General introduction and outline of thesis
Introduction

COPD

In the 2005 update of the Global initiative for chronic Obstructive Lung Disease (GOLD) guidelines, chronic obstructive pulmonary disease (COPD) is defined as: ‘a disease state characterized by limitations in lung airflow that are not fully reversible. The airflow limitation is usually both progressive and associated with an abnormal inflammatory response of the lungs to toxic particles or gases’ (1).

In the western world, COPD is most commonly caused by exposure to tobacco smoke (2), but occupational dusts and air pollution also may cause COPD (3-5). In developmental countries indoor pollution by cooking on biomass fuels is an important additional cause (6). The patients’ first symptoms of COPD are dyspnoea on exertion, coughing, and increased sputum production. COPD is a major health problem worldwide: the prevalence of COPD in adults above 40 years of age is approximately 9-10% (7). COPD is currently the fourth leading cause of death in the world (8), and its mortality is still rising (9). COPD exacerbations have an important impact on morbidity and mortality. The in-hospital mortality for patients with an COPD exacerbation is 8-11% and the mortality in the first year following hospitalisation is 23-43% (10;11).

Maintenance treatment with inhaled steroids

The mainstay of treatment of COPD are bronchodilators (1). Several trials in patients with GOLD stage II-IV have investigated the long-term effects of maintenance treatment with inhaled corticosteroids on lung function, quality of life, and exacerbation rates. Inhaled steroids lead to a 30% reduction of exacerbation rates (12) and improvement of symptoms. They have only modest effects on lung function: at best, treatment with high dose inhaled corticosteroids reduces the annual decline in forced expiratory flow in the first second (FEV₁) with 9.9 ml, which by itself is generally deemed not to be clinically relevant (12;13). Therefore, inhaled steroids are currently indicated only in patients with more severe airway obstruction and frequent exacerbations (14).

In a recent meta-analysis of inhaled corticosteroids in stable COPD, anti-inflammatory effects were documented as observed by a reduction in total number of airway cells, neutrophils, lymphocytes, and a trend in eosinophils (15). Although it is generally perceived that the beneficial effects of inhaled corticosteroids should be due to their anti-inflammatory effects, a direct relationship between reduction in airway inflammation and clinical improvement has not been reported so far.

Treatment with the combination of inhaled steroids and long-acting bronchodilators improves lung function decline, symptoms and health related quality of life compared with placebo treatment, but reports on its effects compared with its mono-components alone are conflicting (16;17). The combination therapy reduces sputum neutrophil%, sputum eosinophil counts, and biopsy CD8 lymphocytes in stable COPD (18).

In summary, the beneficial effects of inhaled steroids in the treatment of COPD are perhaps modest, and certainly not as large in asthma. Despite the modest
beneficial effects, withdrawal of inhaled steroids leads in many patients to a deterioration of symptoms, and often to an exacerbation (19-21). When one considers stopping inhaled steroid treatment of a patient with COPD, this should be monitored carefully.

COPD exacerbations

During COPD exacerbations, a sudden deterioration of COPD symptoms for 24 hours or more occurs, i.e. increased dyspnoea, productive cough with an increased volume and/or more purulent sputum, and less specific symptoms such as malaise, fatigue, and insomnia. There is no consensus about the definition of COPD exacerbations though several have been proposed (see table 1). Symptoms worsen already a few days before the actual exacerbations starts, and it may take longer than a month before patients have fully recovered, and some patients do not return completely to their original health status (22). Frequent occurrence of exacerbations is an important feature of COPD: exacerbations of COPD have a large impact on morbidity and mortality, as well as on the quality of life of the patient (23;24). Approximately 56% of costs for COPD in the Netherlands are due to the treatment of patients with exacerbations (25). Since additionally the prevalence of COPD is rising, there is an important and growing need from the perspective of patients, doctors, and society to develop interventions to optimally prevent and treat exacerbations of COPD.

Exacerbations of COPD have different causes: infections of bacterial or viral origin have been identified as the most important causes of exacerbations: approximately 50-70% of exacerbations are associated with airway infections (26). Air pollution has been described to cause about 10% of exacerbations (5). However, in up to 30% of exacerbations, the cause remains unknown (27). During COPD exacerbations, the inflammation in the airway is increased, involving increased numbers of eosinophils, neutrophils and lymphocytes (28-30).

It is difficult to obtain information on airway inflammation during COPD exacerbations. An elegant non-invasive method to assess the inflammation in the airways is sputum induction. Sputum is induced by the inhalation of nebulised saline in an isotonic or hypertonic concentration. This both facilitates and standardises the coughing-up procedure of sputum, and the results of the gained samples reflect the level of inflammation in the airways (31-33). However, there is also a downside to this procedure: inhalation of isotonic and certainly of hypertonic saline causes a bronchoconstrictive response in many patients with COPD (34-38). Since this decrease in FEV\textsubscript{1} is generally transient and more severe adverse effects do not occur, sputum induction is considered to be safe even in stable mild to severe COPD (35;38). However, whether sputum can also be induced safely during exacerbations of COPD is less clear.

COPD exacerbations are generally treated with systemic corticosteroids and short-acting bronchodilators, with or without antibiotics. The beneficial effects of systemic steroids are evidence based (39;40), however the effects are modest
and certainly not as evident as in the treatment of asthma exacerbations. Furthermore, the use of systemic steroids might cause adverse effects like hyperglycaemia, osteoporosis, and mood swings \((39;41;42)\). Treatment of COPD exacerbations with inhaled corticosteroids might be an alternative for systemic treatment avoiding some adverse effects. Inhaled corticosteroids have been shown to be capable of reducing airflow limitation during hospitalisations for COPD exacerbations \((43)\). Steroids might exert their beneficial effect in the treatment of COPD exacerbation by decreasing the eosinophils, since in stable COPD steroids reduce the levels of airway eosinophils \((15;18)\), and since sputum eosinophilia predicts a better response to a short-term steroid treatment in a stable phase of COPD \((44-46)\).

Treatment of COPD exacerbations with antibiotics is still a point of discussion; the most recent GOLD guidelines advocate that antibiotics should be given to patients with exacerbations of COPD with three of the following cardinal symptoms: increased dyspnoea, increased sputum volume, increased sputum purulence, or two of the cardinal symptoms if increased sputum purulence is one of the symptoms. Furthermore antibiotics should be given to all patients with a severe exacerbation of COPD that requires invasive mechanical intervention \((1)\).

**Carbon monoxide**

Based on reports of *in vitro* and *in vivo* studies, it has become clear that carbon monoxide has potent anti-inflammatory and anti-oxidant capacities. Carbon monoxide is endogenously generated by the degradation of heme, which is induced by tissue injury or inflammation \((47-49)\). This degradation is catalyzed by the stress inducible enzyme heme oxygenase-1 (HO-1). *In vitro* studies have shown that carbon monoxide downregulates pro-inflammatory cytokines by inhibiting the mitogen-activated protein kinase pathway \((50;51)\). *In vivo* studies in several animal species consistently show that inhaled carbon monoxide has a protective effect against ischemic injury, hyperoxic injury, graft versus host reactions, and pulmonary inflammation \((52-60)\). These capacities might be of therapeutic use in respiratory inflammatory diseases \((48)\). The next step is to establish these beneficial effects in humans. The HO-1 expression in alveolar macrophages in smoking COPD patients is decreased compared to smokers without COPD \((61)\), and the HO-1 expression in ex-smokers with COPD is decreased compared to healthy ex-smokers \((62)\). This suggests that the HO-1 is insufficiently up regulated in patients with COPD. In ex-smokers with COPD, there is an ongoing inflammation even after smoking cessation. This inflammation is characterized by increased numbers of eosinophils and neutrophils in the sputum of ex-smokers with COPD compared to healthy ex-smoking subjects \((63-66)\). It is unknown what causes the ongoing inflammation after smoking cessation. We hypothesize that the decreased HO-1 expression, resulting in an abnormal inflammatory response to particles and gases in the air, might contribute to the ongoing inflammation. Correction for the HO-1 impairment, by inhalation of CO, might therefore reduce the numbers of inflammatory cells in the airways.
Outline of the thesis

In this thesis, the results of two clinical trials exploring the effects of two inflammation modifying therapies for COPD are described, one in COPD exacerbations, and one in stable COPD but with possible future bearings for exacerbations.

Chapter 2 is a review of observational studies on the changes in airway inflammation from a stable phase of COPD to the onset of an exacerbation. The aim of this review is to give insight in the increases in the different types of inflammatory cells, causes of the exacerbation, and whether these inflammatory changes lead to a decline in lung function. With this insight, we review the inflammation modifying therapies which are currently used, and speculate on future therapies to treat COPD exacerbations.

The first of the two trials is the Symbexco-study, which stands for SYMbicort in the treatment of EXacerbations of COPD. The aim of this trial was to compare the anti-inflammatory effects of combined budesonide and formoterol versus placebo in the treatment of COPD exacerbations. The design of this trial is shown in figure one.

At the first visit, we included patients with COPD in a stable phase, measured inflammation in induced sputum and blood, performed lung function tests, and assessed the patients' health state. After this visit, inhaled corticosteroids were withdrawn if used. At the second visit, these measurements were repeated provided the patients were still in a stable phase of COPD. However, we noticed that many patients deteriorated after the steroid withdrawal, and several even experienced an exacerbation. Chapter 4 describes the changes after inhaled steroid withdrawal, aiming to elucidate the inflammatory mechanisms which cause the exacerbation.

After visit 2, we waited per protocol for the patients to report an exacerbation. When they reported an exacerbation, they were asked to come to the hospital for a third visit. At this visit, we repeated the measurements, including sputum induction, and started the randomised-controlled treatment. We were concerned about performing sputum induction in exacerbated patients, since the nebulised saline used during sputum induction can cause increased airflow limitation, and patients who have a COPD exacerbation already experience increased shortness of breath. Therefore we induced sputum by a more cautious protocol than usual in patients who had a more severe airflow limitation. Chapter 3 describes the safety of our method to induce sputum in patients with COPD experiencing an exacerbation.

Chapter 5 describes the changes in inflammation from a stable phase of COPD (visit 2, no inhaled steroids), to the onset of an exacerbation. It aims to study which inflammatory changes identify a bacterial cause of the exacerbation. The identification by inflammatory markers of a bacterial cause of an exacerbation
could be helpful in the clinician’s decision of an early and useful initiation of antibiotics in the treatment of COPD exacerbations.

At visit 3, patients were randomised for 14 days of one of three treatments: combined budesonide and formoterol, oral prednisolone, or placebo. Chapter 6 describes the results of these treatments. It aims to compare the anti-inflammatory effects of combined budesonide and formoterol to placebo treatment. Secondary objectives are to assess the effects of the budesonide/formoterol combination versus placebo on lung function, symptoms and health status, and to compare the side effects of the combination versus active control with prednisolone.

The second trial was designed to study the anti-inflammatory effects of inhaled low dose carbon monoxide in patients with COPD in a stable phase (see figure 2). Chapter 7 describes the results of this pilot. This is the first exploration of the effects in humans and hence the group studied is small. Investigating the anti-inflammatory effects on airway inflammation in stable COPD patients is a first step towards exploring the therapeutic effects of carbon monoxide.

Table 1: Definitions of a COPD exacerbation.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Description</th>
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<tr>
<td>Davies et al (67)</td>
<td>A history of increased breathlessness and at least two of the following symptoms for 24 h or more: increased cough frequency or severity, increased sputum volume or purulence, and increased wheeze.</td>
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<td>Rodriguez-Roisin (68)</td>
<td>A sustained worsening of the patient’s condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD.</td>
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<td>Anthonisen et al (69)</td>
<td>A disease state characterized by an increase in symptoms of dyspnea, sputum volume and sputum purulence. Exacerbation types graded on the basis of combinations of major and minor symptoms.</td>
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<td>Madison et al (70)</td>
<td>An acute tracheobronchitis, generally infectious in aetiology, that occurs in a patient with established COPD.</td>
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<tr>
<td>Pauwels et al (71)</td>
<td>Increased dyspnea, cough, or sputum expectoration (quality or quantity) that led the subject to seek medical attention</td>
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<tr>
<td>Seemungal et al (72)</td>
<td>An exacerbation was diagnosed if the following symptom patterns were experienced for at least two consecutive days: either two or more of three major symptoms (increase in dyspnea, sputum purulence, and increased sputum volume); or any one major symptom together with any one of the following minor symptoms: increase in nasal discharge, wheeze, sore throat, cough, or fever.</td>
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<td>GOLD 2006 (1)</td>
<td>An event in the natural course of the disease characterized by a change in the patient’s baseline dyspnea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD</td>
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Visit 1: Inclusion. After the visit, inhaled steroids are withdrawn if used.
Visit 2: Stable phase without steroids. After this visit, long-acting bronchodilators if used are replaced by short-acting. Patients are instructed to contact the research center when experiencing an exacerbation as soon as possible.
Visit 3: Exacerbation. Patients are randomised for double-blind treatment with Symbicort, prednisolone, or placebo for 14 days. All patients receive antibiotics (doxycycline), and both ipratropium, and terbutaline as rescue medication.
Visit 4-6: Visits to evaluate the treatment effects. After visit 6, patients re-start using their long-acting bronchodilators if used before.
Visit 7 and 8: Follow-up visits. Patients are contacted by telephone to evaluate their status of COPD. When a new exacerbation occurs, patients are treated open label, and this is the end of study. If the patients do not have a next exacerbation within 90 days after the start of the exacerbations, this is the end of study.
**Figure 2:** Design of carbon monoxide in COPD-study.
CO: carbon monoxide inhalation for 2 hours per day; Pla: placebo inhalation for 2 hours per day; LF: lung function; PC20: provocative concentration of methacholine causing a 20% fall in FEV1. Lung function, sputum and blood were assessed 17 hours after the last inhalation of CO or placebo.

**References**


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Davies L, Angus RM, Calverley PM. Oral corticosteroids in patients admitted to hospital with exacerbations of chronic obstructive pulmonary disease: a prospective randomised controlled trial.


