Novel Therapies to Inhibit Mucus Synthesis and Secretion in Airway Hypersecretory Diseases

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Key Words

Asthma · Chronic obstructive pulmonary disease · Mucus · Mucin · Airways · Epidermal growth factor receptor · MARCKS · Bio-11006 · Macrolides

Abstract

Background: In asthma and chronic obstructive pulmonary disease (COPD), airway mucus hypersecretion contributes to impaired mucociliary clearance, mucostasis and, potentially, the development of mucus plugging of the airways. **Summary:** Excess mucus production can be targeted via therapies that focus on inhibition mucin synthesis, via reducing expression of mucin (MUC) genes, and/or inhibition of mucin secretion into the airways. **Key Messages:** This review discusses a number of therapeutic approaches to reduce airway mucus in asthma and COPD, including the use of synthetic and natural products. In particular, it highlights areas where clinical trials of inhibitors of particular target molecules are lacking. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors are an example of a targeted therapy that has been researched to reduce mucus synthesis, as have inhibitors of EGFR's downstream signalling pathways, for example, mitogen-activated protein kinase-13 and hypoxia inducible factor-1. However, their efficacy and safety profiles are cur-

rently not up to the mark. There is clinical potential in Bio-11006, which reduces mucus secretion via the inhibition of myristoylated alanine-rich C-kinase substrate and is currently in Phase IIb trial.

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1 Introduction

Airway mucus is an aqueous solution of lipids, proteins and mucous glycoproteins (mucins) [1]. The latter are released from intracellular granules stored in epithelial goblet cells and submucosal glands of the airways [1]. Regulation of synthesis and secretion of mucus both merit investigation, since they are complementary processes but are controlled separately.

Mucus exists as a liquid bilayer: an upper 'gel' layer traps inhaled airborne particles and pathogens, which is moved by the cilia of airway epithelial ciliated cells, and beneath is a more watery layer that 'lubricates' cilia and ensures that mucus spreads over the epithelium (fig. 1) [1, 2].

Mucins can be classified as either membrane-bound mucins or secreted mucins; the former are involved in pathogen binding and cellular adhesion, whereas the lat-

Fig. 1. Organisation of the airway lining. Mucins are secreted by goblet cells and submucosal glands and form mucus on the airway surface. Mucociliary clearance is the process by which mucus is moved

by cilia to aid the passage of foreign particles up from the lower airways towards the pharynx to be unwittingly swallowed or, especially in mucus hypersecretory conditions, expelled via coughing.

ter are important for mucus viscoelasticity (the ability to absorb and store energy) [3, 4]. Mucins are encoded by MUC genes and synthesised in both goblet cells and submucosal glands [1].

The main mucins found in airway secretions are the gel-forming mucins MUC2, MUC5AC and MUC5B [1]. Expression of mucin genes varies: MUC2 is expressed in both the epithelium and submucosal glands, MUC5AC is mainly expressed in the epithelium, and MUC5B is expressed mainly in sero-mucous glands [1, 4].

Asthma and chronic obstructive pulmonary disease (COPD) are common clinical conditions, affecting approximately 300 and 210 million people respectively [5], with mucus hypersecretion being a clinical feature in many patients (fig. 2). The World Health Organisation lists COPD as having risen to the third leading cause of death globally (causing over 3.1 million deaths in 2012); the aging population has contributed to this, since COPD is a disease appearing in later life and it is strongly associated with the cigarette smoking habit and its long-term effects. Most ar-

eas of the world are seeing rising total death rates, with poorer countries having the higher mortality rates [6].

Overall, the prevalence of asthma is increasing, but prevalence varies largely between countries; 2% of Vietnam's population had asthma in 2010 compared to 33% of Australia's population [6].

Existing therapies for asthma and COPD are available, but have varying effectiveness. Bronchodilators do not specifically target mucus hypersecretion but act to dilate the airways. These include anticholinergics such as ipratropium bromide – which antagonise muscarinic receptors, and thereby reverse parasympathetic-induced bronchoconstriction – and β_2 -adrenoceptor agonists, such as salbutamol, which directly dilate the airways [7]. Glucocorticosteroids, such as fluticasone, are anti-inflammatory and effective in treating non-severe asthma but poor at targeting the underlying inflammation of COPD due to the differing pathophysiology of the diseases. Corticosteroid resistance is also a gradually developing problem in severe asthma [8].

Fig. 2. Mucus overproduction in asthma and COPD. Excess mucus (M) is seen partially occluding the large airways (arrows) of asthma and COPD patients. The excess mucus could be due to excess synthesis or hypersecretion, or both. Original figures from collection of author (D.F.R.).

Table 1. Potential targets and inhibitors of mucus synthesis and secretion

Target	Inhibitor(s)
<i>Mucin synthesis</i>	
CCR3	Anti-CCR3 monoclonal antibodies
EGFR tyrosine kinase	BIBW 2948, BIBX1382, BIBX1522, cetuximab, gefitinib
GABA-A receptors	GABA _A receptor antagonists
hCLCA1	Niflumic acid
HIF-1	YC-1
Hyperplasia (goblet cells)	Ellagic acid, statins
MUC5AC expression	Macrolides, statins
<i>Mucin secretion</i>	
HSP-70	MAL3-101
MARCKS	Bio-11006, MANS peptide
Munc proteins	Munc-18 inhibitors
P2Y ₂ receptors	P2Y ₂ receptor agonists (mucus hydration) and antagonists
SNARE proteins	Modified bacterial neurotoxins of <i>Clostridium botulinum</i>

Therefore, alternative novel therapies to treat this condition need to be investigated. Strategies to improve mucus hydration are relatively effective in cystic fibrosis [9]. However, mucus dehydration is not a notable pathophysiological feature of asthma and COPD. Under these conditions, focussing on limiting mucus hypersecretion, both mucin synthesis and secretion, could be helpful in aspects of both these hypersecretory diseases (table 1).

2 Pathophysiology of Asthma

A common clinical characteristic of asthma is 'wheezing'; this condition is characterised by impaired ability to exhale (and, in more severe cases, inhale) due to bronchoconstriction – narrowing of the bronchi and bronchioles via contraction of airway smooth muscle – and also excess mucus release in the airways (fig. 2). Other clinical features of asthma include coughing, shortness of breath, re-

current bronchitis, persistent cough with colds, and history of chest tightness. The day-to-day symptoms are particularly provoked by encounters with allergens (patients are hypersensitive to antigens and may also have eczema and rhinitis in response), although some asthmatics have asthma that is more exercise-induced [10–12].

2.1 Airway Inflammation and Remodelling

At the cellular level, asthmatics have raised levels of eosinophils, basophils and mast cells (measured in sputum), contributing to the inflammation of asthma [13].

A key feature of asthma is airway remodelling: chronic inflammation leads to thickened basement membrane and bronchial smooth muscle, as well as increased submucosal gland mass and goblet cell number. The amount of airway remodelling that takes place is proportional to the amount of inflammatory mediators present [10].

2.1.1 Inflammatory Mediators

Regarding the mediators involved in asthma and its clinical features, certain pro-inflammatory cytokines (IL-4, IL-5 and GM-CSF) are all produced in higher quantities than in a healthy person. These products attract and activate eosinophils that release further cytokines (multiple interleukins and tumour necrosis factor- α (TNF- α)); this leads to airway inflammation [14]. Increased IL-4 levels play a role in increasing differentiation towards a hypersecretory phenotype with higher levels of mucin expression. IL-4 also causes IL-8 release by influencing the differentiation of epithelial cells; this contributes to inflammation via stimulation of neutrophils [15, 16]. Certain inflammatory mediators, such as histamine, act on smooth muscle to induce bronchoconstriction – this requires lower amounts of mediators in asthmatics than in non-asthmatics [13].

2.2 Mucus in Asthma

Some of the mediators mentioned earlier also stimulate mucus hypersecretion [16], and asthmatics have higher levels of MUC5AC, one of the main gel-forming mucins, in their airways, as well as increased total mucus levels [1, 2]. MUC5B mucin is also present more in asthma patients than in healthy patients [17].

Airway obstruction in asthmatics is not only due to bronchial hyperresponsiveness; patients can occasionally die from airway occlusion characterised by mucus plugging. The raised mucus levels in asthmatics result from goblet cell hyperplasia as well as submucosal hypertrophy; this results in less effective mucociliary clearance,

which significantly impairs breathing ability and can result in formation of a mucus plug [11, 17].

Mucus hypersecretion is also linked to the overexpression of the cytokine IL-13 – it has been shown to increase the goblet cell number in both mouse and human airways, which will increase the amount of mucus being synthesised within the cells [11].

Increased release of mucus from the granules within goblet cells is stimulated by the increased presence of mast cells due to inflammation. The infiltration of these mast cells not only causes granule release but also produces cytokines such as IL-4 and IL-13 which, as previously mentioned, contribute to the detrimental effects of asthma [18].

3 Pathophysiology of COPD

COPD comprises chronic bronchitis, small airways disease and emphysema, although these do not exclusively occur in COPD. Impaired breathing ability due to COPD is normally irreversible, since lung destruction and enlarged airspaces caused by emphysema are permanent [19]. Chronic bronchitis defines the long-term airway inflammation causing mucus hypersecretion (since the airway epithelium is irritated). The main clinical symptoms of many COPD patients are those pertaining to chronic bronchitis: persistent cough with sputum production and breathlessness, causing lack of energy [19, 20]. The Medical Research Council has proposed that these symptoms should appear ‘for at least 3 months of the year on 2 consecutive years’ in order to diagnose chronic bronchitis [21].

3.1 Inflammation and Remodelling

The small airways in a COPD patient undergo tissue remodelling – specifically, fibrosis of the airways occurs as well as smooth muscle hypertrophy [22]. COPD patients have increased macrophages, neutrophils and CD8+ T lymphocytes (differing from the cell types that become more prevalent in asthma) throughout the airways, lung parenchyma and vasculature. Similar to asthma, the increase in these cells correlates with the severity of airflow obstruction [23].

3.1.1 Inflammatory Mediators

Macrophages contribute to the chronic inflammation since they release pro-inflammatory mediators such as TNF- α , IL-1, and IL-8 in response to cigarette smoke and other irritants [24, 25]. TNF- α binds to one of its 2 recep-

tor subtypes and enhances migration and adhesion of inflammatory cells to endothelia [24]. IL-1 increases production of adhesion molecules, which permit neutrophils and lymphocytes to attach to endothelial cells within the airways, while IL-8 recruits and stimulates neutrophils [26]. The neutrophils themselves release oxidants and further cytokines. Elastase is also produced from neutrophils, and this enzyme breaks down elastin in the connective tissue of the lung, contributing to emphysema. CD8⁺ T cells are increased in number and are cytotoxic, and so can participate in alveolar wall destruction [23].

3.2 Mucus in COPD

Cigarette smoking is the leading risk factor for COPD, and cigarette smoke induces the epidermal growth factor receptor (EGFR) cascade, which results in the production of mucins and goblet cell hyperplasia [27, 28]. EGFR expression is also increased by TNF- α , and smokers and COPD patients have increased EGFR expression in their airways, with associated increased mucin production and goblet cell hyperplasia [28].

Mucus hypersecretion in COPD, like in asthma, results from submucosal gland hypertrophy and goblet cell hyperplasia; this impairs mucociliary clearance, which exacerbates mucostasis (fig. 2) [17, 27]. There is an association between higher increases in goblet cells with lower FEV₁ values, supporting the hypothesis that increased mucus results in decreased breathing ability. The hypertrophy of submucosal glands in COPD differs from that of asthma, where the gland enlarges while keeping the same distribution of mucous and serous cells; in COPD patients, the ratio favours mucous cells [27].

Like asthma, mucins MUC5AC and MUC5B are found in higher amounts in COPD patients [3, 17]; however, COPD differs in that there is also a slight upregulation of MUC2, the other gel-forming mucin found in airway secretions, in goblet cells. However, MUC2 is normally only present in small amounts in airway secretions of COPD patients [1, 17]; so this small phenotypic difference (between asthma and COPD) may not be of great importance.

4 Mechanisms of Mucin Synthesis

Mucin proteins are translated from mRNA sequences coded by MUC genes. These mucins then undergo post-translational modifications such as glycosylation before being released as mature molecules.

4.1 Key Molecules

4.1.1 EGFR

EGF and its tyrosine kinase receptor, EGFR, are implicated in the regulation of mucin synthesis (fig. 3) [29]. Ligands that bind to this receptor include EGF, transforming growth factor- α (TGF- α), and vascular endothelial growth factor (VEGF) – these are proteolytically cleaved by metalloproteases before the active growth factor can be released to bind to EGFR.

Studying mucin synthesis in human asthmatics found increased EGFR expression in both submucosal glands and surface epithelium – increased mucin staining was correlated with increased EGFR immunoreactivity [29]. TNF- α induces EGFR expression in rat airway epithelium [28]; the level of EGFR expression positively correlates with the level of goblet cell hyperplasia [30]. Since asthmatics have increased TNF- α levels from increased eosinophils and infiltrating neutrophils, they have associated higher expression of EGFR, causing excess mucin synthesis via its activation.

Similarly, cigarette smoke upregulates EGFR mRNA and induces phosphorylation, thereby upregulating MUC5AC [29]. Neutrophil-derived reactive oxygen species can also activate EGFR to increase mucin production; since smoking can cause oxidative stress, antioxidants partially prevent experimentally ‘smoking’-induced goblet cell hyperplasia [28].

4.1.2 IL-13

IL-13 is a key cytokine involved in airway inflammation; as mentioned earlier, it contributes largely to increased goblet cells and mucin production [11]. IL-13 regulates airway epithelium signalling via γ -aminobutyric acid (GABA); although it is the main inhibitory neurotransmitter in the central nervous system, its effects in the bronchial epithelium are actually excitatory and its signalling contributes to mucus overproduction [31]. This is due to intracellular chloride ion levels exceeding those in the airway lumen; opening of GABA channels results in Cl⁻ efflux, making the cell membrane potential less negative and easier to excite [15, 30]. IL-13 increases GABA expression in mouse airway epithelial cells in vivo [32]. Nicotine increases expression of GABA_A receptors and nAChRs in rhesus macaque bronchial epithelial cells: nAChR activation increases signalling in GABAergic systems; therefore, nicotine-induced increased nAChR expression increases airway GABA_A channel activity further [16, 31].

IL-13 also activates signal transducer and activator of transcription 6 (STAT6), which contributes to excess MUC5AC expression; STAT6 downregulates FOXA2, a

Fig. 3. Example of a signalling cascade leading to mucin protein production following EGFR activation by ligand binding. Ligands bind to the extracellular domain of EGFR, causing the tyrosine kinase domain to phosphorylate part of the receptor located in the cytoplasm. This causes recruitment of effector molecules such as JAK and MEK proteins. These are involved in pathways that

modulate DNA synthesis; JAK phosphorylates STAT protein, which binds to specific DNA motifs to regulate expression of genes; in this case, mucin (MUC) genes, leading to mucin synthesis. ERK = Extracellular signal-regulated kinases; JAK = Janus kinase; MEK = mitogen-activated protein kinase; P = phosphate group; PI3K = phosphoinositide 3-kinase.

negative regulator of MUC5AC [2]. Targeted genetic deletion of FOXA2 in mice causes excessive production of MUC5AC; its downregulation can be brought about by airway inflammation via EGFR signalling and high expression of IL-13 and IL-4 [3].

4.1.3 Hypoxia Inducible Factor-1

Hypoxia inducible factor-1 (HIF-1) is a transcription factor, stimulated downstream of EGFR activation, that upregulates many genes including VEGF, which ac-

tivates EGFR to induce mucus production [2]. HIF-1 also binds to the MUC5AC promoter to increase MUC5AC expression [33]. HIF-1 is also activated by IL-13 and responds to inflammation; it could therefore partially contribute to mucin overproduction in inflammatory diseases such as asthma and COPD [2]. Cigarette smoke increases HIF-1 production and activity in human bronchial epithelial cells, and other HIF-1 signalling pathways, encouraging goblet cell hyperplasia [33, 34].

Inhibition of HIF-1 activity by the use of anti-HIF-1 α siRNA in human bronchial epithelial cells reduced MUC5AC expression following cigarette smoke exposure [33]. Knockout of *HIF1A* gene in vivo in a mouse model of asthma (ovalbumin-induced) reduced airway hyperresponsiveness, goblet cell hyperplasia and decreased the release of several cytokines, including IL-4 and IL-13 [35]. This indicates a role for HIF-1 in mucin synthesis and airway disease.

4.2 Goblet Cell Hyperplasia

In hyperplasia, the lack of inhibitory effect of meta-phase-blockers suggests that the formation of new goblet cells does not occur via mitosis [28]. Instead, these cells form via differentiation of existing, non-granulated secretory cells. This hyperplasia is dependent on IL-13 expression and FOXA2 downregulation mentioned earlier [29]. Increased goblet cell number is associated with increased total amount of mucins synthesised, contributing to airflow obstruction.

5 Mechanisms of Mucin Secretion

Mucins are stimulated for release from their intracellular granules upon irritation of the airways [1]. Unlike constitutive exocytosis that depends on the rate of synthesis of secretory products, mucin secretion is regulated exocytosis: secretory machinery within cells must be activated by ligands [3].

5.1 Myristoylated Alanine-Rich C-Kinase Substrate

Myristoylated alanine-rich C-kinase substrate (MARCKS) enables the intracellular mucin granules in airway secretory cells to localise near the plasma membrane, which also requires MARCKS-mediated cytoskeletal remodelling (fig. 4) [34]. The importance of MARCKS in mucin secretion was demonstrated when a lipidated peptide was introduced into human bronchial epithelial cells in vitro to interfere with MARCKS function; mucin secretion was significantly reduced [36].

5.2 Neural Control

Cholinergic agonists (e.g. methacholine), mimicking part of the parasympathetic nervous system, cause mucin secretion from murine goblet cells in vivo – there is uncertainty about the exact mechanism, but it is suggested to be indirectly via nucleotide release induced by smooth muscle contraction [37]. The nucleotides bind to P2Y purinoceptor 2 (P2Y₂) receptors (responsive to ATP and

UTP, implicating their importance in secretion regulation), which generate intracellular second messengers to activate the exocytic machinery that releases mucins into the airway lumen [2, 3]. ATP release is induced by mucous cells undergoing inflammation, mechanical deformation or irritation [3].

Submucosal glands are innervated parasympathetically; parasympathetic stimulation of human airways increased the volume of secretions and also glycoprotein output [38]. These effects can be reproduced by mAChR agonists and blocked by muscarinic antagonists [39], hence, the possible therapeutic benefit of anticholinergics in existing therapies for asthma and COPD.

6 Inhibition of Mucin Synthesis

There are many aspects of mucin synthesis that have been therapeutically targeted (fig. 5) to reduce mucin levels in the airways and to theoretically improve the symptoms of asthma and COPD. A number of inhibitors of these targets are in the process of being developed (fig. 6), and are discussed in this section.

6.1 EGFR Tyrosine Kinase Inhibitors

Inhibition of EGFR tyrosine kinase has been a major interest as therapy for airway hypersecretory diseases. Inhibition reduces mucin synthesis and goblet cell hyperplasia [17, 29]. Analysis of MUC5AC in human cells pretreated with TGF- α found increased expression that was blocked by selective EGFR tyrosine kinase inhibitors (EGFR-TKIs) such as BIBX1522 [28, 29]. These inhibitors also prevent IL-13-induced goblet cell hyperplasia and neutrophil recruitment. Furthermore, upregulation of EGFR mRNA and its induced phosphorylation are prevented by EGFR tyrosine kinase inhibition [29]. BIBX1382 is a similar EGFR-TKI that inhibits the intracellular domain of EGFR but failed a Phase I trial due to low bioavailability and an undesirable benefit: risk ratio [40].

Gefitinib is an EGFR-TKI used as an anti-cancer drug and has shown to reduce goblet cell hyperplasia in mice, suggesting its potential use in reducing mucus synthesis in hypersecretory phenotypes [41]. However, there are adverse effects to many EGFR-TKIs including gefitinib, such as papulopustular eruptions, as well as lack of successful trials on human asthma and COPD patients [42].

A safety and efficacy trial of an EGFR antagonist (BIBW 2948) published in 2010 found that this EGFR-TKI had no significant efficacy in reducing mucin stores in airway epithelial cells of COPD patients, and was not well tolerated

Fig. 4. Mechanism of action of MARCKS. Upon activation, MARCKS is phosphorylated by PKC, weakening the electrostatic bonds between the phosphorylation site domain and the acidic head groups of the phospholipid bilayer cell membrane. This allows MARCKS to move from the membrane to the cytoplasm to attach to intracellular mucin-containing granules via affinity be-

tween granule membrane proteins, including CSP and HSP-70 bound to MARCKS in the cytoplasm. MARCKS is then dephosphorylated and binds to actin-myosin systems to mediate transport of granules to the cell membrane, to which they are then docked via interactions between SNARE proteins on the target and vesicle membranes.

among patients. Twenty four percent of the BIBW 2948 subjects discontinued their treatment due to adverse events (compared to 4.3% of the control group) [43].

Therefore, development of EGFR-TKIs with improved efficacy and side effect profiles is needed before they can be considered treatment options for use in mucus hypersecretory patients.

6.2 Mitogen-Activated Protein Kinase-13 Inhibitors

Mitogen-activated protein kinase-13 (MAPK13) can be activated by human calcium-activated chloride channel-1 (hCLCA1), and this IL-13-induced signal transduction stimulates MUC5AC gene expression and therefore, mucin synthesis [44]. Consequently, suppression of MAPK13 expression using siRNA significantly reduced

Fig. 5. Potential therapeutic targets to reduce synthesis of mucins. A variety of drug classes aimed at targeting a variety of different cell-surface or intracellular molecules on/in airway epithelial cells have been developed in order to inhibit synthesis of mucins (see text for details). GABA_AR = γ -Amino butyric acid A receptor.

the expression of MUC5AC in human lung epithelial cells [44].

Inhibition of p38 kinases, a family including MAPK13, prevents mucus hypersecretion and airway hyperresponsiveness induced by allergens [45]. This suggests a potential pharmacotherapy for asthma, which is largely exacerbated by allergens (e.g. pollen, dust), however, perhaps less effective in COPD therapy since its pathophysiology depends less on environmental allergens.

Currently, there is lack of clinical trials describing safety and efficacy of MAPK13 inhibitors in therapy for mucus hypersecretion – this is due to an absence of sufficiently specific and potent inhibitors. BIRB-796, an inhibitor of the MAPK family, blocks MAPK14, although is too weak for MAPK13, despite the structural homology between the 2 [44]. BIRB-796 formed the basis of developing compounds with combined potency for MAPK13 and MAPK14, which highlighted the need for drugs with higher selectivity between the kinases. Further development will potentially improve drug specificity and maximise inhibition of mucin synthesis.

6.3 CLCA1 Inhibitors

The CLCA1 protein mentioned earlier has been identified as an important factor in mucus hypersecretion, although with an unknown exact mechanism: increased

CLCA1 expression in mice increased goblet cell hyperplasia [17] and COPD patients have higher CLCA1 levels [44]. Furthermore, its transduction into humans induced mucus production and MUC5AC expression, while its blockade suppresses mucin expression. This agrees with increased hCLCA1 expression found in bronchial epithelia of asthmatics and COPD patients [46]. However, a causative relationship between CLCA1 and increased mucin synthesis has not been demonstrated.

Niflumic acid is a non-selective experimental human CLCA1 inhibitor that significantly reduced mucus production and MUC5AC mRNA levels in human airway mucosal cells in vitro [46, 47]. It has also been shown to reduce IL-13-induced goblet cell hyperplasia in epithelial cells from asthmatics in vitro [48].

Contrarily, a murine study published in 2005 [49] stated that Gob-5, the mouse ortholog of CLCA-1, was not required for mucin overproduction. Knockdown of Gob-5 did not result in altered MUC5AC production and had no effect on inflammation in allergic mouse models [49]. The discrepancy may be due to the non-selectivity of niflumic acid for CLCA1 and suggests that novel therapy targeting hCLCA1 may not be the most useful therapeutic approach.

Fig. 6. Chemical structures of drugs intended to inhibit mucin synthesis (see text for details).

6.4 HIF-1 Inhibitors

As discussed earlier (section 4.1.3), the HIF-1 transcription factor contributes highly to mucin synthesis; so is another potential therapeutic target to reduce EGFR signalling and MUC5AC gene expression.

YC-1 (3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole) inhibits HIF-1 following transcription; this drug has been studied for treatment of hypertension, atherosclerosis and as an anti-cancer drug [50]. Experiments using YC-1 as a HIF-1 α inhibitor found that levels of MUC5AC expression and secretion were significantly reduced in human bronchial epithelial cells treated with YC-1 in hypoxic conditions [51].

Currently, there have been no clinical trials analysing the effect of YC-1 in asthmatics or COPD patients. However, due to its anti-oncogenic function, YC-1 may not be suitable for use in reduction of mucin synthesis via HIF-1 inhibition, as it blocks cell proliferation and increases cell death in hypoxic situations [52].

6.5 Immunotherapy

Monoclonal antibodies (mAbs) acting against the extracellular domain of the EGFR could potentially block EGFR activity on mucin synthesis via the immune system of an asthma/COPD patient. Cetuximab is an example of a mAb against EGFR and is used to treat various human cancers [53]; however, there are no data available about its effect on airway mucus production.

MABs acting against various chemokine receptors are also potential therapeutics for hypersecretory airway diseases: for example, anti-CCR3 mAbs were shown to reduce the expression of MUC5AC and EGFR mRNA in asthmatic mouse airway cells [54]. However, clinical trials of this mAb have not yet been conducted on humans or published – generally, studies on mAbs have not had robust results, for reasons such as infections during treatment [55].

6.6 Receptor Antagonists

Importance of GABA in IL-13-induced inflammation and mucin production in asthma was highlighted in section 4.1.2, and GABA_A receptor antagonists (bicuculline, picrotoxin) reduce mucin production in airway rhesus monkey epithelial cells [16, 31]. However, non-selective GABA blockade is unlikely to be an appropriate therapy for asthma or COPD, since its inhibitory effects are too widespread in the body and its blockade would bring about detrimental or undesirable effects (such as convulsions, hypertension, and insomnia); many are due to brain overactivity.

6.7 Statins

Simvastatin is an effective HMG-CoA reductase inhibitor (statin) that reduces cholesterol synthesis [56]. It significantly inhibits airway mucus production in vitro [57] and, upon systemic administration, decreases goblet cell hyperplasia by 33% ($p < 0.005$) in OVA-mouse models of asthma [58]. IL-13 inhibition may be responsible for the effect on goblet cells [59].

In OVA-challenged mouse models of asthma, lovastatin suppresses airway mucus secretion via reduced goblet cell hyperplasia and also inhibited MUC5AC mRNA expression [60]. Rosuvastatin is an additional statin that inhibits mucus hypersecretion via antagonism of the GABA_A receptor in murine asthma models [61]. Rosuvastatin has not yet been featured in trials of asthmatics, but the RODEO randomised controlled trial (RCT) of 2012 on 99 COPD patients did not find improvements in pulmonary function [62].

The STATCOPE RCT assessed the effects of 40 mg simvastatin daily on risk of exacerbations in moderate-severe COPD patients; no significant effects were found on the frequency of exacerbations or lung function [56]. However, this differs from findings of other studies, which suggest statins do reduce frequency of COPD exacerbations [63] and also slow the rate of lung function decline in smokers [64]. The STATCOPE trial may have been perhaps affected by its early termination, and did not critique the previous studies to suggest reasons for the inconsistency. STATCOPE's lack of improvements on lung function using simvastatin was similar to that found in the RODEO trial using rosuvastatin.

As statins are already recommended for certain cardiovascular issues, a lot of data exist regarding their safety. A systematic review of statin safety from 2006 [65] looked at 4 sources (cohort studies, RCTs, notifications of adverse events of patients taking statins, and 152 case reports) to assess the risks associated with statins. The authors concluded that statins are safe and the benefits much outweigh the low risks, although polypharmacy must be considered due to certain drug–drug interactions.

6.8 Ellagic Acid

Ellagic acid (EA) occurs naturally in a number of fruits, and decreases levels of Th2 cytokines (including IL-4, IL-5 and IL-13) and eosinophils in a mouse model of asthma [66]. In concert with this reduction in inflammation, EA also inhibits goblet cell hyperplasia in similar models in a dose-dependent manner, thereby indicating that EA is a candidate for reducing mucin synthesis [67]. Mice

Fig. 7. Potential therapeutic targets to reduce secretion of mucin. A variety of drug classes aimed at targeting a variety of different cell-surface or intracellular molecules in airway epithelial cells have been developed in order to inhibit secretion of mucin (see text for details).

treated with EA (10 mg/kg) had 80% less mucus secretion upon inflammatory stimulus compared to controls [67]. To the knowledge of the authors, there are no data regarding the efficacy of EA on human airway epithelial cells. Further research is merited to assess the minimum doses needed to see the above effects.

6.9 Ginkgolide B

Ginkgolide B (GKB), extracted from *Ginkgo biloba* leaves, interferes with the MAPK pathway [68]. GKB significantly inhibited the activation of MAPK in OVA-induced mouse models of asthma, and reduced asthma-induced goblet cell hyperplasia [68]. However, the paper gave no numerical comparison of goblet cell count or statistical tests used to determine the significance of these findings.

Similar to EA, GKB reduces secretions of inflammatory cytokines IL-5 and IL-13 in ovalbumin-induced mouse models of asthma; secretions were much lower than the control ovalbumin-sensitised mice that did not receive ginkgolide treatment [68].

Ginkgolides have been studied in randomised placebo-controlled trials for research into Chinese herbal medicine, including a 1997 trial on 61 asthmatics [69], which found a statistically significant improvement in lung function (15% increase in FEV₁, $p < 0.05$) in the ginkgolide group compared with the control. However, there are several problems with this trial, such as lack of blind-

ing and randomisation, which could have confounded results or introduced bias. Furthermore, the use of GKB may be irrelevant to the present review, as the improved lung function is likely more due to reduced airway inflammation rather than direct effects on synthesis or secretion of mucins. Current literature on GKB does not describe if it has indirect effects on MUC5AC expression via inhibition of MAPK13.

7 Inhibition of Mucin Secretion

Another method of reducing airway mucus levels is to reduce its secretion (via regulated exocytosis). Several molecules involved in this process, especially MARCKS (section 5.1), have been targeted by novel therapies (fig. 7 and 8). However, it should be noted that the inhibition of secretion might lead to intracellular mucin accumulation, which upon an 'unexpected' or 'overly-powerful' stimulus, may lead to the release of this accumulated mucin, with potentially catastrophic flooding of the airways.

7.1 MARCKS Blockade

The myristoylated N-terminal sequence (MANS peptide) is complementary to the first 24 amino acids in the N-terminal region of MARCKS; MANS peptide introduction to mouse cells reduced airway obstruction and

Fig. 8. Chemical structures of drugs intended to inhibit mucin secretion.

mucin secretion in a concentration-dependent manner [70]. A similar result was seen in human bronchial epithelial cells [17]. By blocking MARCKS, the mucin-containing intracellular granules cannot be linked to the apical membrane for release. MANS peptide-treated mice also showed lower increases in pro-inflammatory cytokine levels and neutrophil migration than the control [70]; this implies that MARCKS may also have a role in inflammation in asthma and COPD.

Bio-11006, a shorter analogue of MANS and also an inhibitor of MARCKS, reduced mucus hypersecretion in airway cells of COPD patients and improved their lung function in a randomised double-blinded controlled trial (the Breath-1 study) [71]. However, this study has a comparatively small sample size ($n = 172$) and the treatments (Bio-11006 or half-normal saline as the control) were administered to patients only for 21 days, which is an insufficient period to analyse the effects of a long-term disease,

such as asthma and COPD or to identify true side effects. BioMarck Pharmaceuticals, who manufactured Bio-11006, funded this study that could have potentially introduced bias in the data selected to be published. Since this study was published as an abstract in 2011, there have been no peer-reviewed published data further describing the effects of Bio-11006 on mucus hypersecretion, although BioMarck is set to carry out a Phase IIb trial in 2015.

MARCKS movement to the membrane, and therefore, its function in aiding exocytosis, can be inhibited by inhibition of protein kinase C (PKC) [46]; this could be an indirect therapeutic target to reduce the secretion of mucus. However, it is unlikely that non-selective PKC inhibition could be beneficial as PKC phosphorylates many substrates for a number of physiological functions – for example, its inhibition would impair smooth muscle contraction of many human systems.

7.2 Heat Shock Protein-70 Inhibitors

Heat shock protein-70 (HSP-70) is a key molecule aiding regulated exocytosis of mucin granules via MARCKS (fig. 4), and is inhibited by MAL3-101 [72]. MAL3-101 reduces the binding of HSP-70 to MARCKS and cysteine string protein (CSP) in normal human bronchial epithelial cells. Upon phorbol 12-myristate 13-acetate stimulation of mucus secretion, MAL3-101 inhibits the translocation of MARCKS between the plasma membrane and cytoplasm, significantly inhibiting mucin secretion [72]. This movement was also inhibited by the use of HSP-70 siRNA.

However, there have been no clinical trials assessing the safety and efficacy of MAL3-101 in asthmatics or COPD patients; these are required if MAL3-101 is to progress as a potential new treatment for hypersecretory airway disease.

7.3 Soluble NSF Attachment Protein Receptors Cleavage

Another potential novel therapeutic is the use of bacterial neurotoxins, for example, those of *Clostridium botulinum*, which cleave soluble NSF attachment protein receptors (SNARE) proteins [17]. As mentioned (fig. 4), SNARE proteins in airway secretory cells aid fusion of mucin-containing granules to the apical membrane; so targeting these cells to impair SNARE function could directly reduce release of mucus. However, SNARE proteins are also involved in docking of vesicles containing neurotransmitters [17]. Thus, administering these neurotoxins might also limit acetylcholine release, which would impair neurotransmission and cause a number of unwanted side effects (e.g. tachycardia). Consequently, these toxins would need to be targeted specifically at, for example, goblet cells to be effective and without side effects.

7.4 Munc Inhibitors

Munc proteins are important in the SNARE complex formation that aids vesicle docking and exocytosis (fig. 3). Therefore, inhibitors of Munc proteins (particularly Munc18) could suppress mucin secretion [17]. However, targeting vesicle exocytosis may not realistically be the best approach since it is vital in other processes, for example, neurotransmission, and there are no therapeutics of this type yet in studies.

7.5 P2Y₂ Agonists/Antagonists

The P2Y₂, mentioned in section 5.2, has also been targeted for mucus-regulating drugs. Since P2Y₂ receptors interact with ATP and UTP to bring about mucin secre-

tion, P2Y₂ antagonism could inhibit mucin hypersecretion [17, 73]. However, this system also regulates water secretion, which aids mucociliary clearance; P2Y₂ agonists (such as diquafosol) have also been studied to improve mucus hydration. Although these drugs will not inhibit synthesis or secretion of mucin, they may still help treat airway hypersecretory diseases through mechanisms of mucus clearance [73].

7.6 Macrolide Antibiotics

Macrolides are antibiotics, including azithromycin (AZM) and clarithromycin (CAM), which interfere with bacterial protein synthesis [74], but also reduce airway inflammation via mechanisms unrelated to their antibacterial properties. For example, macrolides release small amounts of mucin from isolated airway submucosal glands via partial agonism at muscarinic receptors, but conversely suppress stimulated mucin release by interfering with intracellular calcium handling [75].

CAM reduced airway mucus production in OVA-induced rats in vivo in a dose-dependent manner [76]. This study also specifically looked at MUC5AC secretion in human cells (NCI-H292 line) and found that it was decreased with both CAM and erythromycin (at concentrations equal to, or above 10⁻⁶ mol/l). MUC5AC mRNA expression was also significantly lower in macrolide-treated NCI-H292 cells and human nasal epithelial cells than controls [76]. Significantly, these results for CAM and AZM have been reproduced in a separate study [77]. The reduced MUC5AC mRNA transcription was suggested to be via macrolide inhibition of IL-13 signalling pathways [78].

In an RCT of 1,117 COPD patients, AZM reduced the frequency and total number of acute exacerbations [77, 79], which could potentially be due to impaired mucus synthesis and secretion, although the study did not measure mucin levels in the patients' sputum or nasopharyngeal samples. The smaller (n = 99) AZISAST trial, measured similar primary endpoints, but this time in asthmatic patients – however, AZM did not significantly reduce acute exacerbations [80]. A recent meta-analysis of AZM treatment in bronchial asthma analysed 8 RCTs and found that AZM did improve peak expiratory flow and asthma control test scores and was well tolerated [81].

A major hindrance to using these antibiotics is the emergence of bacterial resistance; macrolides are commonly used to treat bacterial infections and the use of macrolides in patients with chronic airway disease could reduce their anti-bacterial efficacy.

8 Conclusions

The impact of airway mucus hypersecretion and mucus plugging, coupled with impaired breathing ability, in asthma and COPD patients highlights the role of excessive mucus production in pathophysiology and clinical manifestation of these respiratory conditions. Existing therapies focus more on reducing inflammation (via glucocorticosteroids) and inducing bronchodilation, rather than specifically targeting airway mucus hypersecretion. Consequently, there is an unmet need to develop pharmacologically active compounds designed to target excess mucin synthesis and secretion.

Many features of both mucin synthesis and secretion have been discussed in this study that could be therapeutically targeted for airway hypersecretory diseases. Some targets, such as EGFR (involved in mucin synthesis), and SNARE and Munc proteins (involved in mucin secretion), have not advanced significantly due to how widespread their physiological effects are. For example, blocking proteins involved with exocytosis may reduce mucin secretion, but would also possibly block neurotransmission and other homeostatic secretory processes.

Other aspects, such as blockade of MARCKS (involved in secretion), may be more promising for future therapies. The upcoming Phase IIb trial of Bio-11006, a

MARCKS inhibitor (section 7.1), will be a helpful indication as to whether this form of novel therapy will be efficacious and safe in COPD patients, although there are yet to be similar investigations among asthmatics.

Many of the novel compounds discussed here (fig. 6 and 8) would be more likely to progress clinically if they had improved selectivity as inhibitors of mucin synthesis and/or secretion specifically in airway mucin-producing cells. However, to develop this level of selectivity is a challenge, and would likely also require high financial and research input. Nevertheless, although seemingly still in the early days for these novel therapies, properly designed clinical trials will be required to elucidate the contribution of mucus hypersecretion to disease progression in asthma and COPD, and to indicate new pathways for intervention.

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Disclosure Statement

Both contributing authors declare no conflicts of interest.

References

- 1 Rogers DF: Physiology of airway mucus secretion and pathophysiology of hypersecretion. *Respir Care* 2007;52:1134–1146.
- 2 Evans CM, Kim K, Tuvim MJ, Dickey BF: Mucus hypersecretion in asthma: causes and effects. *Curr Opin Pulm Med* 2009;15:4–11.
- 3 Evans CM, Koo JS: Airway mucus: the good, the bad, the sticky. *Pharmacol Ther* 2009;121:332–348.
- 4 Rose MC, Voynow JA: Respiratory tract mucin genes and mucin glycoproteins in health and disease. *Physiol Rev* 2006;86:245–278.
- 5 Ferkol T, Schraufnagel D: The global burden of respiratory disease. *Ann Am Thorac Soc* 2014;11:404–406.
- 6 Burney P, Jarvis D, Perez-Padilla R: The global burden of chronic respiratory disease in adults. *Int J Tuberc Lung Dis* 2015;19:10–20.
- 7 Cazzola M, Page CP, Calzetta L, Matera MG: Pharmacology and therapeutics of bronchodilators. *Pharmacol Rev* 2012;64:450–504.
- 8 Hakim A, Adcock IM, Usmani OS: Corticosteroid resistance and novel anti-inflammatory therapies in chronic obstructive pulmonary disease: current evidence and future direction. *Drugs* 2012;72:1299–1312.
- 9 Tildy BE, Rogers DF: Therapeutic options for hydrating airway mucus in cystic fibrosis. *Pharmacology* 2015;95:117–132.
- 10 McConnell W, Holgate S: The definition of asthma: its relationship to other chronic obstructive lung diseases; in Clark T, Godfrey S, Lee T, Thomson N (eds): *Asthma*, ed 4. USA, Arnold, 2000, pp 7–25.
- 11 Rubin BK, Priftis KN, Schmidt HJ, Henke MO: Secretory hyperresponsiveness and pulmonary mucus hypersecretion. *Chest* 2014;146:496–507.
- 12 McCarty JC, Ferguson BJ: Identifying asthma triggers. *Otolaryngol Clin North Am* 2014;47:109–118.
- 13 Maslan J, Mims JW: What is asthma? Pathophysiology, demographics, and health care costs. *Otolaryngol Clin North Am* 2014;47:13–22.
- 14 Rothenberg ME, Hogan SP: The eosinophil. *Annu Rev Immunol* 2006;24:147–174.
- 15 Nakagome K, Matsushita S, Nagata M: Neutrophilic inflammation in severe asthma. *Int Arch Allergy Immunol* 2012;158(suppl 1):96–102.
- 16 Wang Y, Bai C, Li K, Adler KB, Wang X: Role of airway epithelial cells in development of asthma and allergic rhinitis. *Respir Med* 2008;102:949–955.
- 17 Rogers DF, Barnes PJ: Treatment of airway mucus hypersecretion. *Ann Med* 2006;38:116–125.
- 18 Bradding P, Walls AF, Holgate ST: The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006;117:1277–1284.
- 19 Theander K, Hasselgren M, Luhr K, Eckerblad J, Unosson M, Karlsson I: Symptoms and impact of symptoms on function and health in patients with chronic obstructive pulmonary disease and chronic heart failure in primary health care. *Int J Chron Obstruct Pulmon Dis* 2014;9:785–794.
- 20 Hassett DJ, Borchers MT, Panos RJ: Chronic obstructive pulmonary disease (COPD): evaluation from clinical, immunological and bacterial pathogenesis perspectives. *J Microbiol* 2014;52:211–226.
- 21 Definition and classification of chronic bronchitis for clinical and epidemiological purposes. A report to the medical research council by their committee on the aetiology of chronic bronchitis. *Lancet* 1965;1:775–779.

- 22 van den Berge M, ten Hacken NH, Cohen J, Douma WR, Postma DS: Small airway disease in asthma and COPD: clinical implications. *Chest* 2011;139:412–423.
- 23 MacNee W: Pathology, pathogenesis, and pathophysiology. *BMJ* 2006;332:1202–1204.
- 24 Bradley JR: TNF-mediated inflammatory disease. *J Pathol* 2008;214:149–160.
- 25 Barnes PJ: Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clin Chest Med* 2014;35:71–86.
- 26 de Oliveira S, Reyes-Aldasoro CC, Candel S, Renshaw SA, Mulero V, Calado A: Cxcl8 (IL-8) mediates neutrophil recruitment and behavior in the zebrafish inflammatory response. *J Immunol* 2013;190:4349–4359.
- 27 Williams OW, Sharafkhaneh A, Kim V, Dickey BF, Evans CM: Airway mucus: from production to secretion. *Am J Respir Cell Mol Biol* 2006;34:527–536.
- 28 Nadel JA, Burgel PR: The role of epidermal growth factor in mucus production. *Curr Opin Pharmacol* 2001;1:254–258.
- 29 Nadel JA: Role of epidermal growth factor receptor activation in regulating mucin synthesis. *Respir Res* 2001;2:85–89.
- 30 Takeyama K, Dabbagh K, Lee HM, Agustí C, Lausier JA, Ueki IF, et al: Epidermal growth factor system regulates mucin production in airways. *Proc Natl Acad Sci U S A* 1999;96:3081–3086.
- 31 Fu XW, Wood K, Spindel ER: Prenatal nicotine exposure increases GABA signaling and mucin expression in airway epithelium. *Am J Respir Cell Mol Biol* 2011;44:222–229.
- 32 Glasser MA: GABAergic system as a target for treatment in asthma. *Thorax* 2007;62:1080.
- 33 Yu H, Li Q, Kolosov VP, Perelman JM, Zhou X: Regulation of cigarette smoke-mediated mucin expression by hypoxia-inducible factor-1 α via epidermal growth factor receptor-mediated signaling pathways. *J Appl Toxicol* 2012;32:282–292.
- 34 Polosukhin VV, Cates JM, Lawson WE, Milstone AP, Matafonov AG, Massion PP, et al: Hypoxia-inducible factor-1 signalling promotes goblet cell hyperplasia in airway epithelium. *J Pathol* 2011;224:203–211.
- 35 Crotty Alexander LE, Akong-Moore K, Feldstein S, Johansson P, Nguyen A, McEachern EK, et al: Myeloid cell HIF-1 α regulates asthma airway resistance and eosinophil function. *J Mol Med* 2013;91:637–644.
- 36 Adler KB, Tuvim MJ, Dickey BF: Regulated mucin secretion from airway epithelial cells. *Front Endocrinol (Lausanne)* 2013;4:129.
- 37 Agrawal A, Rengarajan S, Adler KB, Ram A, Ghosh B, Fahim M, et al: Inhibition of mucin secretion with MARCKS-related peptide improves airway obstruction in a mouse model of asthma. *J Appl Physiol* (1985) 2007;102:399–405.
- 38 Rogers DF: Motor control of airway goblet cells and glands. *Respir Physiol* 2001;125:129–144.
- 39 Nadel JA, Davis B, Phipps RJ: Control of mucus secretion and ion transport in airways. *Annu Rev Physiol* 1979;41:369–381.
- 40 Dittrich C, Greim G, Borner M, Weigang-Köhler K, Huisman H, Amelsberg A, et al: Phase I and pharmacokinetic study of BIBX 1382 BS, an epidermal growth factor receptor (EGFR) inhibitor, given in a continuous daily oral administration. *Eur J Cancer* 2002;38:1072–1080.
- 41 Hur GY, Lee SY, Lee SH, Kim SJ, Lee KJ, Jung JY, et al: Potential use of an anticancer drug gefinitib, an EGFR inhibitor, on allergic airway inflammation. *Exp Mol Med* 2007;39:367–375.
- 42 Macdonald JB, Macdonald B, Golitz LE, LoRusso P, Sekulic A: Cutaneous adverse effects of targeted therapies: part I: inhibitors of the cellular membrane. *J Am Acad Dermatol* 2015;72:203–218.
- 43 Woodruff PG, Wolff M, Hohlfeld JM, Krug N, Dransfield MT, Sutherland ER, et al: Safety and efficacy of an inhaled epidermal growth factor receptor inhibitor (BIBW 2948 BS) in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2010;181:438–445.
- 44 Alevis YG, Patel AC, Romero AG, Patel DA, Tucker J, Roswit WT, et al: IL-13-induced airway mucus production is attenuated by MAPK13 inhibition. *J Clin Invest* 2012;122:4555–4568.
- 45 Chung KF: p38 mitogen-activated protein kinase pathways in asthma and COPD. *Chest* 2011;139:1470–1479.
- 46 Hauber HP, Zabel P: Emerging mucus regulating drugs in inflammatory and allergic lung disease. *Inflamm Allergy Drug Targets* 2008;7:30–34.
- 47 Hauber HP, Daigneault P, Frenkiel S, Lavigne F, Hung HL, Levitt RC, et al: Niflumic acid and MSI-2216 reduce TNF- α -induced mucin expression in human airway mucosa. *J Allergy Clin Immunol* 2005;115:266–271.
- 48 Nakano T, Inoue H, Fukuyama S, Matsumoto K, Matsumura M, Tsuda M, et al: Niflumic acid suppresses interleukin-13-induced asthma phenotypes. *Am J Respir Crit Care Med* 2006;173:1216–1221.
- 49 Robichaud A, Tuck SA, Kargman S, Tam J, Wong E, Abramovitz M, et al: Gob-5 is not essential for mucus overproduction in pre-clinical murine models of allergic asthma. *Am J Respir Cell Mol Biol* 2005;33:303–314.
- 50 Yeo EJ, Chun YS, Cho YS, Kim J, Lee JC, Kim MS, et al: YC-1: a potential anticancer drug targeting hypoxia-inducible factor 1. *J Natl Cancer Inst* 2003;95:516–525.
- 51 Zhou X, Tu J, Li Q, Kolosov VP, Perelman JM: Hypoxia induces mucin expression and secretion in human bronchial epithelial cells. *Transl Res* 2012;160:419–427.
- 52 Tsui L, Fong TH, Wang IJ: The effect of 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole (YC-1) on cell viability under hypoxia. *Mol Vis* 2013;19:2260–2273.
- 53 Van Cutsem E, Köhne C, Hittre E, Zaluski J, Chang Chien C, Makhson A, et al: Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009;360:1408–1417.
- 54 Shen HH, Xu F, Zhang GS, Wang SB, Xu WH: CCR3 monoclonal antibody inhibits airway eosinophilic inflammation and mucus overproduction in a mouse model of asthma. *Acta Pharmacol Sin* 2006;27:1594–1599.
- 55 Ross CL, Hansel TT: New drug therapies for COPD. *Clin Chest Med* 2014;35:219–239.
- 56 Criner G, Connett JE, Aaron SD, Albert RK, Bailey WC, Casaburi R, et al: Simvastatin for the prevention of exacerbations in moderate-to-severe COPD. *N Engl J Med* 2014;370:2201–2210.
- 57 Marin L, Traini D, Bebawy M, Colombo P, Buttini F, Haghi M, et al: Multiple dosing of simvastatin inhibits airway mucus production of epithelial cells: implications in the treatment of chronic obstructive airway pathologies. *Eur J Pharm Biopharm* 2013;84:566–572.
- 58 Zeki AA, Bratt JM, Rabowsky M, Last JA, Kenyon NJ: Simvastatin inhibits goblet cell hyperplasia and lung arginase in a mouse model of allergic asthma: a novel treatment for airway remodeling? *Transl Res* 2010;156:335–349.
- 59 Zeki AA, Franzl L, Last J, Kenyon NJ: Simvastatin inhibits airway hyperreactivity: implications for the mevalonate pathway and beyond. *Am J Respir Crit Care Med* 2009;180:731–740.
- 60 Liou CJ, Cheng PY, Huang WC, Chan CC, Chen MC, Kuo ML, et al: Oral lovastatin attenuates airway inflammation and mucus secretion in ovalbumin-induced murine model of asthma. *Allergy Asthma Immunol Res* 2014;6:548–557.
- 61 Zhu T, Zhang W, Wang DX, Huang NW, Bo H, Deng W, et al: Rosuvastatin attenuates mucus secretion in a murine model of chronic asthma by inhibiting the gamma-aminobutyric acid type A receptor. *Chin Med J (Engl)* 2012;125:1457–1464.
- 62 Neukamm A, Høiseth AD, Einvik G, Lehmann S, Hagve TA, Søyseth V, et al: Rosuvastatin treatment in stable chronic obstructive pulmonary disease (RODEO): a randomized controlled trial. *J Intern Med* 2015;278:59–67.
- 63 Blamoun AI, Batty GN, DeBari VA, Rashid AO, Sheikh M, Khan MA: Statins may reduce episodes of exacerbation and the requirement for intubation in patients with COPD: evidence from a retrospective cohort study. *Int J Clin Pract* 2008;62:1373–1378.
- 64 Keddissi JI, Younis WG, Chbeir EA, Daher NN, Dernaika TA, Kinasewitz GT: The use of statins and lung function in current and former smokers. *Chest* 2007;132:1764–1771.
- 65 Law M, Rudnicka AR: Statin safety: a systematic review. *Am J Cardiol* 2006;97(8 suppl 1):S52–S60.
- 66 Zhou E, Fu Y, Wei Z, Yang Z: Inhibition of allergic airway inflammation through the blockage of NF- κ B activation by ellagic acid in an ovalbumin-induced mouse asthma model. *Food Funct* 2014;5:2106–2112.

- 67 Alves Cde F, Angeli GN, Favarin DC, de Andrade EL, Chica JE, Faccioli LH, et al: The effects of proresolution of ellagic acid in an experimental model of allergic airway inflammation. *Mediators Inflamm* 2013;2013:863198.
- 68 Chu X, Ci X, He J, Wei M, Yang X, Cao Q, et al: A novel anti-inflammatory role for ginkgolide B in asthma via inhibition of the ERK/MAPK signaling pathway. *Molecules* 2011;16:7634–7648.
- 69 Li MH, Zhang HL, Yang BY: [Effects of ginkgo leave concentrated oral liquor in treating asthma]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 1997;17:216–218.
- 70 Green TD, Crews AL, Park J, Fang S, Adler KB: Regulation of mucin secretion and inflammation in asthma: a role for MARCKS protein? *Biochim Biophys Acta* 2011;1810:1110–1113.
- 71 Van As A, Kraft M, Hanania NA, Parikh I, Murphy EC: Inhibition of airway mucus hypersecretion and inflammation with Bio-11006, a novel dual action drug, results in improvement of indices of bronchitis and lung function in chronic obstructive pulmonary disease (COPD). *Am J Respir Crit Care Med* 2011;183:A4092.
- 72 Fang S, Crews AL, Chen W, Park J, Yin Q, Ren XR, et al: MARCKS and HSP70 interactions regulate mucin secretion by human airway epithelial cells in vitro. *Am J Physiol Lung Cell Mol Physiol* 2013;304:L511–L518.
- 73 Rogers DF: The role of airway secretions in COPD: pathophysiology, epidemiology and pharmacotherapeutic options. *COPD* 2005;2:341–353.
- 74 Kannan K, Kanabar P, Schryer D, Florin T, Oh E, Bahroos N, et al: The general mode of translation inhibition by macrolide antibiotics. *Proc Natl Acad Sci U S A* 2014;111:15958–15963.
- 75 Lu S, Liu H, Farley JM Sr: Macrolide antibiotics inhibit mucus secretion and calcium entry in Swine airway submucosal mucous gland cells. *J Pharmacol Exp Ther* 2011;336:178–187.
- 76 Shimizu T, Shimizu S, Hattori R, Gabazza EC, Majima Y: In vivo and in vitro effects of macrolide antibiotics on mucus secretion in airway epithelial cells. *Am J Respir Crit Care Med* 2003;168:581–587.
- 77 Shimizu T, Shimizu S: Azithromycin inhibits mucus hypersecretion from airway epithelial cells. *Mediators Inflamm* 2012;2012:265714.
- 78 Tanabe T, Kanoh S, Tsushima K, Yamazaki Y, Kubo K, Rubin BK: Clarithromycin inhibits interleukin-13-induced goblet cell hyperplasia in human airway cells. *Am J Respir Cell Mol Biol* 2011;45:1075–1083.
- 79 Albert RK, Connett J, Bailey WC, Casaburi R, Cooper JA Jr, Criner GJ, et al: Azithromycin for prevention of exacerbations of COPD. *N Engl J Med* 2011;365:689–698.
- 80 Brusselle GG, Vanderstichele C, Jordens P, Deman R, Slabbynck H, Ringoet V, et al: Azithromycin for prevention of exacerbations in severe asthma (AZISAST): a multicentre randomised double-blind placebo-controlled trial. *Thorax* 2013;68:322–329.
- 81 Liu L, Wang GZ, Han D, Li MX: Effectiveness and safety of azithromycin in the treatment of bronchial asthma: a meta-analysis. *Nan Fang Yi Ke Da Xue Xue Bao* 2015;35:83–87.