthy subjects, as the baseline value in healthy subjects is lower so there is greater scope for increase.

Ozone challenge has also been used to cause neutrophilic airway inflammation in healthy subjects (Holz et al. 2010; Lazaar et al. 2011; Kirsten et al. 2011). A short exposure to ozone (typically 3 h is used) coupled with exercise causes transient

neutrophilic airway inflammation in healthy subjects that can be measured using induced sputum. The potential of ozone challenges to evaluate drug effects in early phase clinical trials has been demonstrated in studies using CXCR2 antagonists Holz et al. 2010; Lazaar et al. 2011). CXCR2 is a receptor expressed on the surface of neutrophils that binds to chemokines such as CXCL8, resulting in neutrophil migration. CXCR2 antagonists reduce sputum neutrophil counts in the airways after ozone challenge in healthy subjects.

2.3 Bronchial Hyperreactivity

Many asthma patients have a tendency for excessive bronchoconstriction in response to a provoking inhaled stimulus. This is called bronchial hyperreactivity (BHR). Tests for BHR are commonly used to facilitate the diagnosis of asthma in clinical practice, while in clinical trials, these tests are used to measure drug effects.

BHR testing can be performed with direct or indirect stimuli (Cockcroft and Davis 2009). Direct stimuli include histamine and methacholine; these exert their effects directly on bronchial smooth muscle. Indirect stimuli are thought to be more clinically relevant as they cause the secretion of substances from airway cells that in turn cause bronchoconstriction. Indirect stimuli include exercise, mannitol and adenosine monophosphate (AMP).

BHR testing generally either uses the tidal breathing method involving 2 min of tidal breathing from a nebuliser, or dosimeter method involving a set number of inhalations of a known concentration and dose of a stimulus. Generally these challenge tests are performed using ascending doses or concentrations and are stopped when a decrease in FEV_1 of 20% or greater is observed; this is called the provocation dose or provocation concentration causing a 20% fall in FEV_1 (PD20 or PC20, respectively). It is accepted that changes of one doubling dose or concentration are within the range of normal variation for challenge tests including methacholine, histamine and AMP and that drug effects greater than this threshold are clinically relevant.

Methacholine and histamine challenge testing have a long history of being used to evaluate drug effects. However, these very specific stimuli may not be influenced by novel drugs that act on different pathways. Nevertheless, methacholine challenge testing has been used to evaluate the dose-response effects and bioequivalence of different formulations effects of ICS and beta-agonists (Lee et al. 2004; Houghton et al. 2004). In contrast, indirect challenge agents cause the release of a variety of mediators in the airways that are involved in the pathophysiology of asthma and are thought to be more clinically relevant. AMP challenge testing shows a high degree of sensitivity for the effects of ICS and has successfully been used to evaluate dose-response curves and duration of effect (Taylor et al. 1999; Ketchell et al. 2002). Mannitol challenges are performed using a dry-powder inhaler causing the release of mediators that are also involved in exercise-induced bronchoconstriction (Porsbjerg et al. 2013). This challenge test has also been used to evaluate the effects of inhaled corticosteroids.

3 Biomarkers

Biomarkers are measurements related to a clinical characteristic, including the presence or severity of disease, prognosis or response to therapy. Biomarkers can be extremely valuable tools in phase 2 studies with limited sample sizes that lack sufficient statistical power to evaluate clinical endpoints such as symptoms or exacerbations. Biomarkers in clinical trials can be classified as those that measure pharmacological effects of the drug only (and are not related to disease activity), those that are related to disease activity and those that identify subgroups of patients who respond to treatment. For example, neutrophil counts in blood or the lungs have been used to evaluate the effects of CXCR2 antagonists on neutrophil migration (Holz et al. 2010; Lazaar et al. 2011); this is a biomarker of pharmacological activity. However, an effect on this biomarker does not automatically mean that the drug has a beneficial clinical effect; this requires the use of measurements of disease activity. Disease activity biomarkers ideally should be easily measurable, show good reproducibility and be able to differentiate between patients with disease and healthy controls (Stockley 2014; Cazzola et al. 2008). Importantly, changes in the disease biomarker measurement should also be related to measurable changes in clinical status, increasing the degree of confidence that drug-associated changes in biomarker measurements will predict the clinical efficacy of the drug.

Asthma and COPD are both recognised as heterogeneous conditions, comprising subgroups of patients with distinct clinical characteristics (Agusti 2014; Lötvall et al. 2011; Fahy 2015). A clinical phenotype is a single or combination of disease attributes that describe differences between individuals related to clinically meaningful outcomes (symptoms, exacerbations, response to therapy, rate of disease progression or death). Biomarkers are being increasingly used in asthma and COPD studies to identify phenotypes of asthma or COPD (Faner et al. 2014; Fahy 2015). In clinical trials, there may be an "a priori" decision to include a specific clinical phenotype of patients most likely to respond to a novel drug based on the mechanism of action, but without and clinical data to support such a decision. An alternative approach is to include a wider range of patients in such clinical trials, in order to be able to identify the responder subgroup based on the data collected during the study; this approach was used to identify biomarkers that would predict response to anti-IL-13 treatment in moderate-to-severe asthma (Corren et al. 2011). This approach requires a larger study and comprehensive clinical characterisation of patients so that the unique features of the responder population can be determined.

An endotype is "a subtype of a (clinical) condition defined by a distinct pathophysiological mechanism" (Lötvall et al. 2011). Clinical phenotypes arise due to one or more biological mechanisms (endotypes). Biomarkers that identify endotypes may also therefore be relevant to multiple clinical phenotypes, and the concept of endotypes can be used to target novel drugs towards specific mechanisms that cause disease characteristics recognisable as clinical phenotypes. The most commonly used biomarkers in asthma and COPD studies will now be reviewed.

3.1 Systemic Biomarkers

One of the most well-known examples of a blood biomarker in asthma is the use of serum IgE levels to select patients most likely to respond to treatment with omalizumab; this is a monoclonal antibody directed against the Fc region of IgE. This anti-IgE treatment improves asthma outcomes in patients with severe allergic asthma and elevated serum IgE levels (Bonini et al. 2014).

IL-13 has a number of biological actions that are potentially important in asthma, such as involvement in the airway allergic response, sub-epithelial fibrosis and mucus hypersecretion (Fahy 2015). Periostin is secreted by epithelial cells in response to IL-13 stimulation in a corticosteroid-insensitive manner (Woodruff et al. 2007). Serum periostin measurements are associated with eosinophilic inflammation (Jia et al. 2012) and have been used as a biomarker of corticosteroid-resistant IL-13 activity; anti-IL-13 monoclonal antibody therapy has the greatest benefit in moderate-to-severe asthma patients with high serum levels of this biomarker (Corren et al. 2011).

COPD is a multidimensional condition associated with systemic manifestations and comorbidities. A large number of studies have been performed trying to identify systemic biomarkers in COPD patients, often focusing on cytokines and chemokines known to be involved in disease pathophysiology (Cazzola et al. 2008; Faner et al. 2014). The difficulty of these studies includes low levels of protein near the detection limit of the assay, poor reproducibility and lack of clear differentiation from healthy controls. However, C-reactive protein (CRP) and fibrinogen are biomarkers of systemic inflammation that have been used in the field of cardiovascular disease, and there is some evidence that the levels of these biomarkers are also associated with increased mortality and exacerbation rates in COPD patients (Faner et al. 2014). These biomarkers have been used in COPD clinical trials to assess the impact of novel therapies on systemic inflammation (Betts et al. 2015; Lomas et al. 2012; MacNee et al. 2013). This approach is clearly applicable to orally administered drugs due to high systemic exposure. Inhaled therapies may also impact these measurements, either by reduction in lung inflammation leading to a reduction in associated systemic inflammation, or by systemic absorption, or by both of these mechanisms.

Blood eosinophil counts have been used to identify asthma patients with higher levels of eosinophilic inflammation who are more likely to respond to monoclonal antibody treatment targeted against IL-5, which plays a central role in eosinophil activation and recruitment (Ortega et al. 2014). In COPD patients, higher sputum eosinophil counts are a predictor of response to inhaled corticosteroids in placebo-controlled clinical trials (Brightling et al. 2005; Brightling et al. 2000). However, induced sputum counts are not as practical as blood eosinophil counts and consequently can only be done by specialist centres. Blood eosinophil counts can be used to predict the response to oral corticosteroid therapy given for the treatment of acute exacerbations (Bafadhel et al. 2012a). Further work is required to investigate whether blood eosinophil counts can be used to predict the clinical response to maintenance treatment with inhaled corticosteroids.

Table 1 Typical induced sputum cell counts in asthma and COPD patients

Major cell types	Healthy subjects	Eosinophilic asthma	Neutrophilic asthma	COPD
Neutrophils	≤60%[a]	≤60%	≥61%	>70%[c]
Macrophages	>50%	>50%	<50%	<40%
Eosinophils	<3%	>3%[b]	<3%[b]	Variable[d]

[a]Neutrophil percentage increases with age, usually <50% in younger subjects. 60% is suggested as upper limit of the normal range in healthy subjects (Simpson et al. 2006)
[b]Cutoff point of 2% has also been used
[c]Neutrophil count in COPD patients can vary greatly between individuals
[d]A subset of COPD patients have increased sputum eosinophils (>3%)

3.2 Induced Sputum

Induced sputum is a non-invasive procedure that is generally safe to perform (Pizzichini et al. 2002). However, some caution is needed when performing this procedure on patients with severe airflow obstruction, as saline can cause bronchospasm. Spontaneous sputum sampling can be used in some COPD patients (Moretti 1999; Sapey et al. 2008), but this is only possible in a subset of patients. Furthermore, spontaneous samples have more non-viable cells, which can adversely affect analysis (Khurana et al. 2014). Not all patients or healthy subjects are able to provide an induced sputum sample, with the rate varying between 50 and 90% depending on the type of patient (e.g. chronic bronchitis patients are more likely to provide a sample) and the experience of the person supervising the procedure.

Induced sputum can be used to obtain slides for immune cell counts and supernatant samples for the measurement of inflammatory proteins. The typical induced sputum cell counts observed in healthy subjects and patients with obstructive lung diseases are shown in Table 1. It is recognised that high technical standards are required for sample handling and processing to obtain good quality slides (Pizzichini et al. 1996). The last two decades have seen increasing use of induced sputum in clinical trials, either as a way of predicting who will display a clinically meaningful respond to therapy (Brightling et al. 2005; Brightling et al. 2000) or as a biomarker of response to anti-inflammatory therapy either in the stable state (Grootendorst et al. 2007; Betts et al. 2015; Lomas et al. 2012) or in the context of the challenge methodologies reviewed earlier.

Reproducibility studies of induced sputum (Rossall et al. 2014; Sapey et al. 2008; Boorsma et al. 2007) have generated data that can be used for power calculations for clinical trials. It is important to note that the reproducibility of sputum cell counts varies with the clinical characteristics of the population studied. One way to reduce the variability of induced sputum data in a clinical trial is to collect multiple samples on different days within a treatment period in order to calculate a mean result (Sapey et al. 2008).

Induced sputum is usually processed with dithiothreitol (DTT) in order to disperse the mucins to allow the cellular component to be analysed (Pizzichini et al. 1996). However, DTT interferes with many immunoassays that are used to

measure protein biomarkers in the supernatant fraction (Woolhouse et al. 2002). A two-step protocol can be used to overcome this issue; this involves initial processing with phosphate buffered saline to obtain supernatant, followed by DTT processing to obtain the cellular component (Bafadhel et al. 2012b; Khurana et al. 2014).

3.3 Exhaled Nitric Oxide

There have been many attempts to develop and validate biomarkers in the breath for use in asthma and COPD clinical trials (Cazzola et al. 2008). However, exhaled nitric oxide (eNO) is the only breath measurement method that has demonstrated the required characteristics to be used for this purpose. eNO levels are associated with eosinophilic airway inflammation in asthma (Korevaar et al. 2015) and are responsive to inhaled corticosteroid therapy (Nolte et al. 2013; Anderson et al. 2012). This biomarker can be used in asthma studies for measuring the effects of anti-inflammatory drugs that impact allergic/eosinophilic inflammation (Arron et al. 2013); for example, anti-IL-13 treatment reduces eNO levels in asthma patients (Corren et al. 2011; Hodsman et al. 2013). The application of eNO monitoring in COPD trials is hindered by the inhibition of nitric oxide production by current smoking (Cazzola et al. 2008). eNO levels show good reproducibility (Borrill et al. 2006; Purokivi et al. 2000) and can be measured using different devices, including a relatively easy to use handheld device.

3.4 Bronchoscopic Sampling

Bronchoscopy allows sampling of the bronchial mucosa of the proximal airways. A number of different analyses can be performed on these samples, including investigation of the structure of the bronchial airways, the degree of inflammatory cell infiltration and the levels of inflammatory mediators. This is a potentially very informative and valuable method in clinical trials. It is usually necessary to perform two bronchoscopies for each patient; one at baseline prior to randomisation and another after completion of the treatment period. Bronchial biopsies have been used to demonstrate anti-inflammatory effects of drugs in both asthma and COPD (Barnes et al. 2014a; Barnes et al. 2006; Gizycki et al. 2002; Leckie et al. 2000).

There are a number of important considerations that limit the use of bronchoscopic sampling in clinical trials of obstructive lung diseases. Bronchoscopy is an invasive procedure which many patients refuse to consent to, and this procedure is not suitable for patients with more severe disease for safety reasons. Additionally, there is a degree of variability in mucosal inflammatory cell counts which means that the sample size has to be carefully considered and optimal analytical technical standards have to be achieved (Barnes et al. 2014a; Barnes et al. 2006). Despite these potential problems, bronchoscopy studies remain an integral part of clinical trial programmes investigating the effects of anti-inflammatory drugs; bronchoscopy

sampling can provide unique mechanistic insights into the effects of drug on the airways.

Bronchoalveolar lavage is an alternative method of sampling inflammatory cells from the lungs during bronchoscopy. This is not frequently performed because of safety concerns; it may cause a transient fever and a subsequent chest infection. Furthermore there is a high degree of variability in the measurement of supernatant proteins due to the dilution effect of saline use for lavage.

3.5 Lung Imaging

The use of high-resolution CT scanning has gained much popularity in recent years as a means of identifying the degree of emphysema and airway wall thickness in COPD patients (Gietema et al. 2011; Kim et al. 2014) and air trapping and airway wall thickness in asthma patients (Witt 2014). However, there are concerns with the radiation dosage required for these studies. This has led to the development of magnetic resonance imaging techniques (Morgan et al. 2014; Zhang et al. 2015; van Beek et al. 2009). The challenge for all of these imaging methodologies is the development of modelling techniques to generate quantitative data to determine the effects of drugs. This is a rapidly evolving field which has the potential to provide quantitative assessments of drug effects in different segments of the lung.

4 Clinical Endpoints in Later Phase Studies

4.1 Minimal Clinically Important Differences

A minimal clinically important difference (MCID) is a value that defines whether a treatment has caused a minimum level of perceived benefit (Cazzola et al. 2008; Jones et al. 2014a). A pharmacological treatment may improve a clinical measurement to a degree that is statistically significant, but this may fall below the level required for patients to feel an improvement in their health. The MCID provides a threshold for determining whether an intervention achieves this purpose.

Distribution- and anchor-based methods have been used to determine the MCID value (Jones et al. 2014a; Jones et al. 2012). The distribution-based approach uses the standard deviation or standard error of the mean to guide estimation of the MCID. Alternatively, the anchor-based approach uses the relationship to changes in other health-related measurements to determine the MCID. Both approaches provide values that require a clinical judgement to be made to finally decide on the value of the MCID. Many MCIDs have been established and applied to clinical trials that have compared active treatments to placebo. We are now moving into an era, certainly for COPD treatment, where combination therapies are becoming more widely used and clinical trials are required to compare two active treatments, for example, two bronchodilators compared to one bronchodilator. These studies have often shown that the addition of a second drug causes an incremental benefit that is

lower than expected based on the effect of the drug given as monotherapy (Singh 2015). Consequently, the second drug has caused statistically significant improvements that have failed to reach traditionally accepted MCID values. It has been proposed that an alternative term is used; minimum worthwhile incremental advantage (Jones et al. 2014a, Jones et al. 2012). This is a responder analysis approach which determines the proportion of patients who derive a benefit greater than the MCID.

4.2 Patient-Reported Outcomes in COPD Studies

The multidimensional nature of COPD encompasses a range of symptoms, systemic manifestations and comorbidities in addition to the presence of airflow obstruction. Improving lung function can be expected to improve some symptoms, such as dyspnoea, but not others, such as cough. Furthermore, improving lung function may have little or no effect on systemic manifestations and comorbidities. There is a need to measure clinical characteristics beyond FEV_1 in COPD clinical trials.

Patient-reported outcomes (PROs) evaluate the impact of disease from the perspective of the patient. Properly validated and sensitive measurement instruments for measuring PROs are needed for clinical trials, and regulatory guidance sets the standard to which new PRO instruments should be developed (Jones et al. 2012); crucially, this involves significant patient involvement during the development of the tool. PROs should ideally be easily administered to the patient and be able to both characterise patients at entry into the study and reliably measure changes caused by interventions. There is only a modest association between changes in FEV_1 and the most commonly used PROs in COPD trials (Jones et al. 2011), underlining that PROs provide information on the patient perspective that is not necessarily captured by lung function changes.

Breathlessness is one of the most common symptoms in COPD patients. This symptom has been measured using the Medical Research Council Dyspnoea Scale (Jones et al. 2012). The MRC scale has five points and consequently is very easy to administer. It is useful for describing the characteristics of patients at entry into a study, but lacks sufficient sensitivity to detect treatment effects. The Baseline Dyspnoea Index (BDI) also characterises symptoms at entry into the study, while the subsequent use of the Transition Dyspnoea Index (TDI) has been extensively used in clinical trials to measure the change from baseline; the MCID is a 1-point change (Jones et al. 2012; Jones et al. 2014a, b). These tools measure the degree of breathlessness based on different components: functional impairment, magnitude of task and magnitude of effort. The BDI and TDI have been administered by an interviewer, but this may suffer from bias or variability due to the interviewer. A computerised version of the BDI/TDI has been developed to overcome this problem. There are other methods of measuring dyspnoea, such as the Borg scale which allows patients to select a rating that corresponds to the severity of symptoms (Jones et al. 2012). This method is useful for measuring changes in dyspnoea during exercise.

The St. George's Respiratory Questionnaire (SGRQ) measures the health status of patients with airflow obstruction using three component scores: symptoms, activity and impact (Jones et al. 2012; Jones et al. 2014a, b). SGRQ-C is a COPD-specific version of this questionnaire. A practical drawback of this tool is that it contains 50 items. Nevertheless, SGRQ has been widely used in many clinical trials both to characterise the population at baseline and to evaluate treatment effects; the accepted MCID is a 4-point change. The COPD Assessment Test (CAT) and the Clinical COPD Questionnaire (CCQ) are shorter methods of assessing health status (Jones et al. 2012; Jones et al. 2014a, b), but they have not been used extensively to measure treatment effects.

4.3 COPD Exacerbations

An exacerbation of COPD is an acute worsening of symptoms, which is often caused by a respiratory tract infection. Historically, different definitions for an exacerbation have been used. An expert consensus opinion that is currently widely accepted is that an exacerbation is an increase in patient symptoms beyond the normal day to day variation that requires an increase in therapy (Celli and Barnes 2007). Exacerbations are characterised by increased levels of airway and systemic inflammation. The frequency of exacerbations is therefore used as an endpoint in long-term studies of the effects of anti-inflammatory drugs in COPD.

COPD clinical trials usually grade the severity of an exacerbation according to the degree of healthcare resource utilisation (HCRU; Dransfield et al. 2013; Wedzicha 2014; Wedzicha et al. 2013); mild exacerbations require an increase in bronchodilator therapy, moderate exacerbations require treatment with antibiotics and/or oral corticosteroids, and severe exacerbations require hospitalisation. This grading system relies on the subjective clinical opinion of the treating physician, rather than an objective set of criteria. Furthermore, the reasons for hospitalisation may vary significantly between different healthcare systems and maybe due to a lack of adequate infrastructure for management of these events in the community. Additionally, many exacerbations have a significant cardiac component, which may not be correctly diagnosed. These considerations illustrate the factors that contribute to variability when using a HCRU definition of exacerbation frequency and severity.

It is known that the best predictor of future exacerbations is the exacerbation history in the last year. The ECLIPSE study defined a "frequent exacerbator" subgroup which had $\geq$2 exacerbations each year over a 3-year follow-up period (Hurst et al. 2010). The Global Initiative for Obstructive Lung Disease (GOLD) management strategy for COPD (www.goldcopd.org) subsequently used this cutoff level to define patients as frequent exacerbators and consequently those who would derive most benefit from anti-inflammatory treatments such as ICS. Previous ICS studies in COPD had used an inclusion criteria of $\geq$1 exacerbation in the previous year to enrol a population with a sufficiently high rate of events after randomisation in order to evaluate treatment effects (Dransfield et al. 2013; Wedzicha et al. 2013;

Wedzicha 2014). More recently, an inclusion criterion of two exacerbations in the last year has been used to demonstrate the effects of the PDE4 inhibitor roflumilast on exacerbations (Martinez et al. 2015). It remains to be seen whether future clinical trials will use a cutoff level of two exacerbations to match the current GOLD definition. Nevertheless, it is important to make sure that patients enrolled in studies of exacerbations have an increased likelihood of these events after randomisation. This can be achieved by shortening the run-in period for studies, to avoid only including patients who are stable after run-in as they are more likely to remain stable after randomisation. Additionally, recruiting patients prior to the winter months when exacerbation rates are higher is also a sensible strategy (Wedzicha 2014).

Many COPD exacerbation events are unreported by patients (Jones et al. 2014b). The EXACT-PRO instrument has been developed to monitor patient symptoms on a daily basis in order to capture COPD worsenings that may go unreported. EXACT is a 14-item questionnaire that can be electronically administered allowing clinicians to monitor patients remotely (Leidy et al. 2014). There is evidence that an increased number of events can be detected using EXACT compared to a HCRU definitions (Jones et al. 2014b). An additional application of EXACT is that the duration of the exacerbation can be objectively measured in order to understand the recovery time course.

We currently have no sufficiently specific and sensitive biomarker of exacerbations that can be used in clinical trials, either to predict patients who would suffer with such events in future or to monitor for events. A biomarker in blood would be a practical option, but efforts in this regard have suffered from low specificity for COPD exacerbations, low detection levels or high variability.

4.4 Clinical Endpoints in Asthma Studies

Asthma treatment guidelines focus on two main aims: achieving optimal control of symptoms and reducing the future risk of asthma-related events such as exacerbations (http://www.ginasthma.org/). It is therefore essential that novel asthma therapies demonstrate a clinically meaningful effect on one or both of these parameters. A variety of experimental bronchial challenge models and other physiological endpoints such as lung function and eNO can be used for a preliminary evaluation of pharmacodynamics in phase 2a asthma studies. However, later phase studies need to focus on demonstrating an improvement in asthma control and prevention of exacerbations.

4.4.1 Measuring Asthma Control

The Global Initiative for Asthma (GINA) recommends a simple set of questions that doctors in clinical practice can use to assess asthma control, based on daytime and night-time symptoms, reliever medication use and limitation of activity due to asthma (http://www.ginasthma.org/). While this is an appropriate approach in daily clinical practice, more quantitative and validated PRO tools are needed in asthma

clinical trials. Three of the most commonly used PRO tools in asthma research are the Asthma Control Questionnaire (ACQ), the Asthma Control Test (ACT) and the Asthma Quality of Life Questionnaire (ACLQ; Worth et al. 2014; Barnes et al. 2014a, b). The ACQ-7 assesses seven items related to asthma control from the last week. Some of these items closely align to the GINA questions; symptoms at night-time and upon waking are included in addition to reliever usage and activity limitation. The other items deal with dyspnoea, wheezing and FEV_1. The ACQ-7 uses a scale from 0 (totally controlled) to 6 (severely uncontrolled) for each item and then calculates the mean score. Shorter versions of this questionnaire include ACQ-6, which omits lung function, and ACQ-5 which also omits reliever use. Thresholds of <0.75 and >1.5 have been used to classify patients as controlled and uncontrolled, respectively.

The ACT assesses five items of asthma control over the last 4 weeks: night-time/waking symptoms, reliever medication usage and activity limitation, in addition to dyspnoea and a rating of overall asthma control. Each item has a scale from 1 to 5, and a total score out of 25 is obtained. A score <20 indicates asthma that is not well controlled. While the ACQ and ACT are short questionnaires that are quick to complete, the AQLQ is longer as it contains 32 items in four domains: symptoms, activity limitation, emotional function and environmental stimuli. The extra AQLQ items provide a wider assessment of the patient perspective of quality of life, while ACT and ACQ are strictly focused on asthma control. The MCIDs for the ACQ and ACT are 0.5 and 3, respectively (Barnes et al. 2014a, b).

4.4.2 Asthma Exacerbations

There is no gold standard definition that is used in clinical trials for asthma exacerbations (Virchow et al. 2015). In COPD clinical trials, the HCRU definition of moderate and severe exacerbations described earlier in this chapter has become regularly used, with mild events (that do not require oral corticosteroids and/or antibiotics) being of less interest and so not always documented. This approach has also been used in some asthma studies to capture only events that lead to oral corticosteroid use; milder worsenings have not been measured (Haldar et al. 2009; Ortega et al. 2014). However, asthma clinical trials are increasingly focusing on measuring disease worsenings that have a significant impact on the patient but are not treated with oral corticosteroids. This has been done using composite endpoints with criteria related to asthma control, such as a reduction in peak flow rate, increase in reliever medication use and night-time wakening in addition to an exacerbation definition based on oral corticosteroid use or hospital emergency room treatment for asthma (Virchow et al. 2015; Corren et al. 2011). If a patient fulfils at least one of these criteria, then the event is classified as a significant worsening of disease or "asthma deterioration". The exact criteria used have varied between studies, and harmonisation of these criteria would be an important step forward.

Novel anti-inflammatory drugs may be able to provide a corticosteroid-sparing effect in asthma. Proof of concept asthma clinical trials have utilised this corticosteroid-sparing property in parallel group studies with gradual withdrawal

of corticosteroid treatment, on the basis that the novel drug will ensure that asthma control is maintained (Wenzel et al. 2013). The potential advantage of such a study design is that corticosteroid withdrawal in the placebo arm will provoke asthma deteriorations to a greater degree than would have been observed if corticosteroid treatment was maintained, and so fewer patients and a faster timeline for study completion can be expected due to the higher event rate.

5 Conclusions

There remains a considerable need to develop novel therapies for patients with asthma and COPD. Optimising the clinical development plans for novel anti-inflammatory drugs is challenging, as these drugs often cause small or no improvement in lung function. Measurements that demonstrate drug effects beyond FEV_1 are needed. Furthermore, we now recognise that only subgroups of patients are likely to respond to these novel anti-inflammatory drugs, so using biomarkers to determine the clinical phenotype most suitable for such therapies is important. Clinical phenotypes usually arise due to different biological mechanisms, so an endotype-driven approach may be more helpful in drug development, enabling drugs to be targeted specifically towards specific biological mechanisms rather than clinical characteristics. This requires the development of biomarkers to define endotypes and/or to measure drug effects. This newer approach should continue alongside efforts to optimise the measurement of clinical endpoints, including patient-reported outcome measurements, required by drug regulatory authorities.

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Drug Delivery Devices for Inhaled Medicines

Anne Lexmond and Ben Forbes

Contents

Abstract

Historically, the inhaled route has been used for the delivery of locally-acting drugs for the treatment of respiratory conditions, such as asthma, COPD, and airway infections. Targeted delivery of substances to the lungs has some key advantages over systemic administration, including a more rapid onset of action, an increased therapeutic effect, and, depending on the agent inhaled, reduced systemic side effects since the required local concentration in the lungs can be obtained with a lower dose. Fortunately, when designed properly, inhaled drug delivery devices can be very effective and safe for getting active agents directly to their site of action.

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1 Introduction

In what is arguably one of the most impressive works on asthma ever written, "On asthma: Its pathology and treatment" (1860), physician (and asthma sufferer) Henry H. Salter discusses and classifies all treatments then available for this intriguing disease, which he describes as "paroxysmal dyspnoea of a peculiar character, generally periodic, with intervals of healthy respiration between the attacks". One treatment that particularly stands out today is smoking tobacco. As a depressant, tobacco was claimed to counteract the spasm of the airway musculature (Salter 1868).

Smoking tobacco fell out of grace in the second half of the twentieth century due to its obvious negative effects on the respiratory tract with increasing evidence associating smoking with lung cancer and chronic obstructive pulmonary disease (COPD) (Cornfield et al. 1959, 2009; Auerbach et al. 1966; U.S. Department of Health and Human Services 2010). Nonetheless, smoking has continued to be advocated as an obscure reliever of breathlessness for some patients with asthma. In essence, tobacco may provide some benefit for some patients with asthma, as it has been demonstrated that nicotine can suppress various inflammatory and allergic parameters providing a plausible explanation why some patients with asthma continue to smoke claiming beneficial effects (Mishra et al. 2008). However, the cigarette presents what may be the worst example of a delivery device for administering the nicotine into the body for the "pleasurable" effects sought by smokers as it is associated with the inhalation of many other harmful chemicals producing what are now well-recognised harmful effects on the lungs and elsewhere. Fortunately, when designed properly, inhaled drug delivery methods can be very effective and safe for getting active agents into the body.

2 Inhaled Drug Delivery

The respiratory system offers a unique route for the delivery of drugs to the body. Historically, this route has been used for the delivery of locally acting drugs for the treatment of respiratory conditions, such as asthma, COPD, and airway infections. Targeted delivery of substances to the lungs has some key advantages over systemic administration routes, including a more rapid onset of action, an increased therapeutic effect, and, depending on the agent inhaled, reduced systemic side effects since the required local concentration in the lungs can be obtained with a lower dose (Newhouse and Dolovich 1986; Dolovich et al. 2005).

However, the particular architecture of the lower respiratory tract, with its vast absorptive surface area of approximately 100 m^2 (Weibel 1963), also allows

inhalation of certain substances into the lung to be an alternative portal to the systemic circulation instead of parenteral or oral administration. The lungs can thus function as a non-invasive systemic delivery route, for example when a rapid effect is desired for pain relievers (Aurora et al. 2011; Farr and Otulana 2006; Fulda et al. 2005; Furyk et al. 2009; Silberstein 2012; Xu et al. 2012), or for substances with low (or no) bioavailability after administration via the gastrointestinal tract (Patton and Byron 2007; Siekmeier and Scheuch 2008). Examples of such substances are therapeutic proteins, which are prone to degradation by metabolic enzymes (e.g. pepsin) in the gastric lumen (Lizio et al. 2000; Zijlstra et al. 2004; Laube et al. 1998; Heinemann et al. 2000; Bosquillon et al. 2004), or substances that are metabolised extensively upon first passage through the gastrointestinal wall and the liver (the first-pass effect) (Zheng et al. 1999).

However, the architecture of the lungs also poses the main challenge for pulmonary drug delivery, as it has evolved to prevent foreign matter from reaching the peripheral parts of the lungs. Therefore, aerosols must meet a strict set of physical and chemical requirements for inhaled drug delivery to be successful. Moreover, a device is needed for aerosol generation and facilitation of its delivery to the lungs. This makes inhaled drug delivery much more complex than oral or parenteral administration.

2.1 Aerosol Deposition in the Respiratory Tract

Aerosolised compounds can only exert their effects when they first pass the oropharynx and subsequently come in contact with the airway surface following inhalation into the respiratory tract. Transport of the particles in the aerosol towards these surfaces, i.e. their deposition, results from a balance between the forces that act on the inhaled particles. Four types of forces are involved in particle deposition in the respiratory tract: inertial, gravitational, and diffusional forces, as well as the drag force of the moving air that counteracts deposition (Hinds 1982; Frijlink and De Boer 2004).

Impaction as a result of high particle inertia is the predominant deposition mechanism in the upper airways, where the air velocity is high and the airflow turbulent. Particles of a sufficient mass (sufficiently high inertia) cannot follow the changes of airflow direction at the bifurcations fast enough and collide with the opposing airway surface. The probability of impaction increases with the square of the particle diameter, particle density, and particle velocity. Generally, particles with an aerodynamic diameter larger than 5–10 μm (at particle velocities above 30–50 L/min) have the highest probability of depositing in the throat by inertial impaction.

Deposition by sedimentation is the settling of particles under the influence of gravity. The gravitational force increases with the mass (cubic particle diameter) and the stationary settling velocity (counteracted by the drag force) with the square of the particle diameter. Furthermore, sedimentation is a time-dependent process, which implies that the longer a particle resides in an airway duct, the higher the

probability that it gets deposited by sedimentation. Therefore, this mechanism prevails when both the air velocity is low and of the same order of magnitude as the settling velocity, and the residence time is high, which is the case in the peripheral airways.

Diffusion, or Brownian motion, is the random movement of particles within a gas resulting from collisions with gas molecules. Diffusion increases with decreasing particle diameter and only very fine particles (smaller than 0.5 μm) are deposited by diffusion. Like sedimentation, diffusion is a time-dependent process. Hence, deposition by diffusion occurs mainly in the peripheral airways, although the relatively limited residence time of particles in the respiratory tract in combination with their random movements results in a very low deposition probability and very fine particles are likely to be exhaled instead of depositing.

2.2 The Aerodynamic Particle Diameter and Particle Size Distribution

In the descriptions above, four parameters were identified that determine whether a particle deposits in the respiratory tract and by which mechanism: the particle diameter, the particle density, the particle velocity, and the residence time in the airways.

Small, spherical particles with high density can exhibit the same aerodynamic behaviour as much larger spheres with a lower density. Hence, expressing the size of such particles in their geometric diameter is not useful for predicting their fate after inhalation. Therefore, the concept of an aerodynamic diameter has been introduced, which standardises for particle density and shape (Hinds 1982). By definition, the aerodynamic diameter of a particle is the diameter of a sphere of unit density that settles with the same velocity in still air (thus under the influence of gravity) as the particle in question. Particles with the same aerodynamic diameter exhibit the same inertial behaviour.

In general, particles with an aerodynamic diameter of 1–5 μm are regarded as suitable for inhalation. To express particle size, the mass median aerodynamic diameter (MMAD: the aerodynamic diameter below which 50% of the emitted mass is contained) is commonly used, in combination with the geometric standard deviation (GSD) as measure for the size distribution. Yet this parameter provides no information on how much of the dose is converted into an aerosol. More meaningful parameters are the fine particle fraction (FPF) and fine particle dose (FPD), which express the portion of the dose (in percentage and mass, respectively) with an aerodynamic diameter below a specified size, usually below 5 μm.

2.3 Patient-Related Factors That Influence Aerosol Deposition

Aerosol deposition patterns are not solely determined by the aerodynamic size distribution of the particles. Particle velocity and residence time, which have

already been mentioned as parameters that affect deposition, depend on the inhalation manoeuvre of the patient. Another important determinant is the geometry of the respiratory tract. With decreasing airway diameter, the deposition probability of all three mechanisms increases. This implies that deposition patterns are different in populations with reduced airspaces, such as children (smaller airways), asthma and COPD patients (narrowed and obstructed airways), or patients with bronchiectasis (dilated airways filled with sputum).

Inhalation is the result of expansion of the chest, which creates a pressure difference between the lungs and the atmosphere, in response to which air flows into the lungs. The harder the patient inhales, the faster the particles travel into the lungs, initially with the same velocity as the air that is inhaled. At higher velocities, particle deposition shifts more towards inertial impaction, as more (finer) particles cannot follow the changes in airflow direction at bifurcations.

The inhaled air functions as the medium for aerosol transport into the lungs. To reach the alveolar region, the inhaled volume has to be sufficiently large. This can be accomplished by either exhaling maximally prior to deeply inhaling once, or by tidally breathing in the aerosol over a prolonged period of time. In the latter case, mixing of the freshly inhaled air containing the aerosol with the air that is already present in the lungs is required to enable deposition of the aerosol particles in the peripheral parts of the respiratory tract (Bennett and Smaldone 1987; Nikander et al. 2010).

Patients can be instructed to inhale in the most appropriate way. However, not all patients have the capacity – either cognitive or physical, or both – to follow these instructions. When an inhalation device is not used properly, device performance is negatively affected, which inevitably results in altered aerosol deposition, and thus in less drug reaching the target area, and possibly increased chance of developing unwanted side effects. It is thus of utmost importance that inhalation devices are user-friendly and optimised for specific patient populations.

3 Delivery Devices for Inhalation

Inhalation products are complex drug delivery systems consisting of a formulation of the drug and a delivery device that converts the formulation into an inhalable aerosol.

Devices for inhaled drug delivery have two basic functions, namely aerosol formation and facilitation of aerosol transport into the lungs. A distinction is made between passive and active devices. A passive device derives the energy required for aerosol formation from the inhaled air stream, i.e. from the patient, while active devices create the aerosol independently of the patient's inhalation. Inhalation devices can be further categorised in various ways, such as single-dose versus multi-dose, or disposable versus reusable. Multi-dose devices may (but do not necessarily) provide benefits for chronic therapy, such as cost reduction, portability, and portability, ease of use and convenience. For irregular administrations and one-time applications, disposable devices may be more suitable. Furthermore,

aspects such as the risk of device contamination acting as a reservoir for microbial growth and allowing the development of antibiotic resistance may affect the choice for a multi- or single-dose device.

Traditionally, three types of inhalation devices can be distinguished: pressurised metered dose inhalers (pMDIs), nebulisers, and dry powder inhalers (DPIs). More recently, new types of devices have emerged, such as the Soft Mist Inhaler (Boehringer Ingelheim), which combine functional characteristics of the traditional classifications.

3.1 Pressurised Metered Dose Inhalers

Pressurised metered dose inhalers are the most often-prescribed inhalation devices for symptom management of patients with asthma or COPD (Laube et al. 2011). pMDIs were developed in the 1950s as the first portable multi-dose inhaled drug delivery system. Their basic design consists of a canister that is closed off by a metering valve, an actuation mechanism, and a mouthpiece. The canister holds a propellant under pressure, in which the drug and any excipients are dissolved or suspended (Smyth 2005).

The principal excipient present in all pMDI formulations is the propellant, which is required for aerosolisation of the drug, but which also acts as solvent or suspension medium. In addition, co-solvents, solubilisers, and stabilisers may be present. The first generation pMDIs contained chlorofluorocarbons (CFCs) as propellants, which have been phased out from use due to their ozone depleting properties since the 1987 *Montreal Protocol on Substances that Deplete the Ozone Layer*. The Montreal protocol has led to replacement of CFCs by hydrofluoroalkanes (HFAs) in pMDIs (Bell and Newman 2007). Some important differences exist between CFC-pMDIs and HFA-pMDIs. On average, the plumes from HFA-pMDIs have a lower velocity and a higher temperature than CFC-pMDI plumes (Gabrio et al. 1999), which may affect patient experience (Laube et al. 2011). In addition, the particles generated with HFA-pMDIs can be smaller, which has led to the development of so-called extra-fine particle products (e.g. Foster (budesonide/formoterol), Chiesi). By virtue of the lower plume velocity and smaller aerosol particle size, HFA-pMDIs show an enhanced lung deposition (Leach et al. 2002; Leach 1998; Goldin et al. 1999; Barnes et al. 2011).

The pMDI is actuated by pressing the canister down, resulting in the release of a fixed amount of the contents that disperses into small particles by rapid expansion of the propellant in the nozzle region. This actuation mechanism is one of the main disadvantages of conventional pMDIs, as actuation takes place independently of the patient's inhalation. Dose release and inhalation should be synchronic, or the entire dose deposits in the back of the patient's throat. Therefore, good actuation-inhalation ("hand-lung") coordination is required, which cannot be taught to all patients. To allow for more generalised use of pMDIs, two alternatives have been introduced: the breath-actuated pMDI (e.g. Teva's Redihaler) and the use of a valved holding chamber (VHC). Breath-actuated pMDIs still require the patient

to comprehend how to perform the desired inhalation manoeuvre. When no comprehension is to be expected at all, for example in very young children, a VHC can be used.

VHCs (e.g. Trudell's AeroChamber, GSK's Babyhaler) are extension devices with a one-way valve incorporated into the mouthpiece, allowing for the patient to inhale a static aerosol instead of a plume. The patient can keep the device in his mouth while breathing in and out, as the exhalation into the VHC is directed away from the aerosol-holding chamber via the one-way valve. This way, the aerosol can be inhaled in multiple breaths. Even though less mouth deposition can be expected when using a VHC, the final lung deposition is still low due to losses in the VHC by various mechanisms, including impaction and sedimentation of the aerosol (Bisgaard et al. 2002). For the sake of an easier name, VHCs are sometimes incorrectly grouped with spacers. Spacers (e.g. GSK's Volumatic) are simpler types of extension devices that have no valve and function solely by increasing the distance between the pMDI and the throat of the patient. This lack of a valve strongly diminishes the spacer's applicability in patients who cannot follow inhalation instructions, as it bears the risk of the patient exhaling into the device and thereby spoiling the aerosol.

3.2 Nebulisers

Nebulisers generate aerosols from aqueous solutions or suspensions of the drug. Their use is mainly confined to situations that do not allow for the use of a pMDI or DPI, for example when the patient is unconscious, or for therapeutic agents for which no pMDI or DPI formulation is available (yet) (Le Brun et al. 2000). Also for drugs requiring high doses, such as antibiotics, nebulisation was the only available delivery option until recently.

Basically, two different nebuliser types exist: jet and ultrasonic. Jet nebulisers produce aerosols with a two-fluid nozzle. The relatively wide size distribution of the droplets from such nozzles is adjusted to the desired range by removal of the largest droplets, which occurs through impaction against a flow body in the aerosol stream (baffle). Many variables can influence the droplet size distribution, including the physicochemical properties of the solution (McCallion et al. 1995; MacNeish et al. 1997; Coates et al. 1997; Le Brun et al. 1999; Lexmond et al. 2013), the jet pressure adjusted for the nozzle (Lexmond et al. 2013; de Boer et al. 2003; Newman et al. 1986; Niven and Brain 1994), and the breathing manoeuvre of the patient, or when tested in the lab, the suction flow rate (Le Brun et al. 1999; de Boer et al. 2003). The lung deposition efficiency of jet nebulisers is low, resulting in long administration times (Le Brun et al. 2000). Other major drawbacks of jet nebulisers are the long preparation and cleaning times, as well as the large residual volumes in the nebuliser cups, which may result in considerable waste of the formulation.

Ultrasonic nebulisers produce droplets by applying high-frequency pulses from an oscillating piezo-element to the solution, thereby creating standing waves on the

liquid surface from which droplets are released. The droplet size distribution depends largely on the oscillation frequency, which is mostly in the order of magnitude between 1.3 and 2.4 kHz. Unlike jet nebulisers, ultrasonic nebulisers do not require rather bulky compressors or other pressurised air systems. In the more recently developed vibrating mesh nebulisers, the piezo technology is combined with a perforated membrane (mesh), which is in contact with the drug formulation. Two different principles are available; those in which the oscillation is applied to the membrane itself and those in which the oscillation comes from a horn transducer that vibrates in the liquid reservoir. Vibrating mesh nebulisers deliver more condense aerosols than jet nebulisers, which increases the output rate and reduces the administration time. They are often equipped with chip technology to adjust the nebulisation procedure to the solution to be administered and to the breathing manoeuvre of the patient (adaptive aerosol delivery), or to monitor patient adherence and compliance (Nikander et al. 2010; Geller and Kesser 2010; Bennett 2005; Fischer et al. 2009; McCormack et al. 2012). Examples of vibrating mesh nebulisers are the I-Neb (Philips Respironics), Aeroneb (Nektar Therapeutics), Micro Air (Omron Healthcare), and eFlow Rapid (Pari).

Nebulisers generally are reusable devices. Consequently, they have to be cleaned and disinfected on a regular basis. Improper cleaning can lead to deterioration of nebuliser performance (Rottier et al. 2009). Moreover, good hygiene is paramount because nebuliser formulations consist of water mostly, and are thus highly sensitive to microbiological contamination.

3.3 The Soft Mist Inhaler

Like the vibrating mesh nebulisers, the Soft Mist Inhaler (SMI) is a more recent development in inhalation devices. The SMI is a nebuliser, as it disperses a solution of the active agent into fine droplets. It differs from the traditional nebulisers in that it is a hand-held, portable device that does not require an external power source, but is actuated by a mechanical spring. The instantaneous formation of the aerosol is comparable to a pMDI; thus, proper actuation-inhalation coordination is necessary (Lavorini 2013). However, it takes longer before the entire aerosol is generated (1.5 s versus 0.21–0.36 s for an HFA-pMDI) and the aerosol is emitted as a slow-moving mist, allowing for a relatively high lung deposition (Dalby et al. 2004).

3.4 Dry Powder Inhalers

Dry powder inhalers are the only inhalation devices that contain the drug in the dry state. DPIs typically consist of a powder formulation, a dose metering mechanism that either contains or measures a single dose of the therapeutic, a powder de-agglomeration principle, and a mouthpiece (Frijlink and De Boer 2004). Most DPIs are passive (breath-actuated) devices, so actuation and inhalation do not have to be coordinated as with pMDIs. Being operated by the breath of the patient means

that a certain minimal inspiratory effort from the patient is required for proper dose release from the DPI.

Various DPIs containing different therapeutics are commercially available. These devices can be classified into three types. The first are multi-dose DPIs, which contain the powder formulation in bulk in a reservoir, from which a dose is metered upon use by the patient. The second category includes devices that contain multiple pre-metered (sealed) doses within the device, which are called multiple unit-dose devices. Lastly, single-dose DPIs exist that are loaded with a single dose of the powder formulation, which is prepared either by the manufacturer (disposable devices) or by the patient immediately before use (capsule-based devices).

Delivery of the powder formulation to the lungs occurs through consecutive processes within the DPI, which are (typically) initiated by the inhalation manoeuvre of the patient. After entrainment of the powder formulation from the dose metering system, de-agglomeration or dispersion takes place, eventually resulting in an aerosol of small, inhalable particles of the active agent (and any excipients). The effectiveness of these processes, and hence the effectiveness of aerosol formation, is dependent on the powder formulation, the DPI (especially the powder dispersion/de-agglomeration mechanism), and the patient's inspiratory effort (inspiratory flow rate and inhaled volume).

Particularly for DPIs, the inhalation manoeuvre is highly important and the inhalation profile that is needed depends on the working principle of the inhaler and the desired deposition site in the respiratory tract (Frijlink and De Boer 2004). Whether a patient is able to perform the inhalation manoeuvre required for a particular type of DPI depends on patient characteristics like age and clinical condition (i.e. type and severity of disease), which may present them with physical limitations or insufficient understanding of how to handle the device (Price et al. 2013; Brocklebank et al. 2001; Lavorini et al. 2008; Pedersen et al. 2010; Lexmond et al. 2014a).

DPIs are versatile and applicable to a wide array of drugs because of the large dose range that can be covered, the dry state of the formulation, and the various formulation approaches that are available. DPIs are also very complex delivery systems, consisting of an inextricable combination of device and formulation. For that reason, the development of DPI products is often a next-level approach and they are generally only available for well-established therapies.

4 Choosing the Appropriate Device

The interaction between patient and device is the most important interaction to acknowledge when prescribing an inhaled drug product, since the device has to be prepared and used correctly to achieve sufficient lung deposition required for the desired therapeutic effect. In other words, an inhalation product is only as good as the patient's ability to use it, or their motivation to use and maintain it correctly. The options are of course limited by the therapies that are available. Asthma and COPD medications are the best-established inhaled therapies, for which numerous

options are available – not only in the number of active substances available, but also in the large number of devices. This is exemplified by the short-acting β_2-agonist salbutamol, where in the UK alone at least four nebuliser solutions are available (two with, and two without a preservative), three HFA-pMDIs, one breath-actuated pMDI, and five DPIs, most of these in various dosages.

When there is ample choice, the prescriber should opt for the therapy that has the highest chance of success. This success is not only dependent on the patient's ability to use a specific device, but also on their preferences, cooperativeness, willingness, and possibly familiarity with the device. If a patient has used a specific device correctly for years, their therapy may not be improved by switching to a device that by itself is better than the one they have been using, because they have to learn and adopt new handling instructions, and possibly also a new inhalation manoeuvre. However, a patient who is competent and willing may very well benefit from putting effort into learning a new technique and switching to the new device.

For all types of devices, a challenge facing inhaled drug development is that a large proportion of patients have been reported to use their devices incorrectly, resulting in suboptimal therapy (Lavorini et al. 2008; Giraud and Roche 2002; Melani et al. 2012). These proportions increase when patients use multiple devices, especially if this includes different types of devices (van der Palen et al. 1999; Price et al. 2012). Therefore, it is advisable to limit the number of (different) devices per patient, if possible. Fixed dose combination products may have therapeutic benefits in this respect, provided that the combination is rational – i.e. the combined drugs have complementary pharmacological effects and comparable dosing frequency, and preferably the same target area in the respiratory tract. Various combination products are already available (e.g. the ICS/LABA combinations; budesonide/formoterol, fluticasone proprionate/salmeterol, beclomethasone dipropionate/formoterol, fluticasone furoate/vilanterol for the maintenance treatment of asthma and COPD, and the LAMA/LABA combinations umeclidinium/vilanterol, aclidinium/formoterol, glycopyrronium/indacaterol, currently only for the treatment of COPD) and there are many others currently in late stage development, including triple therapy for the treatment of patients with COPD treatment (LAMA/LABA/ICS) (Cazzola et al. 2012).

Further to increasing the chance of successful therapy, choice of device also allows the most cost effective products to be used, as pharmacoeconomic considerations are increasingly an important aspect of delivering healthcare. This generally implies that the moment a cheaper alternative product becomes available, patients are often switched to the less expensive product. However, because of the precarious balance between patient use and device performance, switching may not always be in the best interest of the patient. For example, if the effectiveness of the therapy is reduced due to the switch to a cheaper device causing suboptimal control of symptoms, perhaps resulting in hospitalisation, then overall costs of healthcare may even increase over the long term. Optimal healthcare is therefore better looked at from the overall cost-effectiveness of treatment, rather than concentrating on just minimising the direct costs of the medication.

When the choice is limited or there is none at all, it is the physician's – and pharmacist's – duty to facilitate and ensure the patient is receiving the maximal therapeutic benefit from whatever inhaled medicine has been prescribed. Training and regular checking of the technique used by the patient or their carer are essential in this respect (Broeders et al. 2009; Papi et al. 2011). Enabling maximal therapeutic benefit includes prescribing appropriate accessories for specific age groups or devices, such as facemasks for infants and toddlers or VHCs for use with pMDIs.

4.1 Effectiveness; Effect of Training and Compliance

Key to effective inhalation therapy is correct inhaler and inhalation technique, which comprise both correct handling of the device and the performance of a correct inhalation manoeuvre. What makes this so hard is that practically each individual device has its own mode of operation. Overall, the same procedures apply to the different types of inhalation devices, but the steps can vary significantly between devices, especially for DPIs. Therefore, general guidelines on the use of a type of inhaler lack practicality and usefulness and should be avoided.

Correct inhaler and inhalation technique can only be expected when the patient is properly trained in using their particular device. Training is a joint effort involving both the healthcare provider and the patient, which starts with teaching and learning the manoeuvre, followed by repeated demonstration by the patient and, if necessary, adjusting the technique by the healthcare provider (Lavorini et al. 2008; Papi et al. 2011). Needless to say, the healthcare provider must completely understand and master the technique for every specific inhalation device that they deal with. Unfortunately, this is often not the case (Self et al. 2007), which can be attributed at least partly to poor appreciation of the complexities of inhaler technology. This can be resolved by additional training for healthcare providers, as well as appointing physician assistants and nurse practitioners specialised in pulmonary diseases and inhalation therapies.

Besides correct technique, patient compliance is just as important for effective inhalation therapy (Cochrane et al. 2000). Compliance is expressed as the level at which the patient's behaviour complies with the prescribed therapy. Indeed, flawless inhaler technique is useless when a patient does not take their medication. Noncompliance is not necessarily deliberate (it includes forgetting to take medication) and it is not necessarily harmful either. However, if a patient has good reasons not to adhere to their prescribed therapy, for example because they have side effects they cannot tolerate, discussing alternatives with their physician is better than altering the dose or dose regimen themselves. Taking any concerns of the patient seriously and engaging in dialogue can often be the key to improving compliance and thereby the effectiveness of the therapy.

4.2 Off-Label Use

Off-label use is defined as the intentional use of a medicinal product for a medical purpose that is not in accordance with the authorised product information (European Medicines 2012). This definition also applies to medical devices and thus to inhaled delivery devices. Off-label use of inhaled delivery devices, mostly nebulisers, can especially be found in clinical research.

Preparing a formulation for nebulisation can be very straightforward, which is the main reason why nebulisers are often used in the early stages of clinical development, as for example seen in studies on pulmonary vaccination and lung cancer therapy (Wauthoz et al. 2010; Otterson et al. 2010). Also for individualised medicine, pulmonary administration of the therapeutic can be accomplished by nebulisation of a simple solution of the compound (Máiz et al. 2009). It should be stressed though that in such cases, compatibility of the formulation and the nebuliser cannot simply be assumed. Stability, for example, can be compromised when the stresses induced by the nebulisation process may damage the formulation, which can be the case for large molecules or complex particulate systems (Niven and Brain 1994; Khatri et al. 2001; Münster et al. 2000; Albasarah et al. 2010; Elhissi et al. 2006, 2007; Amini et al. 2014; Kleemann et al. 2007; Hertel et al. 2014).

However, it is not just complex molecules that may present challenges when nebulised. Sometimes even the most straightforward small-molecule formulation can be incompatible with the chosen delivery device as shown for adenosine 5′-monophosphate (AMP), which is used as a bronchial challenge agent in asthma research and diagnostics. The high AMP concentrations required in some patients to evoke bronchoconstriction were shown to greatly affect nebuliser performance (Lexmond et al. 2013). This study presents a good example of a simple and fast, but inadequate solution to a problem that is more complex than anticipated and has led to the development of an alternative dry powder formulation of adenosine where much higher doses of this challenge agent can be delivered to the airways (Lexmond et al. 2014b, c).

5 Summary

There have been major advances in the development of effective drugs for the treatment of asthma and COPD over the past two decades. Furthermore, since the introduction of the first pMDI for delivering drugs topically to the airways, there have also been considerable improvements to inhalation devices and to our understanding of the complexity of formulating inhaled medicines. However, with the development of new drug classes, some of which are not traditional low molecular weight compounds, but larger molecules such as peptides (Larche 2014), proteins (O'Byrne 2013), and even oligonucleotides (Fonseca and Kline 2009), major challenges remain to ensure that these novel pharmacological entities can be delivered safely and effectively for the benefit of patients.

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Index

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