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REVIEW ARTICLE

Effects of inhaled therapy on biomarkers of systemic inflammation in stable chronic obstructive pulmonary disease

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Abstract
In chronic obstructive pulmonary disease (COPD) airways inflammation is associated in more advanced stages with systemic inflammation. COPD-associated systemic inflammation syndrome is defined currently with rather non-specific biomarkers such as C-reactive protein (CRP) but there are also other 'organ-specific' biomarkers such as surfactant protein-D which are still not well characterized but might represent more appropriate and reliable alternatives to the non-specific biomarkers. Inhaled therapies are the mainstay in stable COPD and they were demonstrated to reduce airway inflammation and more recently in the case of inhaled corticosteroids alone or combined with long-acting beta-2 agonists to reduce systemic inflammation as well. This paper focuses on current and potential biomarkers of systemic inflammation in COPD and on the systemic anti-inflammatory effects of inhaled therapies in stable COPD.

Keywords: *COPD-biomarkers-systemic inflammation-inhaled therapies*

Introduction

Chronic obstructive pulmonary disease (COPD) is progressive inflammatory disease having smoking as the most common risk factor but exposure to other noxious agents is also recognized as a potential risk factor in COPD.

In COPD inflammation can be found in the airways as well as in the lung parenchyma and the inflammatory cell pattern is characterized by neutrophils, macrophages and T lymphocytes (especially CD8) (The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008). These cells elaborate a large range of cytokines such as interleukin (IL)-6, IL-8 or tumour necrosis factor (TNF)-α which maintain and augment the inflammation locally (Keatings et al. 1996).

The airway inflammation leads to lung function impairment due to obstruction which unlike asthma cannot be fully reversed by bronchodilators and inhaled corticosteroids. Clinically it manifests as respiratory symptoms such as dyspnoea and productive cough which are aggravated as the disease progresses and during exacerbations. The airway obstruction parallels the inflammation in terms of progression and is followed by abnormalities in gas exchange with subsequent respiratory failure and cor pulmonale (O'Donnell et al. 2004).

In COPD not only bronchial and parenchymal inflammation have been reported but also systemic inflammation. For example in the NHANES-3 study low-grade systemic inflammation was found in subjects with moderate to severe airflow obstruction and high levels of C-reactive protein (CRP) were identified as a risk factor for cardiac impairment (Sin et al. 2000).

Furthermore in a systematic review on the systemic inflammation in patients with stable COPD, it was found that markers of systemic inflammation such as CRP, fibrinogen, IL-6, IL-8 and TNF-α were associated at higher levels with more impaired lung function (Gan et al. 2004).

COPD therapy in stable state includes pharmacological treatments which are mainly represented by

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inhaled bronchodilators and inhaled corticosteroids, and non-pharmacological methods such as by smoking cessation which slows lung function decline, and long-term oxygen therapy which prolongs survival (Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008, Antoniu et al. 2007).

Inhaled therapies have been shown to reduce airway inflammation and some, such as inhaled corticosteroids alone or associated with β2-agonists, were found to reduce the levels of some markers of systemic inflammation in stable COPD.

This paper reviews the data on the biomarkers of systemic inflammation in stable COPD and discusses the anti-inflammatory effects of inhaled therapies in this setting.

Biomarkers of systemic inflammation evaluated in COPD

Classically, systemic inflammation syndrome associated with COPD has been defined with biomarkers such as CRP or fibrinogen which are non-specific acute-phase mediators encountered in any type of inflammation whether acute or chronic. Subsequently proinflammatory cytokines directly stimulating the production of these inflammatory proteins such as IL-6 or TNF-α were advocated as potential biomarkers but their non-specificity limits their routine use for characterizing the systemic inflammation found in COPD.

More recently however, the need of having newer more specific biomarkers triggered the search for other molecules and currently surfactant protein-D or Clara cell secretory protein 16 are evaluated in both stable and exacerbated COPD.

These molecules are briefly presented below and their biomarkers properties are discussed as applied to stable COPD.

C-reactive protein

CRP was the first acute-phase protein described and was named based on its ability to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae* (Pepys & Baltz 1983). It is currently considered one of the most sensitive markers of systemic inflammation and tissue damage. It is mostly synthesized in hepatocytes and IL-6 is currently recognized as the triggering stimulus (Pepys & Hirschfield 2003). The plasma half-life of CRP is approximately 19 h and its level denotes how intense is the hepatic synthesis and indirectly how important and persistent is the inflammatory stimulus. Therefore CRP is a very sensitive marker of systemic inflammation and used as such in many chronic inflammatory and infectious diseases.

In COPD patients CRP levels were found to be significantly higher when compared with controls (both smokers and non-smokers) and were not associated with the presence of any clinically relevant form of ischaemic heart disease (Pinto-Plata et al. 2006).

CRP has been more extensively studied as a biomarker of atherosclerosis and other cardiovascular diseases but more recently it has also been evaluated as a biomarker of prognosis in stable COPD. A cohort of 1302 patients with asymptomatic airways obstruction ('at risk for COPD') in the Copenhagen City Heart Study had CRP levels measured at baseline and the subsequent COPD hospitalizations and deaths were recorded over the follow-up period. CRP levels at baseline were found to be significantly increased in patients who subsequently died of or were hospitalized due to COPD. Baseline CRP levels >3 mg l^{-1} were associated with significantly higher risks of hospital admissions and COPD-related deaths (Dahl et al. 2007).

In addition to its predicting properties for COPD morbidity CRP was also identified as a marker of steeper lung function decline: CRP levels correlated with forced expiratory volume in 1 s (FEV1) % predicted annual decline rate (Higahsimoto et al. 2009). However, in patients with more advanced COPD the CRP level was not identified as a predictor of survival/mortality when compared with the BODE index or other outcome measures (De Torres et al. 2008).

In COPD CRP was found to be a sensitive measure of therapeutic outcome. In a randomized, study evaluating the effects of 6 months pravastatin therapy on exercise capacity in 125 patients with stable COPD, it was found that improvement in exercise capacity was correlated with a reduction of CRP levels independently of haemodynamic status or lipid profiles (Lee et al. 2008).

However despite high sensitivity CRP has the major disadvantage of low specificity. For example in the case of smoking-related COPD with comorbid cardiovascular diseases it is difficult to attribute increased CRP levels to systemic inflammation of the pulmonary disease or of the extrapulmonary diseases.

Fibrinogen

Fibrinogen is another acute-phase protein which is synthesized by the liver under the control of the same proinflammatory cytokine IL-6 and its basic function is that of clot generation. It is also a marker of systemic inflammation but in COPD its use as a biomarker of disease seems to be less reliable and limited by its low predictive value for COPD for similar reasons as with CRP. However in a general population cohort of 8955 Danish adults analysed in the Copenhagen Heart Study, higher levels of fibrinogen at baseline were associated with an impaired lung function and an increased risk of

COPD irrespective of the smoking status. Furthermore subjects with fibrinogen level >3.3 g l^{-1} had a significantly increased relative risk of COPD hospitalizations compared with subjects with fibrinogen levels <2.7 g l^{-1} (Dahl et al. 2001). Higher fibrinogen levels were also found in elderly patients with more accelerated lung function decline (Jiang et al. 2008).

Interleukin-6

IL-6 is a potent proinflammatory cytokine produced under the modulating control of IL-17 by T cells and macrophages, and plays a major role in inflammation either directly by activating both inflammatory and structural cells or by inducing increased synthesis of other acute-phase reactants such as CRP or fibrinogen. In COPD high serum levels of IL-6 have been detected in COPD patients with stable disease (Kim et al. 2008). IL-6 is also produced in the skeletal muscles and might be involved in inflammatory muscle wasting associated with COPD or in generation of pulmonary hypertension (Heinrich et al. 2003, Chaouat et al. 2009). Its potential for predicting the outcome in COPD has not been assessed extensively but in some studies IL-6 polymorphisms were found to be associated with more accelerated lung function decline and with increased risk of COPD development in smokers (He et al. 2009).

Given its sensitivity to inflammation irrespective of its aetiology, the IL-6 role as a biomarker in COPD has the same disadvantages and limitations as fibrinogen and CRP.

Surfactant protein D

Surfactant protein D (SP-D) is a glycoprotein which belongs to the collectin protein family and is synthesized mainly in type II pneumocytes in the lungs (Atochina et al. 2004). Apart from modulating surfactant homeostasis it plays an important role as a defence mechanism against infectious agents and allergens, its underexpression increasing the risk of pulmonary infections whereas over expression is a marker of chronic inflammation and has been reported in COPD, asthma or idiopathic pulmonary fibrosis (Cheng et al. 2000, Honda et al. 1995, Sin et al. 2007). Furthermore in one study it was found that increased serum SP-D levels were correlated with reduced lung function, and worsening health status and symptoms (dyspnoea) levels in subjects with more advanced COPD disease (Sin et al. 2007). In a more recent study performed on SP-D, levels were compared in COPD patients, and in smokers and non-smokers without airflow obstruction: SP-D levels were significantly higher in COPD patients when compared with non-smokers and were found to be a predictor of exacerbations. Furthermore, SP-D levels were found to be increased during COPD exacerbations and were subsequently reduced by prednisolone therapy (Lomas et al. 2009). The fact that SP-D originates at the pulmonary level, and therefore reflects the amplitude of this local inflammation, can be an asset which needs to be exploited in order to qualify this parameter as a biomarker of COPD. SP-D could also be a biomarker of disease progression and this particular aspect should be further evaluated at different stages of COPD severity as well as in both smokers and non-smokers populations.

Clara cell secretory protein 16

Clara cell secretory protein 16 (CC-16) is a secretoglobin which exerts a protective role against oxidative stress in the respiratory tract. It is produced by non-ciliated Clara cells in respiratory bronchioles and by their equivalents in larger calibre airways as a result of epithelial damage (Braido et al. 2007). Higher levels of CC-16 were found in sputum in patients with COPD but the significance of high serum levels are still debated as data from studies with small samples of patients are conflicting. One study found a reduced level of serum CC-16 in patients with COPD or lung cancer whereas another found that increased levels acted as a marker of increased permeability of the epithelial barrier (Bernard et al. 1992, Pilette et al. 2001). CC-16 might be a very valuable biomarker of airways damage in COPD but its levels might be influenced by age and renal function (Hermans et al. 2003). More recently, in a larger cohort study CC-16 serum levels were found to correlate more strongly with disease severity in current smokers and much more weakly in former smokers, and were demonstrated to depend on age and gender but not on body mass index or the presence of either emphysema or chronic bronchitis (Lomas et al. 2008).

Tumour necrosis factor-α

TNF-α, also called cachectin, is a potent proinflammatory cytokine which induces tissue depletion (cachexia) playing a major pathogenic role in chronic inflammation encountered in debilitating diseases such as rheumatoid arthritis, psoriasis or Crohn's disease (Kim et al. 2008, Mukhopadhyay et al. 2006). In lungs it is mainly produced by macrophages but mast cells, eosinophils, T cells and epithelial cells can also produce TNF-α (Mukhopadhyay et al. 2006). In COPD TNF-α plays also a major role in lung inflammation, its main action being represented by the maintenance of neutrophilic inflammation locally in the airways and lung parenchyma and systemically by 'inflammatory' weight loss (Di Francia et al. 1994). Higher serum TNF-α levels were found in patients with COPD and weight loss when compared with COPD patients

with stable weight or with age- and gender-matched healthy volunteers (De Godoy et al. 1996). TNF-α could be a biomarker of advanced COPD as its serum levels were found to be inversely correlated with paO2 and increased with dyspnoea severity (Takabatake et al. 2000, Garrod 2007).

Inhaled therapy in stable COPD: effects on systemic inflammation

In stable COPD the mainstay of the pharmacological therapy is represented by the inhalatory therapy which has two main purposes: bronchodilation and reduction of airways inflammation.

In stable COPD inhaled bronchodilators used include short- or long-acting β_2-agonists and anticholinergics. Short-acting formulations are currently recommended to be used on demand, i.e. when symptoms such dyspnoea increase transitorily in order to reduce it promptly, whereas long-acting bronchodilators are recommended to be used regularly, on daily basis, in moderate to very severe disease COPD stages.

Inhaled corticosteroids are the main anti-inflammatory therapy used in COPD. Despite their effectiveness they are not as significant as in asthma, and these agents are used as a maintenance therapy in more advanced stages of COPD usually combined with long-acting β_2-agonists.

Inhaled therapy is aimed at exerting its effects principally at the local (airways) level but surprisingly some of them such as some of the inhaled bronchodilators, even if they are not intended to be used as anti-inflammatory therapies might be demonstrated to possess such activities not only locally but even systemically. These effects on various biomarkers of inflammation are discussed below.

Long-acting anticholinergics

Tiotropium bromide is a potent long-acting anticholinergic which exerts its bronchodilator activity by blocking M3 muscarinic receptors in the airways. Inhaled tiotropium bromidum has been demonstrated to exert a sustained bronchodilating effect, reduce exacerbations rate and improve quality of life, its therapeutic effects being superior to those of its short-acting predecessor ipratropium bromide (Casaburi et al. 2002, Vincken et al. 2002). On a longer-term basis it was also found to reduce lung function decline in COPD subjects with milder COPD (Tashkin et al. 2008).

However its effects on both systemic and airways inflammation are unclear. A placebo-control trial investigating the effects of 1 year treatment with tiotropium on sputum and systemic inflammation biomarkers and on exacerbation rate found that tiotropium therapy was associated with a significantly reduced exacerbations rate compared with placebo but did not impact significantly on the serum or sputum IL-6 or on CRP levels (Powrie et al. 2007).

Experimental data however show that *in vitro* human bronchial epithelial cell line tiotropium was able to attenuate acetylcholine-induced IL-8 release mediated via the M3 muscarinic receptor (Profita et al. 2008), whereas in another study tiotropium was found to inhibit fibroblast and myofibroblast proliferation induced by acetylcholine (Pieper et al. 2007).

Long acting β2-agonists

In stable COPD long-acting β_2-agonists (LABAs) such as salmeterol or formoterol are currently used more frequently as maintenance combined therapy with inhaled corticosteroids in severe or very severe disease, whereas the existing management guidelines recommend that long-acting bronchodilators should be the mainstay of the inhaled therapy in stable COPD irrespective of its severity (The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2008).

LABAs exert their bronchodilator effect by inducing airway smooth muscle relaxation via increase of intracellular cAMP and β_2-adrenoreceptor stimulation (Dougherty 2003).

Both salmeterol and formoterol have a bronchodilator effect lasting about 12 h, although formoterol has a quicker onset of action (Cazzola et al. 1995). Both LABAs when given as a stand-alone therapy improved lung function, respiratory symptoms and quality of life and reduced exacerbation rate and the use of short-acting β_2-agonists in COPD patients on both a short- and long-term basis (Calverley et al. 2003 a,b, Szafranski et al. 2003, Jones & Bosh 1997, Husereau et al. 2004).

LABAs have also been found to enhance *in vitro* the anti-inflammatory activity of inhaled corticosteroids by increasing the activation of glucocorticoid receptor in lung cells (Sin et al. 2007).

Apart from bronchodilator effects LABAs have been claimed to exert a therapeutic effects on other cells having β_2-adrenoreceptors such as neutrophils which play a major pathogenic role in airway inflammation in COPD both in the stable state and during exacerbations. LABAs were found to reduce airway neutrophilic inflammation, this effect being the consequence of a complex anti-inflammatory mechanism: both salmeterol and formoterol inhibited neutrophil endothelial cell adhesion in experimental studies (Bolton et al. 1997, Bowden et al. 1994). LABAs have also been shown to reduce neutrophil accumulation and activation by reducing the release of neutrophil chemotactic factors such as IL-8 or myeloperoxidases and the production

of neutrophil-related radical oxygen species (Jones & Bosh 1997).

The effects of LABAs on systemic inflammation in COPD are however unknown, only the anti-inflammatory effects of their combinations with inhaled corticosteroids being documented so far. These effects are discussed in more detail subsequently.

Inhaled corticosteroids

Inhaled corticosteroids such as fluticasone propionate or budesonide are recommended to be added to the long-acting bronchodilators in more advanced COPD in order to reduce exacerbations rate.

Their anti-inflammatory efficacy in COPD is rather modest when compared with asthma. For example a 3-month therapy with fluticasone propionate did not reduce significantly CD8+, CD68+ cells, or neutrophils counts but reduced the CD8:CD4 ratio in the epithelium and mast cell counts in bronchial biopsies in COPD patients (Keatings et al. 1997, Gyzicki et al. 2002, Hattotuwa et al. 2002).

Previous large-scale studies such as EUROSCOP, ISOLDE and the Copenhagen Lung Study failed to demonstrate any significant impact on lung function decline although a subsequent meta-analysis suggested that higher doses given for longer periods of time might be effective in slowing lung function impairment (Burge et al. 2000, Pauwels et al. 1999, Sutherland et al. 2003, Vestbo et al. 1999).

In a study evaluating biomarkers of systemic inflammation in COPD subjects and in smokers and non-smokers without COPD, CRP levels were found to be lower in COPD patients treated with inhaled corticosteroids than in those not treated raising the question of the systemic anti-inflammatory effects of inhaled corticosteroids (Pinto-Plata et al. 2006).

In a placebo-controlled study the effects of inhaled fluticasone and oral corticosteroids on CRP were evaluated in 41 mild to moderate stable COPD patients. In patients who were previously taking inhaled corticosteroids a 4-week withdrawal before study entry resulted in an increased baseline CRP level by 71%. After 2 weeks of fluticasone therapy (500 μg twice daily) or prednisone therapy CRP levels were found to be reduced by 50% and by 63%, respectively, whereas no significant changes were reported in the placebo arm. An additional 8 weeks of fluticasone therapy further reduced CRP levels when compared with baseline (Sin et al. 2004).

A subsequent randomized placebo-controlled compared the effects of 4-week therapy with inhaled fluticasone alone or combined to salmeterol on several biomarkers of systemic inflammation represented by CRP, IL-6, and SP-D. Compared with placebo inhaled fluticasone alone and in combination did not reduce IL-6 or CRP levels but they reduced SP-D levels significantly (Sin et al. 2008).

Inhaled combinations LABA/corticosteroids

Combination of LABAs with corticosteroids in the same inhalator device in asthma and COPD has the double benefit of improving the adherence to therapy by reducing the number of devices and actuations and of improving the efficacy by reciprocal enhancement of their activities: bronchodilators improve the penetration of inhaled corticosteroids whereas these augment the bronchodilating activity at receptor level (Antoniu et al. 2007).

In COPD combinations such as fluticasone/salmeterol or budesonide/formoterol have been shown to improve respiratory symptoms, reduce exacerbation rate, improve quality of life and in the case of the former combination reduce mortality rate (Calverley et al. 2003a, b, 2006, Szafranski et al. 2003).

Salmeterol/fluticasone combination was found to reduce significantly CD8+, CD4+ CD45+ cell counts, and the number of cells expressing TNF-α and interferon-γ in bronchial biopsies and it also decreased significantly sputum neutrophil and eosinophil counts, these effects being associated with an increase in pre-bronchodilator FEV_1 in COPD subjects with former or current tobacco use (Barnes et al. 2006). In a study comparing the systemic anti-inflammatory effects of fluticasone, its combination with salmeterol, and placebo the largest therapeutic effect on biomarkers of systemic inflammation was reported with the inhaled combination but as salmeterol was not a comparison agent it is not appropriate enough to assume that such an effect was due to synergistic anti-inflammatory effects of the components rather than to enhancing effects of LABA on inhaled corticosteroids (Sin et al. 2008).

Conclusions

COPD is a chronic inflammatory disease of the airways having as the main risk factor cigarette smoking. Apart from local inflammation, a systemic inflammation can also be documented even in the stable state and the presence and amplitude of such pathogenic event might be a feature of disease severity. However the systemic inflammation in stable COPD is not well characterized especially because some of the inflammation markers are non-specific and their high levels might be due also to comorbid conditions commonly associated with COPD such as ischaemic heart disease or hypercholesterolemia, whereas others, which at least seem to be organ specific have not been thoroughly evaluated.

In this setting such biomarkers can be used to characterize the severity of the disease in the stable state or

during exacerbations, to predict the risk of further exacerbations and of mortality and to document the efficacy of potential therapies. CRP is the 'oldest' one and its use is extrapolated from other chronic inflammatory diseases. CRP is the most commonly used biomarker of systemic inflammation in COPD and has demonstrated prognostic properties for COPD-related morbidity and mortality. Others such as CCL-16 or SP-D have been evaluated for biomarker properties in several pulmonary diseases but still need to be further evaluated particularly in COPD. SP-D in particular seems to be an appropriate biomarker in both stable and exacerbated states. Such 'specific' biomarkers were mostly studied in smoking-related COPD and seem to be rather related to smoking-induced inflammation, but in non-smoking COPD the characterization of systemic inflammation and biomarkers is scarce.

Inhaled therapy used to treat stable COPD is mainly aimed at producing bronchodilation and at reducing airway inflammation but surprisingly some of these therapies such as inhaled corticosteroids alone or combined with long-acting β2-agonists are able to reduce not only the airways inflammation but even the systemic inflammation.

Based on the existing data inhaled corticosteroids are able to reduce systemic inflammation in COPD, their anti-inflammatory efficacy being amplified in combination with LABAs.

However the effects of inhaled therapies on systemic inflammation in COPD must be further documented especially with bronchodilators, with newer corticosteroids and with more specific biomarkers.

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