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Chronic obstructive pulmonary disease: patho-physiology, current methods of treatment and the potential for simvastatin in disease management

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Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a severe disease that leads to a non-reversible obstruction of the small airways. The prevalence of this disease is rapidly increasing in developed countries, and in 2020 it has been predicted that this disease will reach the third cause of mortality worldwide. COPD patients do not respond well to current treatment modalities, such as bronchodilators and corticosteroids.

Areas covered: This review article focuses on the patho-physiology of COPD, explores current approaches to alleviate and treat the disease, and discusses the potential use of statins for treatment. Specifically, the mechanism of action and metabolism of simvastatin, the most known and studied molecule among the statin family, are critically reviewed.

Expert opinion: Various cellular pathways have been implicated in COPD, with alveolar macrophages emerging as pivotal inflammatory mediators in the COPD patho-physiology. Recently, emerging anti-cytokine therapies, such as PDE4 inhibitors and ACE inhibitors, have shown good anti-inflammatory properties that can be useful in COPD treatment. Recently, statins as a drug class have gained much interest with respect to COPD management, following studies which show simvastatin to exert effective anti-inflammatory effects, via inhibition of the mevalonic acid cascade in alveolar macrophages.

Keywords: COPD, HMG-CoA reductase inhibitors, mechanism of action, metabolism, simvastatin

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1. Introduction

Chronic obstructive pulmonary disease (COPD) arises as a result of the combined effects of smoking (and airborne pollutant) exposure and genetic susceptibility to the damaging effects of smoking. It is a debilitating disease characterized by four pathologic conditions: chronic obstructive bronchitis, fibrosis, emphysema and mucus overproduction. All these conditions are responsible for small airway obstruction via the combined effects of destruction of lung parenchyma and loss of lung elasticity leading to small airway closure, together with bronchiolar wall fibrosis and mucus overproduction all of which contribute to obstruct airways [1-3].

Chronic bronchitis is defined by the presence of a productive cough of > 3 consecutive months over a duration of > 2 successive years [4]. The cough is due

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to hyper-secretion of mucus and is not necessarily accompanied by airflow limitation [4,5]. The chronic inflammation is characterized by inflammatory cell migration to the small airways, together with fibrosis and smooth muscle cell proliferation, which results in reduced airway diameter and increased resistance [1,3].

The WHO predicts that by 2020 COPD will rise from being the fourth most common cause of death to the third [4,5]. Reasons for the dramatic increase in COPD include reduced mortality from other causes such as cardiovascular diseases in industrialized countries and infectious diseases in developing countries, along with a marked increase in cigarette smoking and environmental pollution. [6]. Such a debilitating and increasingly visible disease has been the subject of several studies. Furthermore, as current therapies usually applied to anti-asthmatic treatments do not seem to be effective in COPD patients, there is a pressing need to investigate and develop new therapeutic approaches in COPD disease management.

Some drug classes such as ACE inhibitors, PDE4 inhibitors and statins have demonstrated anti-inflammatory properties and seem to have an effect in improving COPD conditions [7]. In particular, the statins have been shown to have beneficial effects and seem able to reduce mortality in COPD patients [8,9]. Frost *et al.* observed in a group of COPD patients that those who took a statin dose of > 4 mg/day for 90 days presented a reduced mortality [8].

In a retrospective study, Søyseth *et al.* investigated death cases in a cohort of 850 patients hospitalized with acute COPD exacerbation. They found that statin therapy was successful in decreasing mortality: the mortality rate/1000 person-years was 110 in patients treated with statins versus 191 in patients not treated with statins [9]. Similarly, other studies have demonstrated that the number of exacerbations and intubation cases in COPD hospitalized patients were significantly reduced and that patients treated with simvastatin (SV) showed a reduction in the blood concentration of inflammatory cytokines [10-12]. Recent literature suggests that statins, a popular and safe cholesterol-lowering class of drugs, could be effective in COPD treatment as they target the production of inflammatory mediators rather than effects on smooth muscle.

This review article focuses on the patho-physiology of COPD and explores current approaches to the alleviation of symptoms and treatment of the disease with a focus on the mechanism of action and metabolism of SV.

2. The patho-physiology of COPD

2.1 Alveolar macrophages and the NF-κB pathway

Histopathological studies show that most inflammation in COPD occurs mainly in the peripheral airways (bronchioles) [1]. Alveolar macrophages appear to play a critical role in the inflammation implicated, as these constitute the activated population present at up to 10 times the level at sites of damage [13]. Cigarette smoke activates alveolar macrophages to secrete numerous inflammatory cytokines. The mechanism by which alveolar macrophages can produce chemokines, cytokines and the enzymes involved in chronic lung inflammation has been extensively reviewed [13,14].

NF-κB is a heterodimer protein complex of p50 and p65 which binds κB sites in the promoter regions of genes encoding inflammatory proteins (**Figure 1**) [15]. NF-κB is ubiquitously expressed in eukaryotic cells, including alveolar macrophages, and plays an important role in cellular responses to stress, cytokines, ultraviolet radiation and free radicals. Deregulation of the NF-κB response has been implicated in cancer, sepsis, autoimmune disorders and inflammatory diseases [16].

Cytoplasmic NF-κB exists bound to the inhibitory protein binding kB (IkB). Activated NF-κB requires the phosphorylation and subsequent degradation of IkB by the activation of a specific IkB kinase (IKK) which is strongly activated by irritants and among them cigarette smoke (**Figure 2**). On degradation of IkB, NF-κB migrates into the nucleus and activates the transcription of inflammatory mediators [13,17-18]. In this context, smoke induced macrophage activation mechanisms include an increase in NF-κB mediated chemokine, cytokine and MMP production all of which are vital in the activation and propagation of the inflammatory cascade observed in COPD.

2.2 TNF-α mechanism of action in COPD

The inflammatory cytokines TNF-α and IL-1β, are produced by macrophages/monocytes during inflammation and are involved in a range of signaling events including activation of IKK [15,19].

TNF-α is essential for cellular protection against infection and cancer. TNF-α binds as a trimer to either a 55 kDa cell surface transmembrane receptor (TNFR-1) or a 75 kDa cell surface receptor (TNFR-2), both of which are members of the TNF receptor (TNF-R) superfamily. An extracellular domain consisting of six repeats of cystein rich motifs typifies this superfamily. Once activated, TNF-R recruits TNF-R-associated death domain, receptor-interacting protein and TRAF2 (TNF-R-associated factor 2) (**Figure 1**) [19,20].

Similarly, the cytokine protein, IL-1β, is a member of the IL-1 cytokine family and an important mediator of inflammation, cell proliferation, differentiation and apoptosis. IL-1β is produced by activated macrophages as a proprotein, which is processed to its active form caspase 1. Binding of IL-1β results in the recruitment of TRAF6. Both TRAF2 and TRAF6 induce the phosphorylation and activation of MEKK1 (MEK kinase); in addition, TRAFs activate NIK (NF-κB-inducing kinase). Both MEKK1 and NIK can phosphorylate the IKKs the result of which is an activation of the NF-κB response [21,22]. As a result, cigarette smoke-mediated IKK activation creates an amplifying loop by which macrophages are induced to proliferate and produce more and more inflammatory cytokines [13,15,19,23] (**Figure 2**).

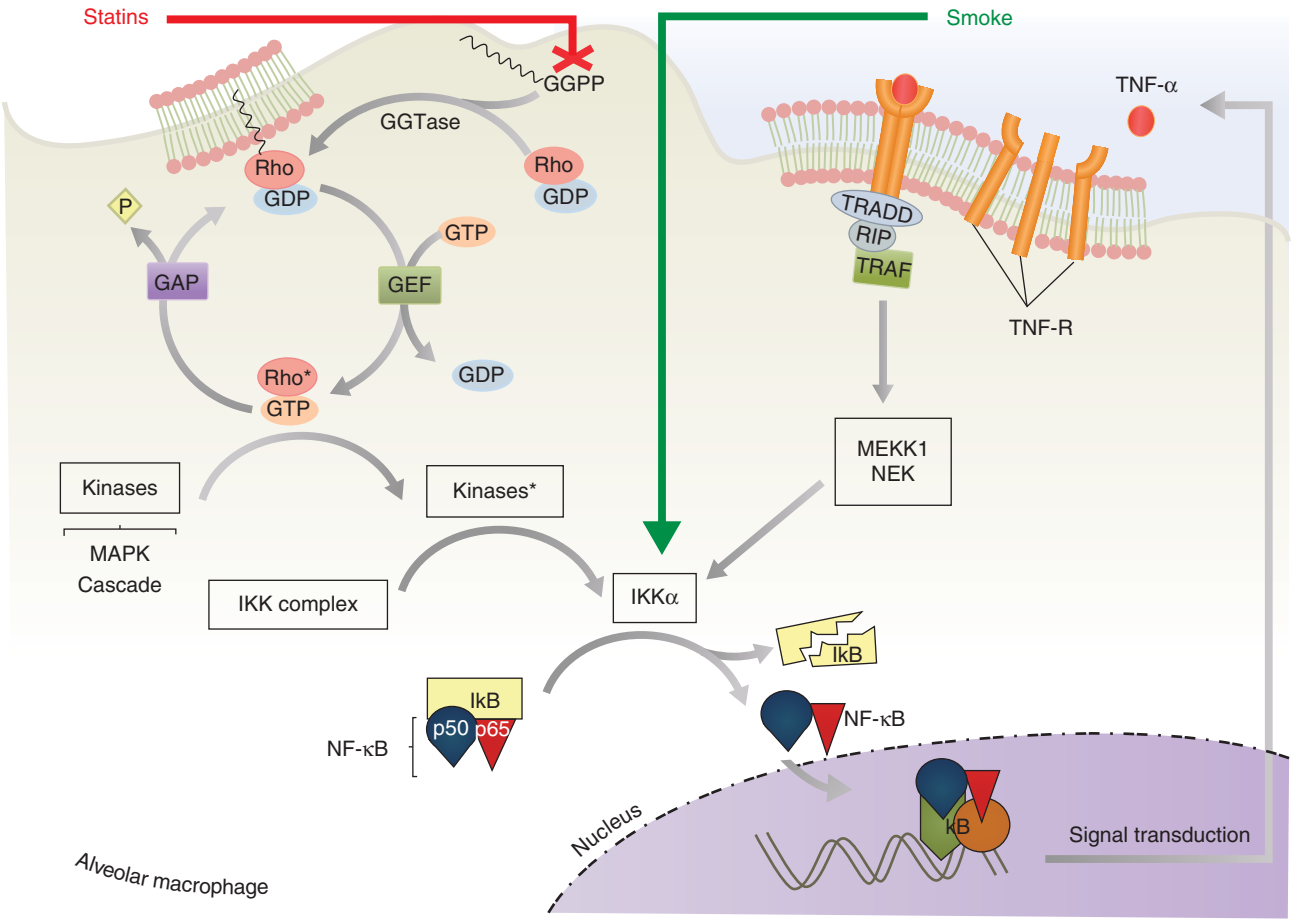

Figure 1. Rho is isoprenylated in the presence of geranylgeranyl pyrophosphate by geranylgeranyl transferase. Rho-isoprenylated can be attached to the membrane where it is activated by guanine nucleotide exchange factors and binds GTP, while GTP Rho can be inactivated with the help of GTPase-activating proteins. Rho activates several kinases as MAPK that leads to the activation of IKK complex. The activation of IKK is crucial; the synthesis of lots of inflammation proteins via NF-κB pathway.

* represents the activated proteins in the pathway.
IKK: Inhibiting protein binding kB kinase.

2.3 Other chemokines and MMPs involved in COPD

Inflammatory chemokines are chemoattractants and play an essential role in the migration of effector immune cells towards sites of infection or tissue damage. Activated macrophages produce numerous chemokines (**Figure 3**) including IL-8, leukotriene-B4 (LTB4) and monocyte chemotactic protein-1 (MCP-1); these chemokines serve to recruit effector cells from the blood stream to the small airways. In particular, IL-8 and LTB4 are chemotactic agents for neutrophils, while MCP-1 increases the number of monocytes that migrate from the blood stream to the site of inflammation where they differentiate into macrophages (**Table 1**) [1]. Moreover, it has been demonstrated that macrophages normally have a low proliferation, but in smokers they proliferate faster than in non-smokers [13,24-25]. This mechanism may partly explain why there is such an increased number of macrophages in the sputum of smokers. In addition, MCP-1 is reported to be chemotactic also for the cytotoxic T-cell lymphocyte $CD8^+$ [26].

The MMPs are zinc-dependent endopeptidases whose dependence on metal ion cofactors and their ability to degrade extracellular matrix distinguishes them from other endopeptidases. MMPs have been shown to be main players in cell proliferation, apoptosis and, importantly, in chemokine and cytokine regulation among other things. Human macrophages are known to produce several MMPs including MMPI, MMPIII, MMPVII, MMPIX and MMPXII [27]; and in the lung the primary isoforms expressed include MMPXII and MMPIX (**Table 1**) [28,29]. Increased MMPs production destroys the alveolar wall attachments resulting in parenchyma destruction and loss of the alveolar integrity observed in COPD patients. This condition leads to reduced

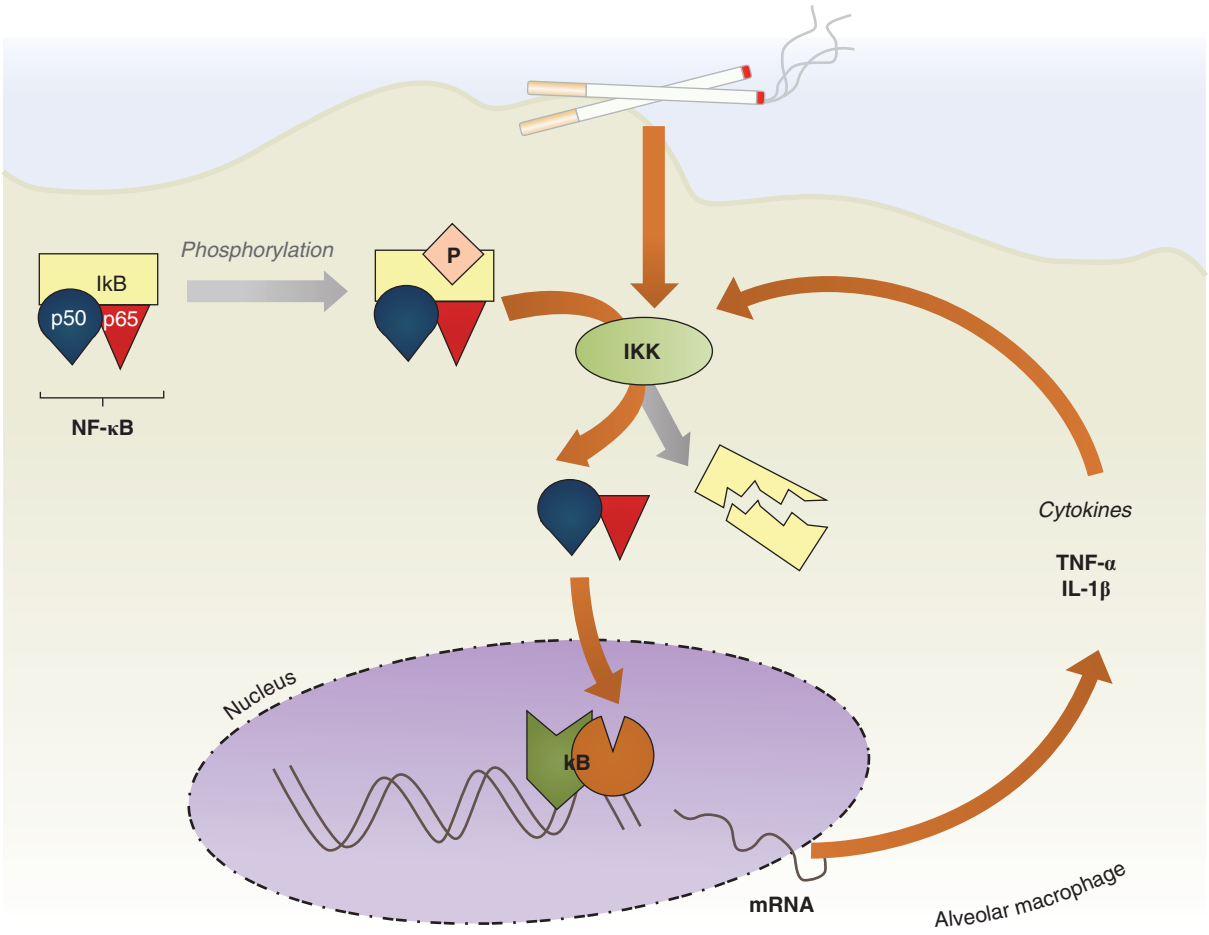

Figure 2. NF-κB pathway. NF-κB is bound by an inhibiting protein (IkB) which can be phosphorylated and subsequently degraded by a specific IKK. After IkB degradation, NF-κB can migrate into the nucleus and activate the transcription of inflammatory mediators including TNF-α and IL-1β. Many irritants such as cigarette smoke can activate IKK but also by cytokines themselves (TNF-α and IL-1β). An amplifying loop is created and alveolar macrophages are induced to proliferate and produce more and more inflammatory cytokines.

IkB: Inhibiting protein binding kB; IKK: IkB kinase.

recoil and collapsed small airway lumens leading to emphysema [4,30-31].

2.4 COPD and epithelial cells

Cigarette smoke also activates the epithelial cells of COPD patients. The epithelium increases production of TGF-b and EGF. TGF-β and EGF activate proliferation of fibroblasts causing airway fibrosis, while activation of the EGF receptor leads to mucin gene expression. Mucus hypersecretion is observed in COPD patients' central airways [4,14].

As a result, the bronchioles are obstructed by mucus, fibrosis and infiltrated with macrophages, neutrophils and T lymphocytes and destruction of lung parenchyma. There is probably a complex interaction between cells and mediators in COPD, resulting in progressive obstructive changes in the small airways and destruction of lung parenchyma.

2.5 Membrane adhesion molecules in COPD inflammation

COPD is characterized by an uncontrolled airway inflammation, where a large number of leukocytes migrate from the blood stream into the airways. Leukocyte migration is due to adhesion of leukocyte membrane to endothelial cells. Specifically, both the blood vessel endocyte walls and the leukocytes membrane express adhesion molecules [32].

The first step of this leukocyte migration consists of the tethering and 'rolling' of the leukocyte cell onto the endothelial cells. In this early phase, selectins play a crucial role as they are transmembrane proteins (with an N-terminal C-type lectin domain, a single EGF domain, a single transmembrane domain and a short cytoplasmic domain) [33] able to bind some glycoproteic ligands expressed on the cells membrane.

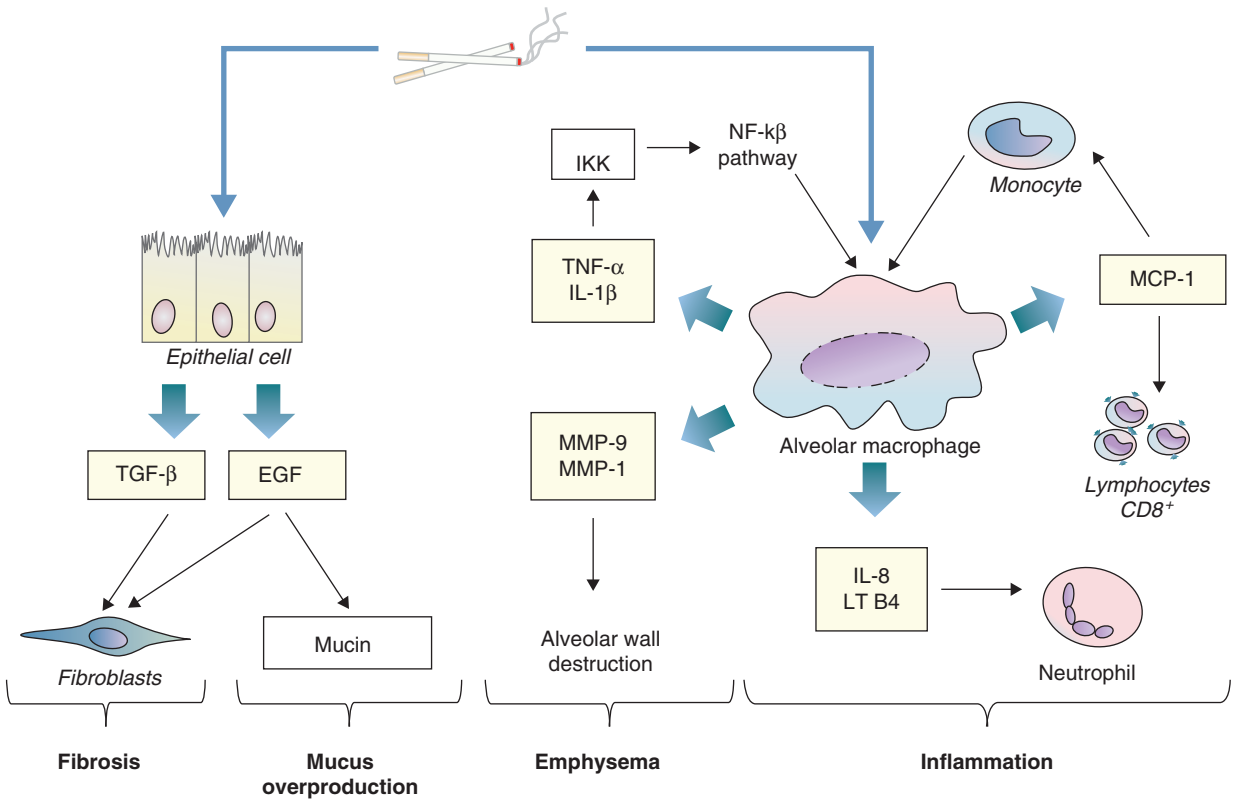

Figure 3. COPD patho-physiology in lung parenchyma. Cigarette smoke activates alveolar macrophages, which are induced to produce: IL-8 and leukotriene B4, which are chemotactic for neutrophils; monocyte chemotactic protein-1 as well as IL-1β and TNF-α, which activate NF-κB pathway; and MMPs including MMPI and MMPIX, which destroy the alveolar wall. Cigarette smoke activates epithelial cells to produce TGF-β and EGF responsible for the production of fibroblasts and mucin.
COPD: Chronic obstructive pulmonary disease.

Table 1. Mediators induced by TNF-α/NF-κB pathway and inhibited by statins.

Mediator class	Mediator	Mediator effect	
Cytokines	TNF-α IL-1β	Both activate macrophages activity creating a response amplification	Loop
Chemokines	IL-8 MCP-1	Neutrophil recruitment Lymphocyte $CD8^+$ recruitment	Inflammation
Selectin	E-selectin	Leukocyte adhesion to the endothelial membrane	
Integrine	ICAM-1	ICAM: create stronger binding and mediate the leukocyte extravasation	
MMPs	MMPIX MMPI	Destruction of the alveolar wall	Emphysema

ICAM-1: Intracellular cell adhesion molecule-1; MCP-1: Monocites chemotactic protein-1.

Three different selectins are involved in this process and their names are derived from the original source of identification. L-selectin (leukocytes) is expressed on the leukocyte membranes while endothelial cells can express P-selectin and E-selectin (platelet and endothelial) [33]. L-selectin is constitutively expressed on leukocyte membranes while P-selectin is stored in endothelial cells and can be mobilized to the surface after thrombin or histamine stimulation. E-selectin expression on the endothelial cells is induced by certain cytokines including TNF-α and IL-1 [32]. All of these selectins can create adhesion forces with glycoproteins, modified with sialyl Lewis x or a, such as CD34 and P-selectin glycoprotein ligand-1 which are also expressed on the endothelial cells' and leukocytes' membranes (**Table 1**) [32].

This weak leukocyte adhesion initializes the second step of interaction with endothelial cells, which results in a stronger adhesion via integrin receptors. Integrins are heterodimeric surface receptors of α and β subunits. Different integrins are constitutively expressed on leukocyte membranes and can interact with ligands expressed on the endothelial cell surface. Different ligands can be found on endothelial cells membrane including VCAM-1 (vascular cell adhesion molecule 1) whose expression can be induced by cytokines. ICAM-1 and ICAM-2 (intracellular cell adhesion molecules 1 and 2) are constitutively expressed on the endothelial surface but induced by TNF-α and IL-1.

Finally, platelet endothelial cell adhesion molecule 1 is expressed on both leukocytes and endothelial cells, localized at cell–cell borders suggesting an implication in leukocyte extravasations. TNF-α has a role in inducing some adhesion molecule ligands synthesis such as E-selectin, VCAM-1 and ICAM-1 (**Table 1**) [32].

3. Current and new treatments for COPD

Current COPD therapies are mainly focused on symptom alleviation, reduction in exacerbations and reduction in lung function decline in order to improve the quality of life. Approaches generally include the use of bronchodilators and corticosteroids, administrated directly to the lung via inhalation. Drugs that need to be effective in the lung are often administered by inhalation in order to have a rapid onset of action, a reduced dose and minimized side effects [34]. The major drawback with inhalation therapy is that many patients are unable to inhale the drug properly, mostly because of an incorrect technique of using a pulmonary device [34]. However, recent developments in formulation and device design have, to some extent, begun to resolve these drawbacks [34]. Many COPD treatments are based on a combined therapy regime containing both corticosteroids and β-agonist as primary medications.

The real effectiveness of these drug classes in reducing mortality is still a controversial matter. Two important studies (TORCH and UPLIFT) have shown that combined corticosteroids/long-acting β_2-agonist (LABA) and long-acting anticholinergic agents (LAACs) can reduce the rate of lung function decline, with the most profound effects seen in those with GOLD II disease [35,36]. However, another study by Calverley *et al.* showed that a combination of high-dose inhaled corticosteroid (ICS)–LABA compared to the placebo, did not present a significant improvement in reducing COPD mortality [37]. Data concerning effectiveness of current treatments in reducing COPD mortality are controversial and new therapeutic approaches are of increasing interest [38-40].

3.1 Bronchodilators

COPD patients' airways are partly obstructed by mucus hyper-production, destruction of alveolar wall and fibrosis. As airflow obstruction is partially reversible, bronchodilators can be effective for the maintenance treatment of COPD [41].

3.1.1 *Long-acting β_2-agonist*

The use of LABA therapy is successful in relieving bronchoconstriction in asthma and used in patients with COPD. Although this class of drugs acts by relaxing airway smooth muscle, they do not seem to have an anti-inflammatory effect. When comparing COPD to asthma, bronco-dilatation is not as important, as the disease is characterized not only by bronco-obstruction, but also by small airway fibrosis and emphysema [39].

Salmeterol is the most prescribed LABA in COPD patients and it has been demonstrated that, combined with fluticasone, can reduce inflammation [38].

In literature, there is evidence indicating LABAs are able to increase skeletal muscle mass and strength, improving muscle weakness in COPD patients [38,42]. Moreover, the increased FEV_1 (forced expiratory volume in the first second) in patients taking LABAs is combined with larger changes in lung volumes, leading to a reduction in perceived breathlessness [39]. Conversely, LABAs have side effects, mostly on the cardiovascular apparatus. Pro-arrhythmic effect is observed in those taking LABAs for COPD treatment. Even if this aspect does not seem to be relevant in mortality risk, it has been observed that LABA treatment in COPD may be associated with an increased mortality [43].

In summary, although LABAs are widely used in COPD treatment, this class of drugs are basically symptom relieving, with no effect on the cause of the disease.

3.1.2 *Long-acting anti-cholinergic drugs*

Anti-cholinergic drugs are effective bronchodilators as they are competitive inhibitors of muscarinic cholinergic receptors. They can, therefore, inhibit parasympathetic driven bronchoconstriction and bronchial hypersecretion [41].

Recently, tiotropium bromide, the first LAAC, was introduced for once-daily maintenance treatment of COPD patients [44]. Recent studies demonstrate that tiotropium is able to reduce COPD exacerbations and related hospitalizations, improving quality of life and symptoms, and slowing FEV_1 decline [44]. LAACs used for COPD treatment appear to be more effective than LABAs, contrary to what has been observed in asthmatic patients [41,44].

3.2 Inhaled corticosteroids

ICS are also used alone or in combination with β-agonists to inhibit airway inflammation and potentiate bronchodilatory effects of LABAs. In COPD, the inflammation is strongly neutrophil driven rather than via the T_H2 inflammatory response of asthma, where activated lymphocytes are thought to play a central role [40]. Though it has been demonstrated that ICS/LABA can reduce exacerbations and improve the quality of life, it seems that the effect is mostly due to the LABA contribution rather than ICS [35,38,37]. Moreover, Sin *et al.* have demonstrated that there is no improvement in inflammation markers such as IL-6 and C-reactive protein in those taking ICS [45]. Also, ICSs, such as fluticasone in

combination with salmeterol, have been demonstrated to do little for neutrophilic inflammation [37,46]. This may be due to an active resistance mechanism linked to a reduction in HDAC-2 expression [38].

Still, ICS is used as treatment in COPD and consequently, it not surprising that COPD is characterized by a progressive decline in lung function and premature death despite these treatments.

3.3 Antibiotics

Mucus overproduction is an important COPD characteristic. Furthermore, epithelial cell damage results in a reduction in the efficiency of the cilia clearance mechanism. This 'stagnant' viscous mucus becomes a perfect environment for localized bacterial growth and, therefore, it is unsurprising that a high percentage of COPD patients may encounter severe bacterial lung infection on multiple occasions during disease progression. Subsequently, antibiotic therapies may be routinely used to treat infection. Interestingly, acute exacerbations of COPD may be also due to viral infection and pollutants. In fact, many exacerbation cases are due to viral infections of the upper respiratory tract making antibiotic treatment, in some cases, un-warranted [47].

Bacteria, viruses and other pollutants are inhaled via inspiration into the respiratory tract where they can adhere to the mucus, produced in high amount. Bacteria have a strong affinity with mucus and this is the most likely explanation for a high incidence of infection in COPD patients [47]. Currently, COPD patients affected by lung infections are treated with oral antibiotics for mild to moderate exacerbation. For instance, erythromycin has been associated with a decrease in exacerbations in COPD [48]. The treatments consist mainly of trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, macrolides as clarithromycin or azithromycin and fluoroquinolones as levofloxacin. In moderate to severe exacerbations, treatment consists of intravenous administration of cephalosporins as ceftriaxone, antipseudomonal penicillin, levoxacin or an aminoglycoside [49].

In recent years *Haemophilus influenzae* and *Streptococcus pneumoniae*, two of the most known bacteria causing lung infections, have shown a dramatic rise in resistance against some antibiotics [47]. Also, it has been shown that lung infections treated with antibiotics given orally resulted in poor outcomes for patients. [50]. Recently, new antibiotics have been used. These include quinolones (such as moxifloxacin and gatifloxacin), which inhibit nucleic acid synthesis and other new antibiotic classes that inhibit protein synthesis such as ketolides (telithromycin), oxazolidinones (linezolid or eperezolid) and streptogramins (quinupristin or dalfopristin) [47].

Interestingly, the majority of antibiotics are given orally or intravenously. Such a systemic route may not be an optimum approach to treat bacterial infection that is at the epithelia initially. Subsequently, inhaled antibiotics have attracted some attention in recent times as local delivery may enhance bactericidal affect, reduce dosing and minimize drug resistance. The most important example of inhaled antibiotic approaches is in the treatment of cystic fibrosis (CF) [51]. CF is currently treated in hospitalized patients with tobramicin-based aerosol (TOBI®) [52]. Moreover, ciprofloxacin has also developed as a dry powder for inhalation (DPI) for the CF treatment [51] and some new studies are focused on developing new DPI products based on antibiotics [52-54].

3.4 Selective PDE4 inhibitors

As previously described, there are a large number of chemokines and cytokines involved in mediating inflammation in COPD and these, therefore, constitute potential therapeutic targets. The main objective is to find drugs able to inhibit chemokine/cytokine pathways and chemokine/cytokine synthesis in order to have a disease-specific treatment more effective than a symptomatic treatment (as the case of LABAs and ICS which improve the quality of life but do not treat the pathology) [7].

PDE4 are under investigation as potential therapies for inflammatory airway diseases. PDE4 is expressed in inflammatory cells involved in the patho-physiology of COPD. One of the major anti-inflammatory effects of PDE4 inhibitors is their ability in increase cAMP levels. PDE4 degrades cAMP which is a secondary messenger involved in pro-inflammatory mediators. cAMP activates protein kinase A that is able to phosphorylate proteins, inhibiting many inflammatory cells. As a result, an increased cAMP level leads to an anti-inflammatory effect via reduced PDE4 cAMP degradation [55]. Cilomilast and roflumilast are the most advanced selective PDE4 inhibitors and these have been studied as modulators of inflammatory cells implicated in COPD pathogenesis [55].

3.4.1 Theophilline

Although theophilline has been used for > 70 years for the treatment of airway disease, its mechanism of action and its effectiveness in COPD is still not exactly known. Theophilline is a weak non-selective inhibitor of PDEs and it has also been observed that therapeutic theophylline concentrations can inhibit only 5 – 10% of the total PDE activity in human lung extracts [56]. It is likely that the effectiveness of theophilline is related to the increased activity of the HDAC [57,56].

3.5 ACE inhibitors and angiotensin II type 1 receptor blockers

The inhibition of renin–angiotensin system produces an anti-inflammatory action in COPD patients [58]. Angiotensin II (Ang-II) is not only a potent vasoconstrictor, but it also induces pro-inflammatory genes and other pro-inflammatory substances [58]. This explains why modulators of the rennin–Ang-II system, such as ACE inhibitors and angiotensin II type 1 receptor blockers, have a positive outcome in COPD treatment. Some studies showed that inhibition of Ang-II could not only lower incidence of cardiovascular co-morbidity, but also reduce hospitalization and mortality due to COPD [59,60]. The increasing interest in this innovative

treatment for COPD is evident in the literature. A retrospective cohort study by Mortensen *et al.* evaluated the influence of both statins and ACE inhibitors in COPD patients' mortality within 90 days. In this study, the authors observed that 12.4% of the patients hospitalized with COPD exacerbation died; among the patients observed, 20.3% were taking statins, while 32% were treated with ACE inhibitors. The authors found that both statins and ACE inhibitors had significantly decreased mortality after 90 days [61].

3.6 HMG-CoA reductase inhibitors

HMG-CoA reductase inhibitors, most commonly known as statins, are commonly used as cholesterol-lowering drugs and are able to decrease mortality from cardiovascular disease [62]. They act by inhibiting HMG-CoA reductase which is the key enzyme converting HMG-CoA into mevalonic acid (MA) [62]. It is largely known that MA is a precursor of cholesterol, as it is converted to farnesyl pyrophosphate (FPP). Two molecules of FPP are converted into presqualene-diphosphate, which is then converted to squalene by a two-stage enzymatic reaction catalyzed by squalene synthase. This pathway leads to the production of cholesterol [63]. Statins are potent inhibitors of HMG-CoA reductase and for this property they are currently widely used as cholesterol-lowering agents.

Recently, statins have received increasing interest for their anti-inflammatory effect. In fact, by inhibiting the MA pathway they also inhibit the production of some isoprenoid intermediates such as FPP and geranylgeranyl pyrophosphate (GGPP), which have a pivotal role not only in cholesterol synthesis, but also in the post-translational modification of many proteins, in particular the G proteins. Both γ subunit of heterotrimeric G proteins and small GTP-binding proteins require prenilation to translocate from the cytoplasm to the plasma membrane. Statins can also inhibit activation of small GTP-binding proteins including Ras and Rho leading to a reduced production of inflammation mediators [64].

Statins are emerging as effective therapies in COPD management. Recent studies, conducted in both animals and on cell lines, confirmed that statins could decrease the production of inflammatory mediators, such as MMPIX, and reduce parenchyma destruction (**Table 2**) [65-68]. This inhibition of such mediators makes stains an enticing prospect as a potential treatment for the patho-physiology of COPD.

4. Statins and COPD

Statins have been demonstrated to reduce some inflammatory mediators. Moreover, some studies on COPD patients have already been performed. For example, Rezaie-Majd *et al.* have demonstrated that hypercholesterolemic patients, treated for 6 weeks with SV, showed a reduction in systemic concentrations of cytokines including IL-8, IL-6 and MCP-1 [10]. Moreover, Blamoun *et al.* observed the number of exacerbations and intubations occurring in a 185 COPD patient group reported promising findings [11]. Specifically, patients treated with statins (90 patients were taking one of the following statins: atorvastatin, pravastatin, fluvastatin, SV, lovastatin) showed a maximum of two exacerbations and no requirements for intubation compared to the control group (no statin medication), which reported six exacerbation episodes and > 50% of the patients requiring one or more intubations [11]. As previously reported, Mortensen *et al.* found in a retrospective study a relationship between statins treatment and reduction in COPD mortality [61]. Another cohort study included 803 elderly men whose FEV_1 and forced volume vital capacity were measured between 1995 and 2005. Those who did not use statins had an estimated decline in FEV1 of 23.9 ml/year, while patients who took statins had an estimated 10.9 ml/year; moreover, the study considered four different smokers groups (non-smokers, long-time quitters, recent quitters and smokers) and in all the categories the authors found that statins had beneficial effects [12].

4.1 Statin mechanism of action

Statins are cholesterol-lowering drugs because they strongly inhibit HMG-CoA reductase; the enzyme that converts HMG-CoA into MA initiates the cholesterol synthesis pathway [69]. Statins, and among them SV, are able to bind HMG-CoA reductase and change the enzyme conformation. As a result, the enzyme is no longer active and HMG-CoA cannot bind the reductase active site. Moreover, SV and other statins can inhibit the reductase in nanomolar concentrations, while the biological substrate binds in micromolar concentrations. SV is, therefore, a potent inhibitor of HMG-CoA reductase [70].

The mechanism by which statins could be used for the treatment of COPD patients seems to be the same as that observed for cholesterol lowering. In fact, MA is involved not only in the cholesterol synthesis pathway, but also in the synthesis of isoprenoid intermediates such as FPP and GGPP (**Figure 4**). Both FPP and GGPP are important lipid attachments for the post-translational modification of a variety of proteins, including Ras and Rho GTP-binding proteins.

Inactivation of Rho Rac has shown to reduce the NF-κB migration into the nucleus and accordingly DNA-binding and gene transcription [19]. GGPP and FPP are hydrophobic molecules, having prenyl groups (3-methyl-2-buten-1-yl). These molecules can be attached to proteins by two enzymes called farnesyl transferase and geranylgeranyl transferase. Protein isoprenylation can facilitate protein attachment to cell membranes [71].

Rho proteins are GTP-binding proteins belonging to the Ras superfamily including members of the Cdc42, Rac and Rho subfamilies; they are able to switch on and off many cellular processes. These proteins need to be prenylated in order to migrate close to the cytoplasmic membrane where they can exert their function. Rho proteins can assume two conformations, an inactive GDP-bound state and an active GTP-bound state [72].

Table 2. Summary of some studies on murine/human cells demonstrating statins activity in COPD treatment.

Ref.	Parameter analyzed	Stimulation	Response to stimulation	Treatment	Response to treatment
Lee *et al.* [65]	35 Sprague-Dawley rats MMPIX level	Exposure to the smoke of 10 commercial cigarettes per day for 16 weeks	All rats survived and MMPIX levels increased	SV 5 mg/kg orally once a day per 16 days	SV can inhibit MMP production
	Average interalveolar septal wall distance MLI and alveolar S/V	As above	Enlargement of airspaces MLI was 174% S/V in the smoke exposed group was 50% of the control group	As above	In the SV treated group MLI and S/V values were comparable to the control group
Kim *et al.* [66]	Rat's AMs MMPIX	AM exposed to 25% of CSE	Significant increase in the expression of MMPIX mRNA was observed 24 and 48 h after CSE treatment, with maximum induction at 48 h	SV 10 μM	Inhibition of both MMPIX and mRNA MMPIX. Dose-dependent with a maximum at 10 μM
Ou *et al.* [67]	Male Sprague–Dawley rats Wall airway thickness measured as airway wall area/basement membrane perimeter squared (Pbm2) (¥600)	Rats were placed inside a ventilated smoking chamber (70 × 50 × 50 cm) and exposed to the smoke produced by 15 cigarettes for 16 weeks	Chronic cigarette smoke exposure caused distinct small airway wall thickening	SV = 20 mg/kg 0.5, 5 and 20 mg/kg, once a day for 16 weeks	Compared with the normal small airway structure in control rats, SV attenuates small airway thickening
Kamio *et al.* [68]	Human fetal lung fibroblast strain (HFL-1) MMPs	Stimulation by cytokines (TNF-α 5 ng/ml and IL-1β2 ng/ml)	After cytokine stimulation, MMP levels were increased	Atorvastatin (2.5 μM), fluvastatin (1 μM) or SV (0.5 μM)	All of the statins reduce MMP levels

AM: Alveolar macrophage; COPD: Chronic obstructive pulmonary disease; CSE: Cigarette smoke extract; MLI: Mean linear intercept; S/V: Surface:volume ratio; SV: Simvastatin.

Guanine nucleotide exchange factors catalyze the activation of the GTPases by exchanging GDP for GTP [73], while inactivation is due to an intrinsic GTPase activity catalyzed by GTPase-activating proteins [71] (**Figure 1**).

Perona *et al.* demonstrated that the human RhoA, CDC42 and Rac-1 proteins induce the transcriptional activity of NF-κB by phosphorylation of IKKα and consequent degradation of IKB; as a consequence, NF-κB translocates into the nucleus [74]. The relationship between Rho activation and NF-κB activation explains the mechanism by which statins can have a role in COPD treatment. In fact, statins can inhibit Rho activation by inhibiting the MA pathway with a consequent reduction of isoprenoid synthesis.

4.2 Statin transport

Many statins are transported into the hepatocyte via the organic anion-transporting polypeptide (OATP), in particular OATP1B1, which is expressed exclusively in the basolateral membrane of the hepatocyte. However, this mechanism is true for hydrophilic statins but not for lipophilic statins. SV does not appear to be transported into the hepatocyte via OATP while its active hydroxy acid metabolite is a possible substrate [75]. Lipophilic statins, including SV, with a high octanol:water partition coefficient, are most likely to enter the cell by passive diffusion across the membrane [76-78] (**Figure 5A**).

4.3 Metabolism of SV

SV is a pro-drug and requires activation for its inhibiting function on HGM-CoA-reductase. Once in the hepatocyte, SV is activated to its active form by microsomial carboxylesterase (mCES) that is highly expressed in the liver and intestinal wall [79]. This enzyme belongs to the serine-hydrolyze-class, defined as a functional related class of hydrolytic enzymes containing a serine. In mammals, there are four major carboxylesterase (CES) isozymes, CES-1 – CES-4 but human carboxylesterases (hCES) are mainly hCE-1 (CES1A1) and hCE-2 (CES2) [80].

The CES enzymes are able to hydrolyze many substrates as carboxyl ester, amide and thioester bonds to a variety of drugs. For these reasons, CES enzymes are able to activate

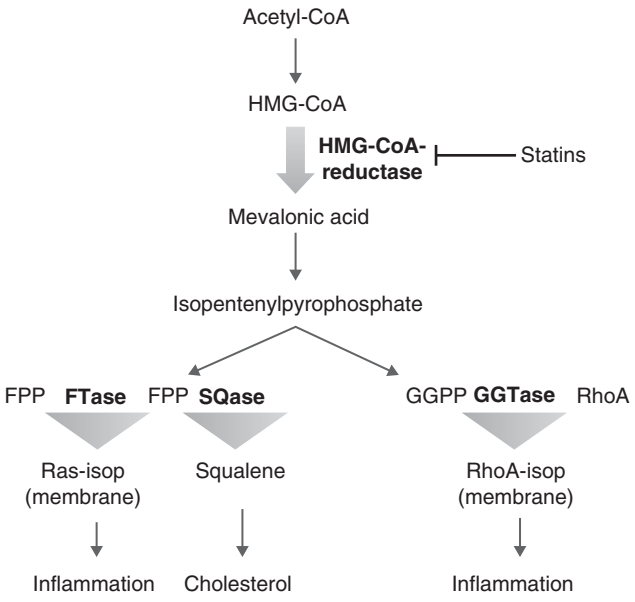

Figure 4. Statin mechanism of action. Statins inhibit HMG-CoA-reductase, the enzyme that can convert HMG-CoA into mevalonic acid. The mevalonic acid pathway leads to cholesterol production but also to isoprenoids farnesyl pyrophosphate and geranylgeranyl pyrophosphate. The enzymes geranylgeranyl transferase and farnesyl transferase isoprenylate two small G proteins (Rho and Ras) and this allows them to be activated by the attachment to the membrane.

pro-drugs and this is exactly the mechanism by which lipophilic statins such as SV are hydrolyzed into their active metabolite (**Figure 6**).

Statins, and among them SV, are both substrates and inducers of the cytochrome P450 (CYP) isoenzymes (**Figure 5A**). These enzymes are expressed in several tissues, but liver and small intestine are the sites of major interest regarding drugs and other xenobiotics [81]. Specifically, CYP3A4 is the enzyme responsible for SV metabolism [78,82-83]. CYP3A4 metabolizes SV by oxidization of the lipophilic ring. The metabolite products are 6′β-hydroxy-SV; 3′-5′-dihydrodiol-SV; 6′-exomethilene-SV and 3′-hydroxy-SV (simvastatin metabolites) [83]. The four known metabolites display activities ranging from 20 to 90% of SV-hydroxy acid (SVA the most active metabolite), but are relatively hydrophilic and a large majority are eliminated with bile [77,84].

Prueksaritanont *et al.* demonstrated in a study that CYP3A4 is the major isoenzyme (≥ 80%) responsible not only for SV but also for SVA metabolism. In fact, both the pro-drug and the hydroxy acid are converted by this CYP isoform into four hydrophilic metabolites (SMs) that are still active (**Figure 5A**) [82]. In the lung, the most widespread CYP isoform is CYP3A5 rather than CYP3A4, but it has been demonstrated that both SV and SVA can be metabolized, albeit to a lower extent, also by CYP3A5 and CYP2C8 [82].

In 2002, Prueksaritanont *et al.* described the SV metabolic pathway. SV is activated by CES into SVA. This hydroxy acid is glucuronidated by UDP-glucuronosyl transferase in presence of UDP-glucuronic acid [85]. Metabolized and glu-conjugated-statins are then excreted into bile. The amount of non-metabolized SV can reach the systemic circulation, but because of its high lipophilicity, it is strongly bound to plasma proteins: 95 – 98% of SV is plasma-protein bound [86]. Lipophilic statins largely undergo first pass metabolism and are strongly bound to plasma proteins, and as a result SV bioavailability is very low with < 5% of the active principle available to the site of action [86].

4.4 Side effects of statins and safety

Research trial evidence and clinical practice experience have demonstrated that statins are generally well tolerated [87]. However, it is still important to note that some side effects have been observed, mostly in the liver, kidney and muscle tissue. The most common hepatic side effect is asymptomatic elevation in aminotransferases. Some clinical trials have shown that statins use can increase amminotransferase levels up to three times the normal level [88,89]. However, the incidence of this hepatic side effect is < 3% and it is also dose-dependent [88,89].

Myotoxicity is the most known adverse effect caused by statin administration. Toxicity manifests as myalgia or muscle weakness with an increased level of creatine kinase (CK) up to 10 times the normal upper limit. The worst, rare myotoxicity is represented by rhabdomyolysis (CK greater than 10 times the normal upper limit), which is a severe destruction of muscle fibers with a consequent release of myoglobin into the bloodstream [90,91].

There are different hypothesis explaining the mechanism by which patients taking statins display myopathy: one

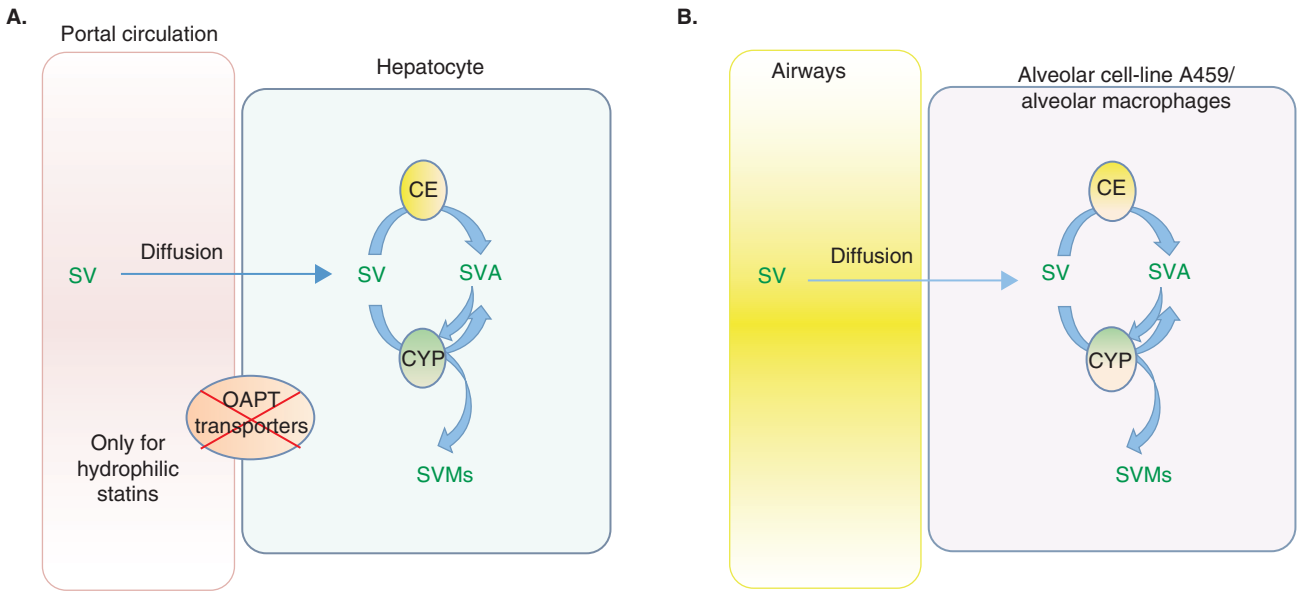

Figure 5. SV transport and metabolism in hepatocyte A. SV does not need an active transporter (OATP), but it can diffuse into the hepatocyte because of its lipophilicity. Once in the cell, SV is activated into SVA by a nonspecific CES; both SV and SVA are metabolized by CYP, in particular CYP3A4, into four more hydrophilic metabolites (SMs). **B.** The authors speculate a possible SV behavior in the lung: SV can diffuse into lung cell because it does not need a transporter. CES is a ubiquitous enzyme also largely present in the lung. In the lung, there is a CYP isoform (CYP3A5) which is also able to metabolize SV but in a really low percentage.

CES: Carboxylesterase; OATP: Organic anion transporting polypeptide; SV: Simvastatin; SVA: Simvastatin hydroxy acid.

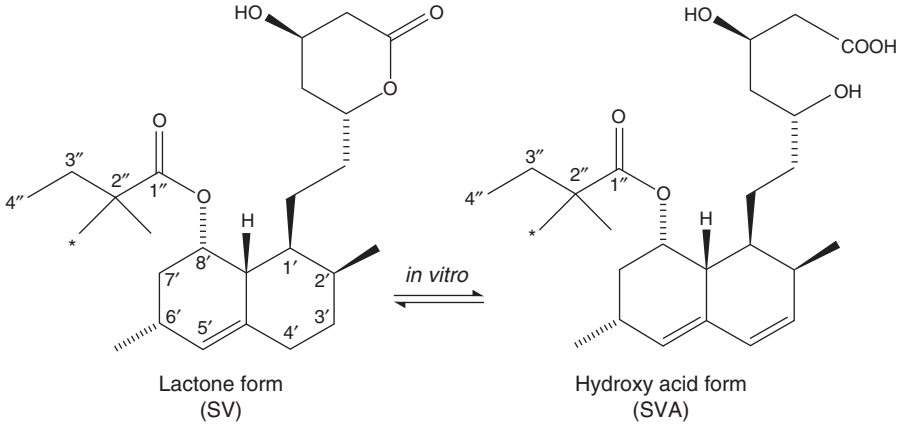

Figure 6. Chemical structures of simvastatin (lactone form) and simvastatin activated (hydroxy acid form).

explanation is due to the inhibition of MA pathway. Statin inhibits MA synthesis, which is an intermediate for the coenzyme Q_{10} (CoQ_{10}) synthesis. CoQ_{10} is a steroid isoprenoid that plays an important role in the cellular energy transduction in the mitochondrial electron transport system [90,92].

In some cases, statins can cause proteinuria since the inhibitory effect of statins reduces the prenylation and the consequent inhibition of protein reabsorption in renal proximal tubules [93]. Proteinuria is characterized by a loss of proteins in the urine; moreover, a low protein level causes a drop of the osmotic pressure and a consequent edema [94]. All these statin-related diseases are dose-dependent and normally marketed dosages are quite safe for the patient.

4.5 Speculation on statins pulmonary administration

Historically, statins have always been considered mostly as oral therapies. Now, the new drugs classes for the treatment of COPD, such as ACE inhibitors, PDE4 inhibitors and statins,

are all showing promise as future treatments for lung pathologies, in particular for COPD. For these reasons, alternative delivery routes are starting to be investigated. Specifically, two very recent papers have presented PDE4 inhibitors as a possible new therapy delivered by inhalation for obstructive lung pathologies [95,96].

Based on recent literature, the authors would like to speculate that statins also, which seem to be effective in COPD treatment, could be administered directly into the lung.

If this were the case, then it would be appropriate to make some considerations concerning statins transport and activation in the lung cells.

4.6 Statin transport and activation in the lung

4.6.1 Transport

It is known that in the liver, the OATP1B1 is responsible for statin active transport. This polypeptide is exclusively expressed in the basolateral membrane of the hepatocytes. It is also known that SV is a lipophilic drug and able to diffuse into the hepatocytes. Because SV is able to diffuse across membranes due to its lipophilicity, with no need of uptake specific transporter [77,75], it is proposed that, if delivered directly to the lung, SV would be able to penetrate into both pulmonary epithelial cells and macrophages readily by diffusion [76].

4.6.2 Activation

The alveolar surface contains numerous serine proteases. Specifically, Munger *et al.* have observed the presence of CES in broncoalveolar lavage fluid. This enzyme is a 60-kDa serine hydrolase. Partial amino acid and complete DNA sequencing have shown it to be identical to human liver carboxylesterase (mCES) [97]. In particular, Hosokawa demonstrated that CES1A1 is the major isozyme of the CES1A subfamily expressed in liver and in the lung [98,99]. Subsequently, it could be assumed that lipophilic statins, such as SV, could be activated in the lung by alveolar macrophages and epithelial cells (**Figure 5B**).

Also, CYP3A has been shown to be involved in the metabolism of statins. Specifically, Anttila *et al.* reported the presence of CYP3A5 in the ciliated and globet cells of the bronchial wall, bronchial glands, bronchiolar columnar and terminal cuboidal epithelium, type I and type II alveolar epithelium, vascular and capillary endothelium as well as alveolar macrophages. CYP3A4 has been found in bronchial glands, bronchiolar columnar and terminal epithelium, type II alveolar epithelium (A549) and alveolar macrophages [100]. Interestingly, recent studies have reported CYP3A4 to have lower expression in the lungs of smoker patients, suggesting a depressive function of smoke on CYP3A4 production [101].

5. Conclusions

Despite the mounting evidence for statins as a potential therapeutics for the treatment of COPD, the clinical relevance and efficacy of this class of drugs still remains focused on its effects on cholesterol lowering. In this review, we highlight that this drug class could be a potential new therapeutic avenue for the treatment for COPD.

Although current treatments for COPD have shown positive effects, such as decreasing the exacerbations, improving the quality of life and relieving symptoms, their efficacy in reducing mortality has not been proven as yet [38-40]. On the other hand, some retrospective studies has shown that some drug classes, already on the market for the treatment of other diseases, such as ACE inhibitors, PDE4 inhibitors and statins, seem to be effective in reducing COPD mortality [7-9].

Furthermore, recent studies have also proposed PDE4 inhibitors as potential inhalable therapeutics for COPD [95,96].

From the literature and data presented in this review, the use of statins for inhalation have significant potential for treatment of COPD; however, more studies are required in order to demonstrate the real effectiveness and the mechanism of action of this class of drugs.

6. Expert opinion

COPD is a very severe disease caused principally by cigarette smoke, together with pollutants and subjective predisposition. This pathology has a very high mortality worldwide and it is expected to become the third cause of death in developing countries in the next few years. Symptoms are debilitating and lung damage is irreversible, particularly at the alveolar wall.

For these reasons, many research groups are interested in studying and developing new treatments able to minimize COPD exacerbations and associated deaths. The role of statins, among them SV, has largely been demonstrated to be crucial in reducing COPD mortality [8,9].

The focus of this review has been to identify the specific biochemical pathways involved in the disease patho-physiology and understand how SV can be effective in the treatment of COPD. Studies performed up to now, concerning SV as a potential COPD treatment, have been focused on SV administered as an oral treatment in a solid dosage form. This is basically due to the fact that SV has been marketed for many years as a treatment of hypercholesterolemia.

In this review, the authors focus on the potential use of statins, specifically SV, for pulmonary administration in the treatment of COPD. Delivery of SV by the inhalation route could have many advantages including the bypassing the extensive first pass metabolism attributed to a low SV bioavailability. Evidences presented in this review and based on recent literature show that there are similarities between SV transport and metabolism administered orally and that observed by pulmonary administration.

What emerges in the present review is that, due to the high lipophilicity, the mechanism by which SV penetrates cells (specifically, in hepatocytes) is diffusion, with no transporter

involvement. Once in the hepatocyte, SV being a pro-drug, is activated by a nonspecific CES, which is ubiquitous, into its active metabolite (SVA).

Following the same basic assumption, SV delivered to the lung should be able to penetrate the epithelial cells and macrophages (strongly involved in COPD development) by diffusion. Furthermore, CES is largely expressed in the lung [97], in particular, in the alveolar epithelium and in macrophages.

Future studies in this field should be directed towards the mechanism by which SV penetrates the lung, performing experiments such as transport studies into cells and activity studies. Furthermore, since it has emerged from the literature that there is a link between the statin pleiotropic effect and COPD mortality reduction, it would also be interesting to perform studies concerning statin pulmonary administration in order to obtain a possible inhalation product useful for the treatment of such an important pulmonary disease.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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