THE UNIVERSITY OF HULL

TITLE:

DIAGNOSIS AND TREATMENT IN AIRWAY INFLAMMATION

Being a Thesis submitted for the Degree of Doctor of Medicine

In the University of Hull

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February 2007

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ACKNOWLEDGEMENTS

These studies were undertaken during my time as a Clinical Research Fellow (2001-2002) and Clinical Lecturer in Respiratory Medicine (2002-2004) at the Division of Academic Medicine, Postgraduate Medical Institute, Castle Hill Hospital, University of Hull.

I would like to acknowledge the financial support received during my year as Clinical Research Fellow from Innovata Biomed Ltd, St Albans, Hertfordshire.

I confirm that I am responsible for all the work contained in this thesis except for the contributions listed below which I gratefully acknowledge.

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Sincerest thanks to all those who supported me throughout my research period and to those who assisted with my studies.

Professor Alyn Morice and Dr Anthony Redington, my research supervisors.

Dr Jack Kastelik, Dr Josephine Ojoo, Dr Caroline Everett, Dr Joseph Hogg and Dr Richard Teoh.

The Clinical Trials Department at Castle Hill Hospital (Caroline, Rachel and Jenny).

Charlotte Waudby and Graham Wright from the Department of Nuclear Medicine, Hull Royal Infirmary.

Ian Hanning from the Department of Biochemistry, Hull Royal Infirmary.

CF specialist nurses Tanya Moon and Julie Mould.

Many thanks to the patients and healthy subjects who participated in the studies.

I also wish to express my deepest gratitude to my family and friends for their unfailing love and support throughout the highs and lows!

PRESENTATIONS & PUBLICATIONS

PRESENTATIONS

1) BTS Winter Meeting December 2002 Oral Presentation

Smoking Salbutamol and the Cough Reflex (CHAPTER 4)

2) ERS Vienna September 2003 & European Cystic Fibrosis Conference June 2003

Poster Presentations & Abstracts

Airway Acidification and Exhaled Nitric Oxide in Cystic Fibrosis (CHAPTER 3)

3) ERS Glasgow September 2004 Poster Presentation & Abstract

Mechanisms of Systemic Side Effects of Inhaled Steroids (CHAPTER 5)

4) BTS Winter Meeting 2005 Poster Presentation & Abstract

Routine Planer Scintigraphy to determine the most appropriate nebuliser for CF patients (CHAPTER 6)

PUBLICATIONS

1) Effect of salbutamol on smoking related cough. **Mulrennan** S. Wright C. Thompson R. Goustas P. Morice A. *Pulmonary Pharmacology & Therapeutics. 17(3):127-31, 2004.*

2) Exhaled breath condensate pH and exhaled nitric oxide in allergic asthma and in cystic fibrosis. Ojoo JC. **Mulrennan SA.** Kastelik JA. Morice AH. Redington AE. *Thorax. 60(1):22-6, 2005 Jan.*

3) Mechanisms of Systemic Side Effects of Inhaled Steroids. **Mulrennan S.** Hogg J. Teoh R. Morice A. *Accepted for publication in British Journal of Clinical Pharmacology 2006.*

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CHAPTER ONE

INTRODUCTION

1.1 INTRODUCTION

Airway inflammation is a feature of many lung conditions. Chronic obstructive pulmonary disease (COPD), asthma and cystic fibrosis (CF) are all associated with airway inflammation (Doring, 1996; Morrissey, 2003; Frieri, 2005; Saetta, 1997; Turato, 2002). This inflammation is a response by the immune system to the environmental agents and intrinsic abnormalities related to these disorders. Novel techniques are required to diagnose, treat and enhance our understanding of airway inflammation and allied diseases.

1.2 AETIOLOGY OF AIRWAY INFLAMMATION

Inflammatory processes within the airway are the result of direct cell injury or the outcome of specific immunological reactions. Respiratory epithelium extends from the lining of the nose to the alveolar portion of the lung (Kumar, 1994). It is the barrier from external irritants and the site of immune response instigation. The respiratory tract is continuously exposed to a number of inorganic and organic particles as well as viruses and bacteria (Boren, 1967; Coffin, 1972). Deposition of these insults within the airways can lead to a number of diseases that occur when the immunological defence mechanisms are overwhelmed or overreact. Underlying genetic conditions can exacerbate this and predispose to the development of airway inflammation and lung destruction (Eriksson, 1965; Talamo, 1977; Agrawal, 2005).

Airway mucosa consists of surface epithelium with underlying supportive tissue known as the lamina propria. The mucosa is covered by mucus and cilia protrude from its surface. Airway mucus and mucociliary transport traps and clears particulate matter. Mucus is composed of water and mucins (proteoglycans, lipids and glycoproteins). It consists of 2 layers, a lower non-viscous periciliary fluid and upper visco-elastic mucus (Yeates, 1975). The upper layer is moved over the lower by beating cilia. Mucus transport is 4-10 mm/min in the trachea and slows in the thinner mucus of the peripheral bronchi. Airways secretions also contain innate defences such as lyzozyme and lactoferrin along with the more specific immunity of immunoglobulin A. All assist in protecting the airway (Tourville, 1969; Masson, 1965). These protective secretory factors coupled with mucociliary clearance acts as the first line of defence against inhaled particulate matter.

Neutrophils, eosinophils and macrophages are cells involved in airway inflammatory processes. Lymphocytes and plasma cells are also present in chronic inflammation (Truitt, 1971; Mackaness, 1971; Kay, 1986). Neutrophils and macrophages phagocytose antigens and process them within phagolysosomes. Reactive oxygen intermediates (superoxide, hydrogen peroxide and hydroxyl radical), bacterial inhibitors and enzymes then destroy the antigens (Weiss, 1989; Meltzer, 1989; Postma, 1988; Lehrer, 1988). Anti-oxidants are found in the airways and protects against tissue damage by uninhibited oxidant activity. (Heffner, 1989)

Eosinophils attempt to damage larger pathogens by external release of cell granules. Neutrophils, eosinophils and macrophages also produce proteinases with the aim of inactivating and degrading antigens and pathogens. (Kay, 1986) Proteinases can however cause tissue damage and anti-proteinases are produced in bronchial secretions to

counteract this (Talamo, 1968). Anti-proteinases are usually produced in excess and thus prevent the harmful effects of proteinases. If there is local inactivation of antiproteinases via oxidation or genetic deficiency, or excess proteinases, then tissue destruction will occur. The delicate balance of respiratory defence mechanisms versus the injurious effects of inflammatory mediators is compromised in many lung conditions and results in both airway and parenchymal inflammation.

Bronchial biopsies and bronchoalveolar lavage (BAL) performed using a fiberoptic bronchoscope have shown that asthma, COPD and cystic fibrosis are all associated with airway inflammation (Armstrong, 1997; Salvato, 1968; Glynn, 1960).

Smoking leads to increased pulmonary macrophages, increased bronchoalveolar neutrophils, cigarette smoke free radicals and reduced activity of lysyl oxidase, which is involved in the repair of elastan. Smoker's macrophages as well as being more numerous are also larger in size and contain more pigment (Niewoehner, 1974). Neutrophils are more numerous in the bronchoalveolar lavage specimens of smokers and patients with chronic bronchitis (Thompson, 1989). Other pathological changes seen in the airways of smokers include disordered epithelial cells with asynchronous or immotile cilia, desquamated mucosal cells and a chronic inflammatory infiltrate within the bronchial wall. Mucus hypersecretion is prominent and related to increased goblet cells and hyperplastic submucosal glands (Ailsby, 1973; Chang, 1957; Wanner, 1977; Reid, 1954). The inflammatory infiltrate includes T-lymphocytes and macrophages. Macrophages cluster in the terminal and respiratory bronchioles and attract neutrophils via macrophage neutrophil chemotactic factors. The release of neutrophil and macrophage proteinases and

radical oxygen intermediates results in mucous hypersecretion and destruction of the elastin and connective tissue if inhibitory mechanisms are lacking (Cosio, 1980; Mitchell, 1976; Hale, 1984; Cantin, 1985; Wright, 1988; MacNee, 1993).

COPD predominantly involves peripheral airways and lung parenchyma. There is bronchiole obstruction and associated fibrosis (O'Shaughnessy, 1997; Di Stefano, 1996). Inflammation is found throughout the bronchiolar and respiratory part of the lung. Lung tissue is destroyed and macrophages and $CD8^+$ cells are seen. Neutrophils are also seen in severe COPD but eosinophils, a prominent feature of asthma are generally not seen except in exacerbations (Saetta, 1998; Fabbri, 1998; Saetta, 1994). The increase in the number of pigmented macrophages and neutrophils in the airways of smokers (Wright, 1988) is associated with bronchitis, bronchiolitis and alveolitis. The alveolitis is caused by recruitment of neutrophils following exposure to cigarette smoke (Cantin, 1985). Activated lymphocytes are found in both asthma and COPD and in COPD there is an excess of CD8 subsets. Inflammatory mediators that predominate in COPD include leukotriene (LT) B4 and Interleukin (IL) 8 (Fuke, 2004; Montuschi, 2003).

In contrast to COPD, asthma involves all airways and not the parenchyma. In mild stable asthma bronchial biopsies have shown mast cells and eosinophils (Beasley, 1989). Thickening of the basement membrane occurs and this is associated with the presence of myofibroblasts. CD4+ lymphocyte activation is also present and associated with IL-5 gene upregulation (Hamid, 1991).

The inflammatory cells that predominate in the airways of asthmatics are mast cells, eosinophils, CD4+ cells and macrophages. Airway epithelial cells, smooth muscle cells,

endothelial cells and fibroblasts are also implicated in the production of inflammatory mediators (Levine, 1995; Saunders, 1997; John, 1997).

The inflammatory reaction that occurs in asthma is mediated in part via allergen binding with specific IgE antibody bound to mast cells and basophils. Mast cell degranulation then leads to release of inflammatory mediators. Histamine, proteoglycans and proteases are released from mast cells following cross linking by allergen of Ig E. T helper type 2 inflammation is also found in asthma leading to CD4 lymphocyte predomination and secretion of the lymphokines interleukin (IL) 4, IL5, IL10 and the cytokines TNF-α and GM-CSF (Bentley, 1993). Other mediators found in asthma include prostaglandin D2, platelet-activating factor (PAF), leukotriene B4 (LTB4), LTC4 and LTD4 (Murray, 1986; Holtzman, 1991; Diaz, 1989).

Inflammatory mediators are central to both the inflammatory cell recruitment and pathological changes found in asthma (Liu, 1991; Diaz, 1989). Histamine, PAF, tryptase and kinogenase lead to vasodilation and vascular permeability and oedema. Histamine, PGD2, LTC4 and LTD4 cause bronchial smooth muscle contraction, mucosal oedema and secretion (Barnes, 1998).

Whilst there is some evidence for inflammation prior to infection there is little doubt the major inflammatory stimulus in CF is infection (Armstrong, 1997; Khan, 1995). CF is a genetic condition that leads to an increase in susceptibility to endobronchial infections and several mechanisms have been postulated to explain the increased susceptibility to infection in CF. These relate to the mucus consistency in the airway and difficulty in preventing bacterial adherence and then subsequent bacterial destruction. The defective CFTR gene causes dehydration in the airways and reduction in mucociliary clearance.

Airway cell surface liquid has reduced antibacterial properties resulting in ineffective removal of bacteria from the airways (Bals, 1998; Smith, 1996). The CFTR mutation also seems to result in increased adherence of Staph aureus and Pseudomonas to epithelium, again preventing efficient bacterial removal (Imundo, 1995; Poschet, 2001; Ratjen, 2003). The CFTR is involved in binding and destroying P. aeruginosa, a role that is defective in CF (Pier, 1996). Mucoid forms of P.aeruginosa produce alginate, which allows enhanced attachment to the epithelial surface and thus resistance to eradication.

Recurrent infections lead to persistent neutrophilic inflammation, epithelial damage and production of proinflammatory mediators and excess elastase (Doring, 1996; Konstan, 1997). These inflammatory mediators and proteinases cause further lung damage and perpetuate the inflammation.

1.3 CLINICAL MANIFESTATIONS OF AIRWAY INFLAMMATION

COPD is strongly associated with smoking and usually presents with symptoms of dyspnoea, wheeze and a productive cough (Fletcher, 1976). Dyspnoea and wheeze is related to airway obstruction and this is assessed with spirometry.

The Global initiative for Chronic Obstructive Lung Disease (GOLD) defined COPD as a condition characterised by airflow limitation that is not fully reversible (reversibility being quantified as ≥ 12% and > 200ml increase in FEV_1 following nebulised/inhaled bronchodilator). It is progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases (Pauwels, 2001). As previously stated the CD8+T-lymphocyte subset tends to predominate in COPD and there are greater numbers of these cells in subjects with airflow obstruction (O'Shaughnessy, 1997).

The severity of airway obstruction can be determined by measuring the FEV_1 and it has been shown that death and morbidity is linked to progressive deterioration in FEV_1 (Peto, 1983). The degree of airway obstruction is useful in predicting health status, exacerbation rate and mortality (Fletcher, 1976; Burge, 2000; Dewan, 2000; Anthonisen, 1986).

Although COPD is defined as airflow limitation that is not fully reversible it is generally considered to be a combination of emphysema and chronic bronchitis. Bronchitis is the feature of COPD that can lead to a productive cough and chronic bronchitis is defined as a productive cough for at least 3 months of the year for at least 2 successive years.

Emphysema is the parenchymal lung destruction caused by proteinases released as part of the immunological response to smoking. This may take years to develop and leads to progressive dyspnoea. It is accelerated in α 1 antitrypsin deficiency, a genetic condition with a relative lack of anti-proteinase (Brantly, 1988).

COPD can eventually lead to the complications of hypoxaemia, polycythaemia, respiratory failure and cor pulmonale (Wright, 2005; Bardsley, 1986).

Asthma is a condition characterised by reversible airway obstruction and linked to allergy and type 1 hypersensitivity. Extrinsic or allergic asthma is asthma triggered by an external allergen. Intrinsic asthma has no obvious external cause. Asthma can also be related to occupational dust exposure (Banks, 2000).

The inflammatory mediators associated with asthma lead to the pathological changes of bronchoconstriction, mucosal oedema and hypersecretion. These pathological changes produce the classical features of asthma, which are intermittent and erratic. The main symptoms are wheeze, dyspnoea, chest tightness and tend to be associated with exercise, allergen exposure and infection (McDowell, 2000).

In allergic asthma there are two phases to the airway obstruction response that occurs following allergen exposure. The early phase reaction occurs within minutes and decays quickly, usually within 60 minutes. Late phase reactions occur 3 to 4 hours following exposure with a maximum effect at 4 to 8 hours and resolves within 12 to 24 hours. It is due to slower acting mediators and accumulated inflammatory cells. The response to allergen can also result in a dual early and late reaction (Perrin, 1991; Pepys, 1977). The early phase presents as an acute asthma attack and the late phase leads to prolongation of the symptoms of airway obstruction.

The FEV_1 will usually be low during an asthma attack. This signifies airway obstruction and along with peak expiratory flow (PEF) rate can vary over time (diurnally and long term) and following treatment. The reversible nature of the disease is demonstrated by

peak flow variability and response to bronchodilators (Hetzel, 1980; Lung function testing: selection of reference values and interpretative strategies. ATS, 1991).

The presence of eosinophils in induced sputum is indicative of the eosinophilic inflammation associated with asthma. It is an objective marker of poor asthma control. The sputum eosinophil count is lowered with corticosteroid treatment and a management strategy aimed at normalising counts has been shown to reduce exacerbations and admission rates (Wark, 2000; Green, 2002).

Chronic inflammation can lead to airway remodelling (Redington, 1997). This structural change results in fibrosis, thickening of the smooth muscle and angiogenesis. The changes can lead to irreversible fixed airway obstruction, which as stated above is a feature generally associated with and indeed used to define COPD. Chronic irreversible airflow obstruction can lead to a reduction in treatment response and adversely affects quality of life (Davies, 2001).

Poorly controlled asthma presents with restriction of physical activity, nocturnal dyspnoea and wheeze, increasing use of short acting bronchodilators and frequent courses of oral corticosteroids. Treatment aims to reduce airway inflammation, minimise symptoms and reduce the risk of permanently altered lung function and premature death. (British Guideline on the Management of Asthma, 2003)

Bronchiectasis is described as dilatation and destruction of the distal bronchi. It can be idiopathic, caused by a genetic disorder or secondary to previous infection.

Cystic fibrosis is a multisystem disorder associated with recurrent respiratory infections, bronchiectasis and bronchial obstruction. Respiratory complications usually present with

a chronic cough and dyspnoea. Respiratory symptoms start early in childhood and are associated with colonisation of the bronchial tree; initially with staphylococcus aureus and haemophilus influenzae and then usually pseudomonas. Although it is unusual for children to produce sputum, adult patients with moderate to severe disease frequently produce purulent sputum on a daily basis.

Many patients have bronchial hyperreactivity with associated wheeze and infection results in airway inflammation (Eggleston, 1988; Heeckeren, 1997).

Pseudomonas thrives in the airways of CF patients and leads to a chronic cycle of persistent infection and continuing inflammation. CF exacerbations propagate the inflammation. The inflammatory mediators and antiproteinases produced lead to further epithelial and parenchymal damage (Rayner, 1991; Armstrong, 1997; Tabary, 1998). The parenchymal damage leads to the typical changes of bronchiectasis.

Lung function tests show both airflow obstruction and a reduction in total lung capacity and gas transfer. This indicates both airway obstruction and parenchymal damage and explains the symptom of dyspnoea. The phenotypic presentation varies and some patients have normal lung function.

The inflammation associated with exacerbations does improve with bacterial elimination (Armstrong, 1997). Treatment with regular physiotherapy, daily nebulised antibiotics, Dnase and high dose oral and intravenous antibiotics during exacerbations aims to limit the damage caused by persistent colonisation and recurrent infections.

Primary ciliary dyskinesia is a condition associated with bronchiectasis, sinusitis and infertility. Abnormal ciliary movement leads to inadequate clearing of mucus within the bronchial tree and subsequent predisposition to respiratory infections and bronchiectasis.

Cough is a symptom associated with all of the conditions described above and can lead to an impaired quality of life (French CT, 2002). It is defined as a forced expulsive manoeuvre usually against a closed glottis and which is associated with a characteristic sound (BTS cough guidelines 2006).

The aetiology of cough in smokers and COPD appears to be related to airway inflammation, bronchoconstriction, reduced cough threshold and pollutant fumes (Turato G, 1995; Wong CH, 1999; Wright JL, 1988). Airway inflammation and bronchoconstriction is present in asthma and bronchiectasis and this explains the frequency of this symptom in these conditions.

Cough challenges are used to measure cough reflex sensitivity and citric acid cough challenge is utilised in this thesis. Reduced cough reflex sensitivity is associated with diminished cough in disease (O'Connell F, 1994).

Specific cough treatment aims to reverse the underlying pathology, for example in asthma steroids and bronchodilators reverse the airway inflammation and bronchoconstriction. Non-specific antitussives such as opiates and antihistamines suppress the cough reflex centrally but tend to have sedating side effects.

1.4 NON-INVASIVE ANALYSIS OF AIRWAY INFLAMMATION

1.4.1 EXHALED NITRIC OXIDE

Nitric oxide (NO) is generated when L-arginine is converted to L-citrulline by the enzyme nitric oxide synthase (NOS) (Palmer, 1988) It can be detected in the exhaled breath of humans via chemiluminescence (Gustafsson, 1991).

It acts by relaxing smooth muscle via cyclic guanosine 3'5' monophosphate (cGMP). It is a potent vasodilator and has been shown to act as a bronchodilator in animal studies (Masaki Y, 1989). Although the evidence for bronchodilation in humans is lacking NO does seem to exert a modulating effect on airway hyperesponsiveness (Ricciardolo, 1996). 3 isoforms of the enzyme NOS are known, endothelial NOS (eNOS or NOS III), neuronal NOS (nNOS or NOS I) and inducible NOS (iNOS or NOS II). eNOS and nNOS as their names suggest are found in the endothelium and brain respectively. They are however also found in other tissues. Inducible nitric oxide synthase activation is independent of calcium but eNOS and nNOS require calcium for activation. All these enzymes are found in many of the cells in the lung including epithelial cells, macrophages, neutrophils and nerves.

The three isoforms of nitric oxide synthase are inherently involved with the regulation of airway function. Constitutive NOS (eNOS and nNOS) affect baseline NO levels that are necessary for normal airway function. eNOS has been found in the endothelial cells of bronchial blood vessels and bronchial epithelium (Kobzik, 1993) (Shaul, 1994). nNOS has been located in airway cholinergic nerves (Fischer, 1993)

Since the initial discovery that nitric oxide (NO) can be detected in exhaled air of humans, (Gustafsson, 1991) reports are now available describing the expression of NO in the respiratory tract in health and disease.

Hamid et al localised iNOS to the bronchial epithelial cells in asthmatics and showed increased expression of iNOS following stimulation of cultured epithelial cells with tumour necrosis factor (Hamid, 1993). Redington et al showed that iNOS has increased expression in the airways of asthmatics (Redington, 2001). This increased expression is regulated by inflammatory cytokines and leads to an increase in exhaled NO levels. NO is therefore implicated in the pathophysiology of asthma and the fractional exhaled concentration of NO (FE_{NO}) is raised in allergic asthma (Lundberg, 1996). It also favours the production of eosinophils by inhibiting the Th1 response and therefore promoting the Th2 response (Barnes, 1995).

The FE_{NO} level is further increased during acute exacerbations of asthma and falls with response to treatment (Massaro,1995). Glucocorticoids by reducing the inflammatory process in asthmatics, reduces the induction of iNOS by cytokines and leads to a reduction in NO. FE_{NO} can therefore be used as a monitoring tool for treatment response as levels will decrease with corticosteroid use (Kharitonov, 1996).

In CF, studies show that expired NO is lower (Lundberg, 1996; Grasemann, 1997) or similar to (Balfour-Lynn, 1996) that in healthy control subjects and does not change during pulmonary exacerbations (Ho, 1998).

COPD is associated with raised FE_{NO} compared to normal subjects but the level is not as elevated as that found in asthmatics (Ansarin, 2001). Higher FE_{NO} is found with severe disease and exacerbations (Maziak, 1998). Exhaled NO can therefore be used as both a diagnostic and monitoring tool in a number of pulmonary disorders.

1.4.2 EXPIRED BREATH CONDENSATE

Expired breath condensate (EBC) is a novel method of sampling airway secretions. EBC is formed by breathing through a cooling system. Custom made devices usually consist of polypropylene tubing connected to a glass condensing system immersed in ice. Commercial devices are also available (Mutlu, 2001). Collection should occur with the subject in a sitting position wearing a noseclip while tidal breathing (Horvath, 2005).

The condensate is a combination of water vapour and aerosol particles from the lower respiratory tract. It contains both volatile and non volatile substances (Manolis, 1983). Substances indicative of oxidative stress and inflammatory mediators are present in EBC. These include prostaglandins, leukotrienes, and H_2O_2 (Montuschi, 2003; Nowak, 1996).

NO derived products such as S-nitrosothiols, nitrite (NO_2) and nitrate (NO_3) can also be measured in EBC. NO_2 and NO_3 can be measured using spectophotometric assay, chemiluminescence, fluorimetric method and ion chromatography.

Concentrations of EBC NO_2 and NO_2/NO_3 are significantly higher in asthma, CF and bronchiectasis compared with healthy controls. Milder asthmatics tend to have normal levels (Ho, 1998; Kharitonov, 2002).

In patients with exacerbations of asthma, higher NO_2 levels have been observed and in contrast stable patients and those treated with inhaled steroids have a significantly decreased concentration (Hunt, 1995; Corradi, 2001).

EBC pH is easily measured with a pH electrode and it has been demonstrated that acidic airway fluid is present in a variety of respiratory disorders (Hunt, 2000; Kostikas, 2002; Tate, 2002). Airway pH in normal subjects is between 7.4 and 8.8 and there is low daily and weekly variation (3.5% and 4.5% respectively) (Vaughan, 2003). It is not affected by hyperventilation, collection duration, oral versus endotracheal sampling or airway obstruction post methacholine.

EBC pH is lower in asthma, COPD, bronchiectasis and acute lung injury (Kostikas, 2002; Tate, 2002; Gessner, 2003).

Patients with acute asthma have airway fluid pH 2 log orders lower than non-asthmatic subjects. The airway acidity causes the conversion of endogenous nitrate to NO and gives a further source of elevated exhaled NO. The pH normalises following treatment with steroids (Hunt, 2000).

These findings demonstrate that EBC has potential use for both diagnosis and treatment monitoring in respiratory disorders. The initial collection is both quick and non invasive and lends itself to use both on wards and in the outpatient setting. Further research will determine the extent of this potential.

1.5 THERAPEUTICS IN AIRWAY INFLAMMATION

Inhaled corticosteroids and β_2 agonists are used extensively in the treatment of COPD and asthma and to a lesser extent in CF. Nebulised antibiotics are an established treatment in CF. For the purpose of this thesis the next section concentrates on the mode of action, administration and effects of these therapies.

1.5.1 CORTICOSTEROIDS

Glucocorticoids have an anti-inflammatory action that is utilised in the treatment of airway inflammation. They act via the glucocorticoid receptor, which is located in the cytoplasm of cells. The binding of steroid to this receptor leads to various intracellular effects, which via several mechanisms (the relative importance of which is debated) result in the suppression of inflammation (Kamada, 1995) (Barnes, 1998).

Topical administration via various inhalation devices aims to deliver the drug to the site of disease and also reduce systemic effects. A number of inhaled steroids and inhalation devices exist and these differ in percentage lung deposition and pharmacokinetics (Barnes, 1998). Inhaled steroids and devices currently available include fluticasone via the accuhaler or evohaler, CFC free beclomethasone dipropropionate via metered dose inhaler and budesonide via the turbohaler (Respiratory System, BNF 50, 2005).

In asthma steroids reduce inflammatory cell counts and bronchial hyperreactivity. They also inhibit production of inflammatory mediators, which in turn reduces the vascular permeability and oedema associated with airway inflammation (Kamada, 1995). The ultimate aim is to reverse airway obstruction, reduce exacerbations, nocturnal symptoms and 'as required' bronchodilator use (British Thoracic Society, 2003).

The effect of inhaled corticosteroids in COPD is more modest than in asthma. At a cellular level there is resistance to corticosteroids (Culpitt, 1999; Keatings, 1997). In clinical practice the use of inhaled corticosteroids in COPD patients with an FEV1 of less than 50% reduces exacerbation rate and rate of decline (Burge, 2000).

There is a role for use of inhaled steroids in CF. A small number of studies have shown reduction in bronchial hyperreactivity, improved respiratory function and slower deterioration of FEV1 between courses of intravenous antibiotics (Van Haren, 1995; Bisgaard, 1997).

Because of the widespread use of inhaled corticosteroids in inflammatory airway conditions, sometimes at doses above the therapeutic dose response curve, that is in asthma 0.8mg beclomethasone equivalent, (Lipworth, 1998) it is necessary to consider the potential side effects of this treatment. High dose inhaled corticosteroids can lead to an increased risk of systemic side effects (Pederson S, 1997; Lipworth, 1999). Systemic side effects include hypothalamic-pituitary-adrenal (HPA) axis suppression, thinning and bruising of the skin, cataracts and osteoporosis (Lipworth, 1999). HPA axis suppression can be used as a marker of the systemic activity of inhaled corticosteroids and the urinary cortisol creatinine ratio can be utilised to demonstrate HPA axis suppression (Kong, 1999; McIntyre, 1995).

1.5.2 β_2 AGONISTS

β_2-agonists are used to treat the symptoms of airway inflammation and are prescribed extensively in the treatment of asthma and COPD. They are useful in CF patients with evidence of bronchial hyperesponsiveness (Holzer, 1981). This drug class act via the β-adrenoceptor, which is linked to adenylate cyclase and produce an increase in intracellular cyclic 3'5'-adenosine monophosphate (cAMP) (Robison, 1967). Cyclic AMP then induces smooth muscle relaxation. The effect is exploited in the treatment of airway obstruction with selective β_2 adrenoceptor agonists. These drugs can be delivered directly to the airways via inhalation and act on β_2 adrenoceptors resulting in smooth muscle relaxation and bronchodilation.

β_2-agonists can be either short acting with duration of action around 4 hours or long acting with a duration of about 12 hours (Ullman, 1988). Salbutamol and bricanyl are short acting and can be administered via inhalers (usually MDI +/- spacer and turbohaler respectively) or nebulisation. Long acting β_2-agonists such as salmeterol and eformoterol can be administered alone or combined with the inhaled steroids fluticasone and budesonide respectively (Respiratory System, BNF 50, 2005). Short acting β_2-agonists are used on an as required basis and long acting are given twice daily to patients whose symptoms are uncontrolled on inhaled steroids alone (British Thoracic Society, 2003). In asthma the main action of β_2-agonists is relaxation and prevention of contraction of smooth muscle via the β-adrenoceptor. This controls bronchospasm and thus assists in ameliorating the symptoms related to asthma. They act as functional antagonists and complement the action of steroids. Long acting β_2-agonists also seemingly inhibit some of the processes involved in airway inflammation (Twentyman, 1990; Whelan, 1993; Jeffery, 2002).

In COPD, long acting β_2-agonists reduce dynamic hyperinflation during exercise and this leads to a reduction in perceived breathlessness (Belman, 1996). Short-acting agents are used on an as required basis to reduce exercise induced dypnoea (Belman, 1996). Long acting β_2-agonists improve health status and reduce symptoms (Dahl, 2001; Jones, 1997). Bronchoconstriction occurs in CF both as a consequence of the disease and as a side effect of nebulised antibiotics. The degree of bronchial reactivity can vary and can worsen during active infection. Treatment in those patients with prominent wheeze is appropriate but should be given following a therapeutic trial and monitoring with lung function in case of paradoxical bronchoconstriction (Macfarlane, 1990). Bronchodilators may be given prior to nebulised antibiotics in those patients who bronchoconstrict following this treatment. Bronchodilators are usually given via inhalers as this is as effective as nebulised treatment but some patients may prefer nebulised therapy (Parkin, 1995).

1.5.3 NEBULISERS and NEBULISED THERAPIES

Nebulised antibiotics and recombinant DNase (rhDNase) are widely prescribed in CF and can be administered via a variety of nebulisers. Nebulisation aims to deposit the drug within the inflamed airways.

Nebulisers produce aerosols by harnessing either compressed air or ultrasonic energy. Jet nebulisers use a driving gas through a narrow aperture (Venturi). Pari jet nebulisers combine continuous nebulisation with an intermittent increase in drug delivery during inspiration. This occurs via means of a valve that allows air to be drawn into the nebuliser during inhalation. The valve closes on expiration and although this reduces drug loss, a significant amount of drug is still wasted during exhalation (O'Callaghan, 1997).

Technological advancement has produced devices of greater efficiency and the recently developed adaptive aerosol delivery technology (AAD) is an example of this. Nebulisers using this technology (Halolite, Prodose) sense respiratory effort and are breath-synchronised devices. They deliver medication only during inspiration and reduce drug loss to that exhaled by the patient. They also adapt to the patients breathing pattern and pulse aerosol during the beginning of inspiration to allow deep lung deposition (Denyer, 2004).

Nebuliser deposition can be assessed by scintigraphy, a non-invasive radionuclide imaging technique. This technique allows visualisation and quantification of drug delivery within the lungs. Scintigraphy can therefore be utilised to demonstrate drug distribution and absorption and show the differences in airway deposition of various nebulisers (Newman, 1993).

The two main nebulised antibiotics used in CF are colomycin and tobramycin and these are employed in the treatment of pseudomonas colonisation. Pseudomonas colonisation of

the airways of CF patients is no longer inevitable and close monitoring for first isolates and rigorous treatment of this is required. Chronic infection can be prevented or delayed by eradication regimes following the first pseudomonas isolate (Littlewood, 1985). Eradication with nebulised colomycin alongside oral ciprofloxacin for 3 months has been shown to significantly reduce the incidence of permanent P. aeruginosa in early colonisation (Frederiksen, 1997). In those with chronic pseudomonas colonisation a regime of twice daily antibiotics (colomycin or TOBI, a preservative free pH-adjusted preparation of tobramycin for inhalation) has been shown to improve lung function, reduce exacerbations and reduce the density of pseudomonas in the lung (Hodson, 1981; Mukhopadhyay, 1996; Ramsey, 1999).

Recombinant DNase has been shown to improve lung function and reduce exacerbation rates in those patients with moderately severe CF (Fuchs, 1994). Trials are given in patients with fixed airflow obstruction, chronic sputum production, good adherence record and willingness to try. Treatment response is monitored via lung function at 1 month and a 10 % increase is generally taken as a positive response and an indication to justify continuation of treatment.

Bronchodilators such as β_2-agonists can also be administered via nebulisation and their effects are described above.

The nebuliser chosen is dependent on several factors and should be tailored to individual needs. The duration of nebulisation depends on the device and faster nebulisers are preferred because of the variety of nebulised therapies required in the treatment of CF. Jet nebulisers have a varied and sometimes prolonged nebulisation time and frequently waste medication. The AAD nebulisers allow efficient delivery of medication by maximising deposition in a shorter nebulisation time and minimising wastage by delivery during

inhalation. In choosing the correct nebuliser for an individual factors such as drug deposition, patient preference and cost all need to be considered.

1.6 AIMS OF THE THESIS

The aims of this thesis are to describe studies on diagnosis and treatment in airway inflammation. The respiratory conditions focused on are chronic obstructive pulmonary disease, asthma and cystic fibrosis. The diagnostic studies describe novel techniques that can be used to diagnose airway inflammation, monitor treatment response and help explain underlying pathophysiological mechanisms. The studies relating to pharmacological intervention concentrate on the use of nebulisers in CF, the treatment of smokers' cough and the side effects associated with inhaled steroids.

Exhaled NO is a quick non-invasive test that can be helpful in the diagnosis and management of asthma. Low levels are found in CF and Primary Ciliary Dyskinesia, conditions associated with bronchiectasis. Portable devices are now available which will allow application of this test at the bedside or in the outpatient department.

In the following chapter the use of exhaled NO in patients with asthma and cystic fibrosis is demonstrated. The relationship of exhaled NO to EBC pH and nitrate/nitrite levels in these conditions is also investigated.

Smoking, COPD, asthma and bronchiectasis are all associated with cough and airway inflammation. The cough associated with smoking can be used as a model of airway inflammation to test various antitussive agents. Chapter 4 describes a study on the antitussive effect of salbutamol on smokers with a chronic cough.

Inhaled steroids remain the cornerstone for the treatment of inflammatory airway conditions such as asthma and COPD. It is well known that oral steroids have systemic side effects but high dose inhaled steroids can also lead to HPA axis suppression. The

mechanisms that may underlie the level of HPA axis suppression associated with different delivery methods are examined.

Cystic fibrosis is a genetic disorder with recurrent infections leading to airway inflammation and nebulised antibiotics are used to treat the respiratory consequences of CF. Various nebulisers are available and differ in their ability to deliver the drug to the large and small airways. Scintigraphy is a method of analysing nebuliser deposition. A study demonstrating the use of scintigraphy in analysing nebuliser deposition in CF patients is described.

CHAPTER TWO

METHODS

2.1 SUBJECTS

Adult patients with Asthma, Cystic Fibrosis and Smokers cough were studied.

Healthy volunteers were recruited from a clinical trials database.

Subjects attended Castle Hill Hospital (CHH) Clinical Trials Department or Hull Royal Infirmary between 2000 and 2004.

The characteristics of the subjects are described in the individual chapters

2.2 SKIN-PRICK TESTING

Skin-prick testing was performed with the following allergens: Dermatophagoides pteronyssinus, Dermatophagoides farinae, mixed grasses, cat allergen, and dog allergen (Allergopharma, Reinbek, Germany). Atopy was defined as a wheal diameter 3-mm or more than the saline control at 15 minutes to at least one of the allergens.

2.3 PULMONARY FUNCTION (spirometry and bronchodilator reversibility)

Spirometry was performed with a Vitalograph compact spirometer (Vitalograph Ltd., Buckingham, UK). 3 forced expiratory breaths from total lung capacity to residual volume were performed. The best of these 3 manoeuvres was recorded as FEV1/FVC. Salbutamol reversibility was defined as $\geq$ 12% and > 200ml increase in FEV1, recorded 15 minutes after 400 micrograms salbutamol via MDI and spacer.

2.4 PEAK FLOW MEASUREMENTS

Peak expiratory flow (PEF) was measured using a Mini-Wright meter (Clement Clarke Ltd., Harlow, UK). Peak flow was measured as the best of 3 blows.

2.5 METHACHOLINE CHALLENGE

The methacholine bronchoprovocation test was used to determine bronchial hyperesponsiveness. It was performed according to ERS Guidelines (Sterk et al. 1993) using an Airway Provocation System (APS) (Jaeger Toennies GmbH, Hochberg, Germany) connected to Masterscope (Jaeger). Subjects attended CHH having withheld long-acting B2 agonists for at least 48 hours and short acting B2 agonists for at least 8 hours. A 32 mg/mL solution of methacholine chloride was prepared by combining 320 mg of methacholine chloride (Methapharm Inc, Brantford, Ontario, Canada) and 10 ml of a sterile diluent; sodium chloride 0.9%, phenol 0.4 % (Allergy Laboratories Inc, Oklahoma City USA)

3 ml of methacholine was placed in the medication chamber of the APS system. Aerosol was produced successively by a dosimeter attached to a nebuliser system incorporated into the APS system. The nebuliser power was 250 microL/min.

Patients received serial doubling of doses of methacholine until the FEV1 fell by 20% or until cumulative dose of 2.8 mg had been delivered. Spirometry and flow volume loop were performed using Masterscope (Jaeger) or Micromedical spirometer (American Thoracic Society 1987). The cumulative dose of methacholine required to produce a 20 % fall in FEV1 (PD20) was determined. Airway hyperesponsiveness was defined as a PD20 < 500 microg.

2.6 CITRIC ACID COUGH CHALLENGE

Concentration-response cough challenges to citric acid were undertaken using a compressed air driven nebuliser controlled by a breath activated dosimeter (Mefar MB3 CE; Bresia Italy) preset to a nebulisation time of 1 sec. The dosimeter output was 0.125 mL per inhalation. Solutions were prepared by serial dilution of 1 M citric acid (Production Pharmacy, Royal Hallamshire Hospital, Sheffield, UK) in sterile solution to obtain concentrations of 1mM, 10mM, 30mM, 100mM, 300mM and 1000mM.

Patients were instructed to exhale to functional residual capacity and then to inhale through a mouthpiece for 1 second until nebulisation had ceased. The number of coughs in the first 10 seconds after inhalation were recorded. There was a 30-second pause between each inhalation. Each concentration of citric acid was inhaled 4 times and incremental concentrations given. 0.9% saline inhalations were given intermittently to increase challenge blindness.

Log concentration response curves were constructed for each test and the concentration of citric acid causing 2 coughs per inhalation (C2). When the specific number of coughs was not reached at the highest concentration of provocant, values were recorded as >1000 mM.

2.7 MEASUREMENT OF FE_{NO}

FE_{NO} was measured using a chemiluminescence analyser (LR2500 series; Logan Research Ltd., Rochester, UK). Nose clips were applied immediately before the manoeuvre to prevent nasal exhalation. Subjects exhaled at a constant flow rate of 250 mL/s in accordance with ERS recommendations.[24] Measurements of FE_{NO} were taken from the plateau at the end of exhalation, as determined by simultaneous monitoring of CO_2. The

procedure was repeated three times and the mean value taken. The machine was calibrated before each use with standard calibration gases.

2.8 COLLECTION AND ANALYSIS OF EBC

EBC was collected using a device which consisted of a mouthpiece and a 2-way non-rebreathing valve connected by polypropylene tubing to a glass Dreschel flask immersed in crushed ice, acting as a condensing chamber. Subjects breathed at a normal frequency and tidal volume for 15 minutes while wearing nose clips, allowing collection of 1.5-2.5 mL of condensate. The pH was measured immediately with a benchtop pH meter (Fisher Scientific Instruments, Loughborough, UK). Vaughan et al have previously shown that, at least in the case of deaerated specimens, EBC pH measurements are highly reproducible and independent of condenser temperature, ventilatory pattern, duration of collection, and degree of airway narrowing. The EBC samples were not deaerated but in preliminary experiments the pH was shown to remain stable for at least 5 minutes after collection. For subsequent assay of NO_2 and total NO_2 plus NO_3 (NO_2/NO_3), EBC was frozen in 250-μL aliquots at -80°C. After thawing, 500-μL samples were spun at 13000 rpm for 30 minutes in a centrifugal filter device with a 10-kDa molecular weight cut-off (Microcon YM-10; Millipore Corporation, Bedford, MA, USA). Concentrations of NO_2 and of NO_2/NO_3 in filtrates were determined in duplicate using a commercially available colorimetric assay kit according to the manufacturer's instructions (Cayman Chemical Company, Ann Arbor, MI, USA). The detection limit was approximately 1-μM. All samples underwent a single freeze-thaw cycle.

2.9 SCINTIGRAPHY

An ADAC Forte (Phillips) gamma camera was used with the two heads positioned at 180 degrees relative to one-another. A low energy general purpose collimator with parallel holes was used and an energy window of 140KeV ±10% to reduce the radiation scatter from the images.

Diethylenetriaminenpentaacetate (DTPA) labelled technetium-99m was used as an indirect label to quantitatively measure the pulmonary deposition of nebulised Salbutamol (5mg in 2.5ml saline solution). Technetium is a gamma emitter; it has a half life of 6.02hrs and energy 140KeV±10% which makes it ideal for lung ventilation imaging. The inert DTPA has similar kinetic properties and viscosity to Salbutamol making it a valid indirect marker.

Static anterior and posterior images of the lungs were taken simultaneously for 240s using the full field of view. The nebuliser chamber was imaged for 10s and the mouthpiece for 30s using a single image resolution. A 256x256x16 pixel image resolution was used. The patients' subcutaneous tissue thickness was measured and used for attenuation correction purposes.

A software plug-in was developed for Image J freeware (National Institutes of Health by Federal Government) to process the gamma camera DICOM scintigraphic images. The original time and activity of the syringe containing the radioactivity and the residual syringe time and activity previously measured were accepted as input. The patient details were automatically transferred from the scintillation images and nebuliser type, image duration, start time and patients' subcutaneous tissue thickness was input into the program. The decay corrected radioactivity was then automatically calculated. Regions of interest (ROI) were drawn around the right and left anterior and posterior lung and

associated background regions were drawn outlining each lung region. The plug-in calculated the number of pixels, mean and total number of counts within each ROI. The software then calculated the background and attenuation corrected geometric mean as a percentage of original starting activity within each ROI. This process was repeated for the percentage of activity that remained un-nebulised in the chamber, deposited in the mouthpiece and filter (Pari only).

ROI drawings were repeated several days later by the same operator and another member of the research team allowing intra and inter contour comparisons and repeatability measurements to be made.

2.10 STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for the Social Sciences version 10 for Windows (SPSS Inc; Chicago IL, USA) or Statview 5.01 for Macintosh (Abacus Concepts, Berkeley, CA, USA) and CIA version 2.1.1 software (University of Southampton, Southampton, UK). Specific statistical tests are described in the individual chapters. A p-value of < 0.05 was regarded as statistically significant.

2.11 ETHICS APPROVAL

Ethical approval was sought for all studies via the Hull and East Riding Local Research Ethics Committee and Hull and East Yorkshire Hospitals NHS Trust R&D Group. The study involving scintigraphy, was also approved by the Administration of Radioactive Substances Advisory Committee (ARSAC).

Subjects gave informed consent.

CHAPTER 3

EXHALED BREATH CONDENSATE pH AND EXHALED NITRIC OXIDE IN ALLERGIC ASTHMA AND IN CYSTIC FIBROSIS

3.1 INTRODUCTION

Nitric oxide (NO) can be detected by chemiluminescence in exhaled air of humans (Gustafsson LE, 1991) and there is now a large body of literature describing the expression of NO in the respiratory tract in health and disease. In the absence of corticosteroid treatment, the fractional exhaled concentration of NO (FE_{NO}) is elevated in adults (Alving K, 1993; Kharitonov SA, 1994; Persson MG, 1994) and children (Lundberg JON, 1996) with allergic asthma. The FE_{NO} in asthma is further increased during acute exacerbations and falls with response to treatment (Massaro AF, 1995). In cystic fibrosis (CF), on the other hand, the FE_{NO} has been reported to be either lower than (Lundberg JON, 1996; Grasemann H, 1997) or similar to (Balfour-Lynne IM, 1996; Dotsch J, 1996; Ho LP, 1998) that in healthy control subjects and not to change during pulmonary exacerbations (Ho LP, 1998; Linnane SJ, 1998).

Endogenous NO may be synthesized during conversion of the amino-acid L-arginine to L-citrulline by enzymes of the nitric oxide synthase (NOS) family (Moncada S, 1993). Three distinct isoforms of NOS, encoded by separate genes, have been cloned and characterized. Neuronal NOS (nNOS) or NOS1 and endothelial NOS (eNOS) or NOS3 generate relatively small (picomolar) amounts of NO that are believed to play a homeostatic role in many physiologic processes. Inducible NOS (iNOS) or NOS2, in contrast, produces much higher (nanomolar) levels of NO at sites of inflammation. Although transcriptionally regulated by proinflammatory stimuli, iNOS is constitutively expressed in normal human airway epithelium (Guo FH, 1995). Consistent with the changes in FE_{NO}, epithelial expression of iNOS mRNA and protein is increased in allergic

asthma (Hamid Q, 1993; Saleh D, 1998; Redington AE, 2001) whereas iNOS immunostaining is reduced in the airway epithelium in CF (Kelley TJ, 1998; Meng Q-H, 1998).

Recent controversy has centred on the potential ability of airway lining fluid pH to regulate FE_{NO} independently of NOS activity. Hunt et al. (Hunt JF, 2000) collected exhaled breath condensate (EBC) from patients hospitalized with acute asthma and found that the pH was over two log-orders lower than that of nonasthmatic control subjects. The degree of acidification was shown to be sufficient to generate NO nonorganically from nitrite (NO_2) in vitro. These authors therefore proposed that pH might be an important determinant of FE_{NO}. In subsequent work, low EBC pH has also been described in cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), and bronchiectasis (Kostikas K, 2002). However, direct comparisons of EBC pH and FE_{NO} have not been made in any of these studies.

In this study, the relationship between exhaled NO and airway acidification in inflammatory airway disease was examined further. The aim was to establish whether alterations in EBC pH and FE_{NO} were necessarily associated or could occur independently of each other. To address this, the study compared FE_{NO}, EBC pH, and EBC NO_2 and nitrate (NO_3) - stable end-products of NO metabolism - in subjects with asthma, subjects with CF, and healthy control subjects.

3.2 METHODS

3.2.1 Subjects

The study population comprised 12 subjects with atopic asthma, 18 patients with stable CF, and 15 healthy control subjects. Asthma was defined according to American Thoracic Society criteria.[22] Subjects in this group had mildly symptomatic and clinically stable disease. None was a cigarette smoker. They were receiving treatment with inhaled β_2-agonists as required but none had taken inhaled or oral corticosteroids, or any other form of preventative anti-asthma medication, within at least 6 weeks of the study. Patients with CF had the diagnosis confirmed by genotype analysis, with the homozygous ΔF508 mutation identified in 12 cases. They were all taking vitamin supplements, pancreatic enzyme supplements, and inhaled or nebulized β_2-agonists as necessary. In addition, 12 CF patients were receiving treatment with nebulized antibiotics (colistin or gentamicin), 3 with nebulized DNase, and 9 with an inhaled corticosteroid (budesonide or fluticasone propionate). One subject in the CF group was a cigarette smoker. Eight of the 18 CF patients were studied on a separate occasion within 3 days of the start of a pulmonary exacerbation. For the purpose of the study, a pulmonary exacerbation was defined empirically on the basis of clinical characteristics (increase in cough, breathlessness, sputum volume, or sputum purulence) in combination with a requirement for intravenous antibiotics, as judged by the supervising clinician. The control group consisted of healthy non-smoking non-atopic volunteers with no respiratory symptoms and no history of lung disease. Subjects then underwent skin-prick testing, spirometry and reversibility, measurement of FE_{NO} and collection and analysis of EBC as detailed in

the methods chapter. All control subjects had normal spirometry and normal airway responsiveness.

3.2.2 Statistical Analysis

Subjects' ages, spirometric measurements and FE_{NO} data were expressed as a mean (SD). Data for EBC pH and for NO_2 and NO_2/NO_3 concentrations were expressed as median [range]. Comparisons between asthmatic and control groups and between stable CF and control groups were undertaken using an unpaired t tests or Mann-Whitney U test, as appropriate. Comparisons of paired data for CF patients in stable disease and in exacerbation were performed with a paired t-test or Wilcoxon test, as appropriate. In the case of NO_2, some values were below the detection limits of the assays and were therefore assigned an arbitrary value of 0 for the purpose of analysis. Correlations were sought using Pearson's test or Spearman's test for normally and non-normally distributed data, respectively.

3.3 RESULTS

The clinical and physiological details of the subjects studied are summarized in Table 3.1. Spirometric values in CF were lower on average during acute exacerbations than in stable disease but the differences were not statistically significant. Asthmatic subjects exhibited varying degrees of airway hyperresponsiveness whereas control subjects all had airway responsiveness in the nonasthmatic range. Methacholine bronchoprovocation was not performed in patients with CF.

The FE_{NO} was significantly greater in subjects with asthma than in healthy control subjects (35 (19) vs. 9 (4) ppb, difference between means 26 ppb [95% CI 15 to 36 ppb]; p<0.001) (Figure 3.1). The EBC pH, however, was similar between the asthmatic and control groups (5.82 [5.19 - 6.33] vs. 6.08 [5.58 - 6.64], median difference -0.18 [95% CI -0.43 to 0.11]; p=0.23) (Figure 3.2). Concentrations of NO_2 in these EBC samples were below the measurable range (<1 μM) except in 5 cases (2 asthmatic and 3 nonasthmatic) where it was present at low levels (Figure 3.3). Levels of NO_2/NO_3 were on average higher in EBC samples from asthmatic subjects, but the difference did not reach statistical significance (21.1 [<1 - 63.3] μM vs. 11.4 [<1 - 67.3] μM, median difference 12.3 [95% CI -3.8 to 28.1] μM; p=0.17). There were no significant correlations between FE_{NO}, EBC pH, EBC NO_2/NO_3 levels, and physiological indices in either the asthmatic or the control group.

In stable CF, the FE_{NO} was significantly lower than in the control group (4 (3) vs. 9 (4) ppb, difference between means -6 ppb [95% CI -3 to -8 ppb]; p<0.001) (Figure 3.1) as was the EBC pH (5.77 [4.81 - 6.99] vs. 6.08 [5.58 - 6.64], median difference -0.34 [95% CI -0.61 to -0.12]; p=0.003) (Figure 3.2). In contrast, levels of EBC NO_2 in stable CF were significantly higher than in control subjects (2.0 [1.1 - 13.5] μM vs. <1 [<1 - 5.4] μM, median difference 1.9 [95% CI 8.7 to 28.2] μM; p<0.001) as were levels of NO_2/NO_3 (29.9 [11.9 - 51.0] μM vs. 11.4 [<1 - 67.3] μM, median difference 19.7 [95% CI 8.7 to 28.2] μM; p=0.002) (Figure 3.3). There were direct correlations between FE_{NO} and both FEV_1 % predicted (r=0.64, p=0.012) and FVC % predicted (r=0.53, p=0.049) (Figure 4), but no significant correlations between EBC pH, NO_2, or NO_2/NO_3 and these physiological parameters. There was a further fall in EBC pH during CF exacerbations

compared with stable disease (5.30 [4.99 - 5.86] vs. 5.77 [4.81 - 6.99], median difference -0.53 [95% CI -1.16 to -0.11]; p=0.017) but no significant change in FE_{NO} (3 (2) vs. 4 (3) ppb, difference between means 0 [95% CI -2 to 2 ppb]; p=0.91). Levels of EBC NO_2 (2.4 [<1 - 4.8] μM) and NO_2/NO_3 (36.6 [13.5 - 96.5] μM) during CF exacerbations were higher than in stable disease but not significantly so.

Table 3.1. Clinical and physiological characteristics of subjects studied.

	†Age (yr)	Sex (M/F)	†FEV_1 (% predicted)	†FVC (% predicted)	†FEV_1/FVC (%)	‡Methacholine PD_{20} (mg)
Control (n=15)	39 (10)	6/9	103 (15)	107 (16)	82 (5)	>1.5
Asthma (n=12)	38 (16)	7/5	**79 (18)	102 (16)	66 (12)	0.068 [<0.0256 - 1.3]
CF Stable (n=18)	**24 (8)	11/7	**68 (25)	*88 (17)	**65 (16)	-
CF Exacerbation (n=8)	28 (9)	4/4	58 (25)	82 (20)	59 (17)	-

Data are presented as †mean (SD) and ‡geometric mean [range].
Comparisons between asthmatic and control groups and between stable CF and control groups were performed using an unpaired t-test. Within-subject comparisons of spirometric measurements for the 8 CF patients studied in exacerbation were undertaken with a paired t-test.
*$p=0.002$ vs. control group **$p<0.001$ vs. control group
Abbreviations: FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; PD_{20}: provocative dose (of methacholine) required to produce a 20% fall in FEV_1.

Figure 3.1 Measurements of FENO in control subjects (n = 15), subjects with mild stable atopic asthma (n = 12), subjects with stable CF (n = 14), and subjects with CF during exacerbation (n = 7). Horizontal bars represent mean values. For technical reasons, data were unavailable for four of 18 CF subjects during stable disease and for one of eight CF subjects during exacerbation. Comparisons were performed using paired and unpaired t tests, as appropriate.

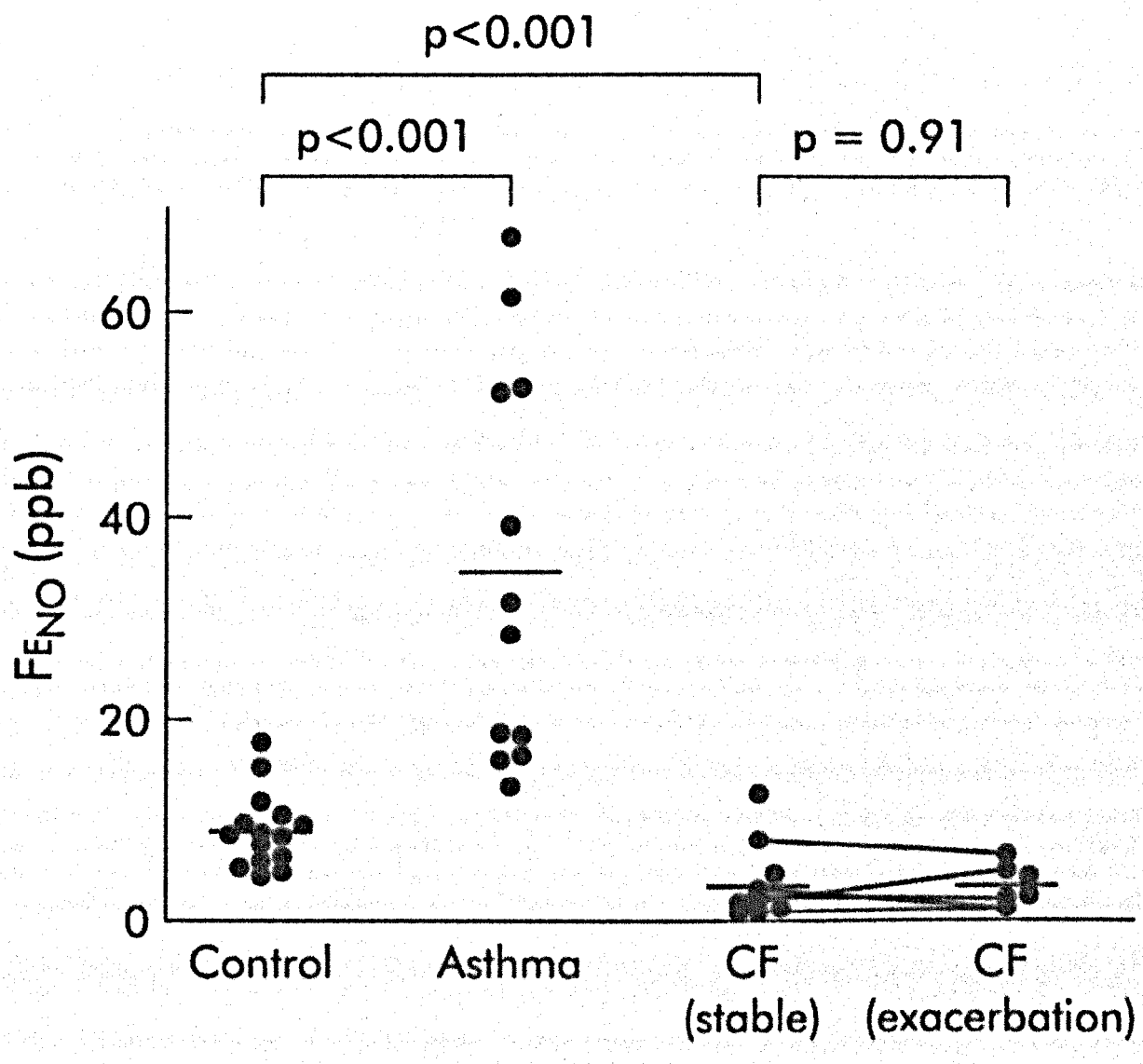


Figure 3.2 Measurements of EBC pH in control subjects (n = 15), subjects with mild stable atopic asthma (n = 12), subjects with stable CF (n = 18), and subjects with CF during exacerbation (n = 8). Horizontal bars represent median values. Paired and unpaired data were compared using the Mann-Whitney and Wilcoxon tests, respectively.

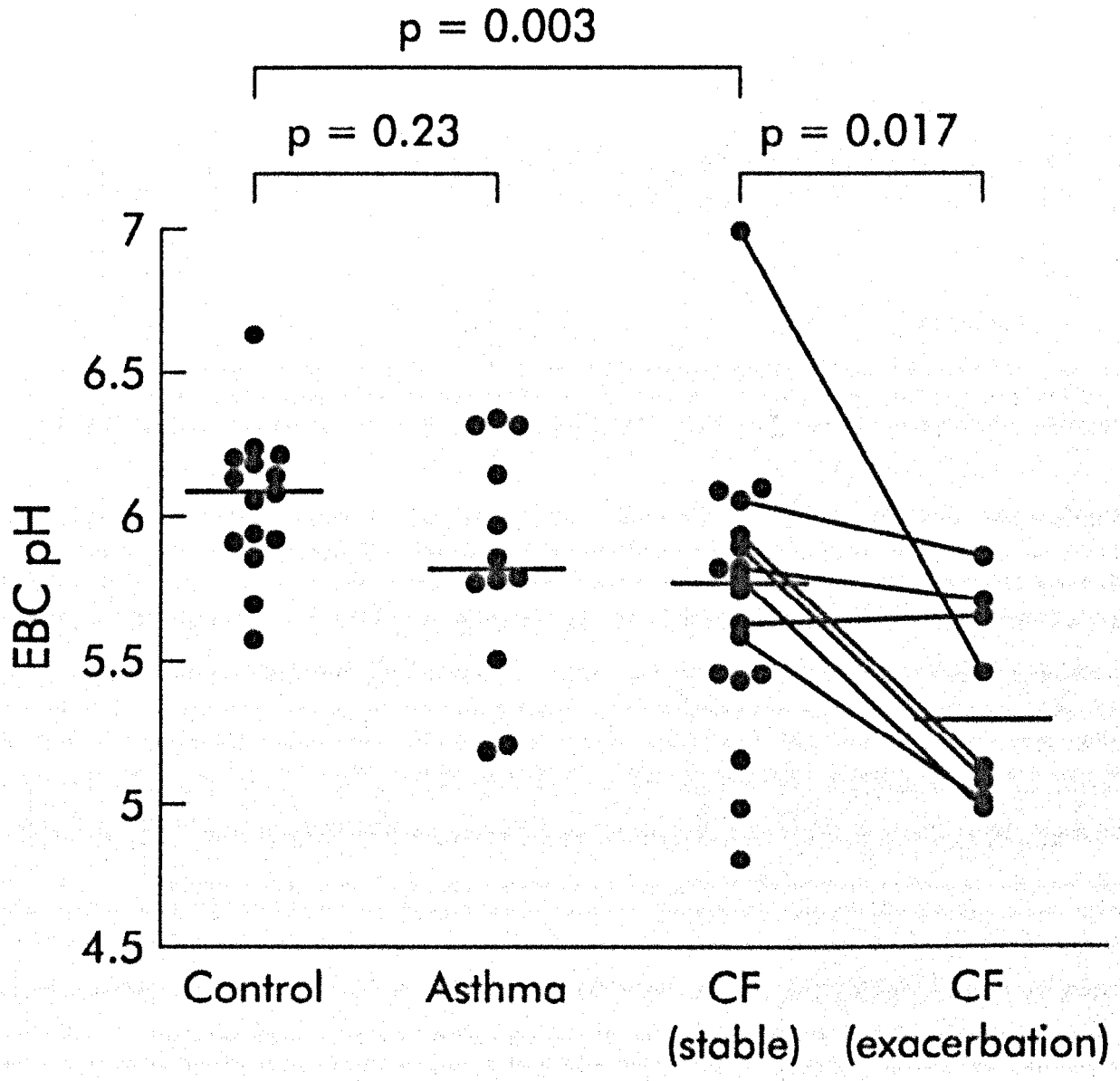


Figure 3.3

Measurements of (A) EBC NO2 and (B) NO2/NO3 in control subjects (n = 15), subjects with mild stable atopic asthma (n = 12), subjects with stable CF (n = 17), and subjects with CF during exacerbation (n = 7). For technical reasons, data were unavailable for one of 18 CF subjects during stable disease and for one of eight CF subjects during exacerbation. Horizontal bars represent median values. Paired and unpaired data were compared using the Mann-Whitney and Wilcoxon tests, respectively.

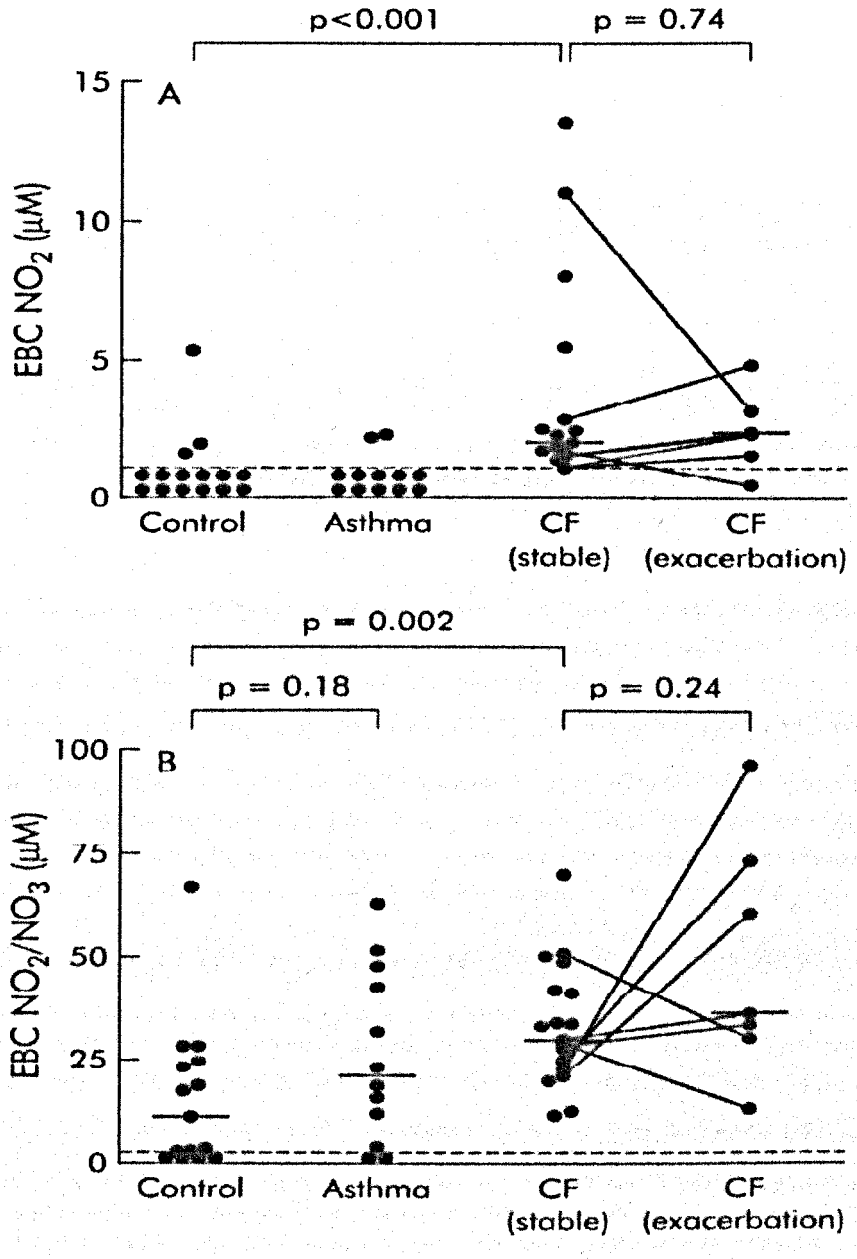
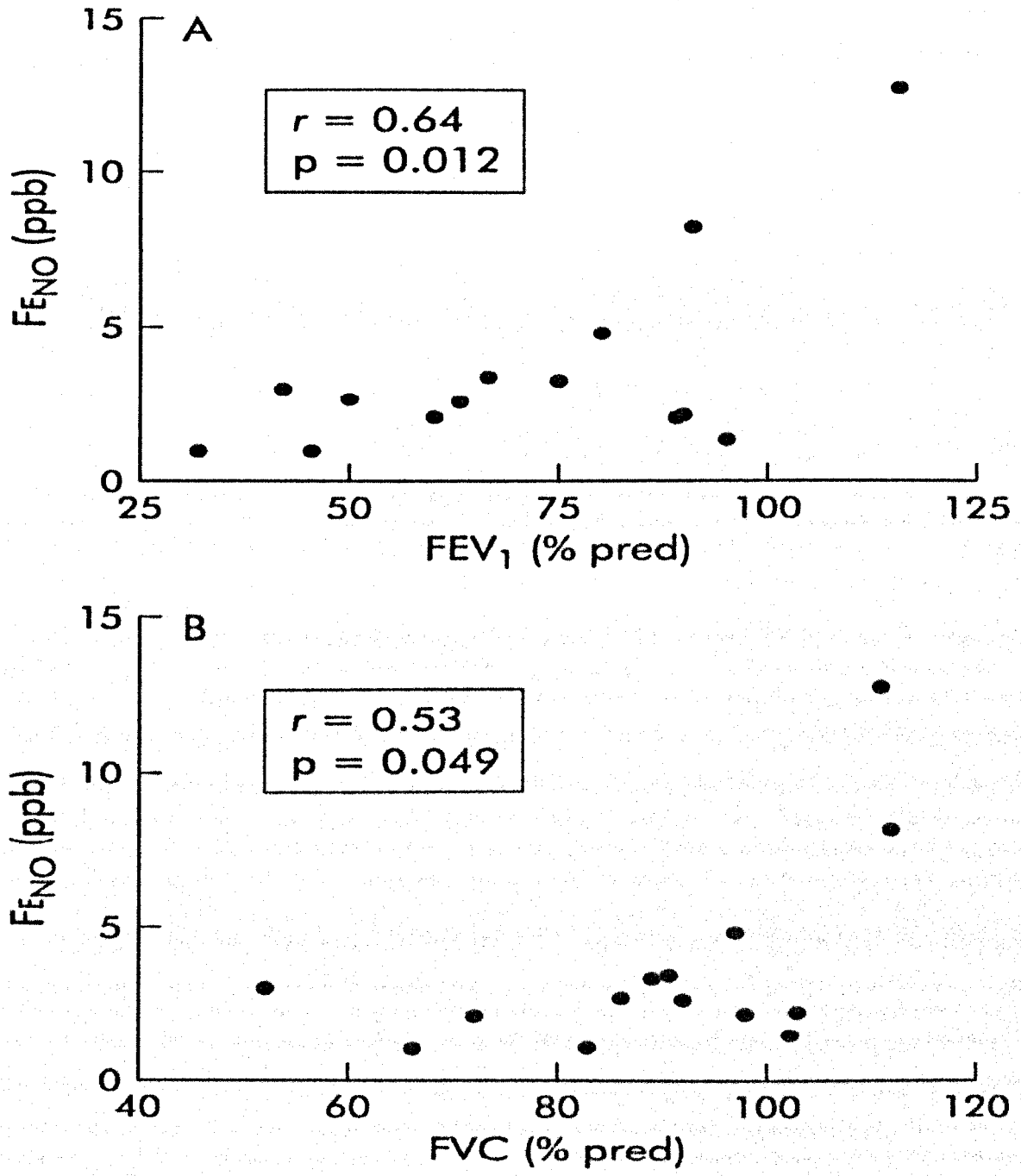


Figure 3.4
Correlations between FENO and (A) FEV1 % predicted and (B) FVC % predicted in subjects with stable CF (n = 14). Statistical analysis was performed using Pearson's test.



3.4 DISCUSSION

Collection of EBC is attracting increasing interest as a novel method to sample lower airway lining fluid. Compared with techniques such as bronchoalveolar lavage (BAL) and sputum induction, EBC offers the advantages that it is safe, inexpensive, noninvasive, may be repeated at frequent intervals, and can be performed by children and the elderly. Previous reports of disturbances of EBC pH in various forms of respiratory disease have led to speculation regarding the relationship between airway pH and exhaled NO. This is however, the first study directly to compare EBC pH and FE_{NO} in the same individuals.

In subjects with mild stable allergic asthma not receiving treatment with corticosteroids, this study showed that the FE_{NO} was elevated and levels of EBC NO_2/NO_3 were also on average higher than those of control subjects. In contrast, the EBC pH was normal. The mechanism of the elevated FE_{NO} in these asthmatic subjects does not therefore appear to be acidification of endogenous NO_2. A more likely explanation is the increased expression of iNOS that has previously been reported in asthmatic airways (Hamid Q, 1993; Saleh D, 1998; Redington AE, 2001). Further support for an enzymatic source of exhaled NO derives from the observation that nonselective NOS inhibitors such as N^G-nitro-L-arginine methyl ester (L-NAME) and N^G-monomethyl-L-arginine (L-NMMA) produce a marked reduction in FE_{NO} in asthmatic (and also nonasthmatic) subjects (Yates DH, 1995).

The increased FE_{NO} in subjects with mild asthma is consistent with a number of earlier studies, (Alving K, 1993; Kharitonov SA, 1994; Persson MG, 1994) and the values measured are in agreement with those previously reported. Some studies have also described significant increases in NO_2 and/or NO_2/NO_3 concentrations in EBC (Hunt JF, 2000; Hunt J, 1995; Ganas K 2001) and induced sputum (Kanazawa H, 1997) in asthma. In the present study, NO_2 was below the limit of detection in most samples and NO_2/NO_3 measurements were variable, with the difference between groups not statistically significant. These findings are consistent with previous observations in BAL fluid where levels of NO_2 and of NO_3 were similar in healthy control subjects, mild non-steroid treated asthmatics, and the same asthmatic subjects following treatment with fluticasone propionate (NO_2: medians 0.74, 0.97, and 0.45 μM; NO_3: 15.1, 16.5, and 15.4 μM) (A. E. Redington; unpublished data). The normal EBC pH in asthma in the present study is consistent with the data of Kostikas et al. (Kostikas K, 2002) who reported that EBC pH was decreased in moderate, but not mild, asthma. Similarly, Hunt et al. (Hunt JF, 2000) found that the low EBC pH in acute asthma rapidly normalized with antiinflammatory treatment. A reduction in EBC pH, and perhaps by inference a contribution of airway acidification to FE_{NO}, appears therefore to be a feature only of more severe disease, not of mild asthma.

In contrast to the situation in asthma, stable CF was associated with acidification of EBC and a further reduction in EBC pH occurred during acute pulmonary exacerbations. Despite the presence of NO_2 and NO_3 reserves in quantities that were elevated compared to control subjects, the reduced pH did not lead to an elevation in FE_{NO}. To the contrary,

FE_{NO} levels in CF were significantly reduced, both in stable disease and during acute pulmonary exacerbations, consistent with previous reports

(Lundberg JON, 1996; Grasemann H, 1997; Dotsch J, 1996; Linnane SJ, 1998). These findings indicate that airway acidification, as assessed by EBC pH, does not necessarily increase NO generation, even in the presence of plentiful NO_2 reserves. They confirm and extend the findings of Tate et al. (Tate S, 2002) who also demonstrated acidification of EBC in CF but did not measure FE_{NO}. Furthermore, the observation that FE_{NO} was reduced in CF whereas levels of NO_2 and NO_2/NO_3 were elevated argues against the possibility that these latter constituents are generated by oxidation of NO ex vivo.

Several mechanisms may be considered to explain the reduced EBC pH in CF. Although its role is still not well defined, cystic fibrosis transmembrane conductance regulator (CFTR) facilitates HCO_3^- secretion in the pancreas and there is also evidence of apical HCO_3^- conductance by CFTR in cultured human airway epithelial cells (Smith JJ, 1992; Devor DC, 2000). Defective epithelial HCO_3^- transport in CF could therefore lead to luminal acidification. However, individual EBC pH data points for one patient with the heterozygous mutation R117H/ΔF508, a genotype associated with significant retention of HCO_3^- conductance, (Reddy MM, 2003) did not represent outliers. Furthermore, a low EBC pH is not specific for CF but appears rather to be a more general feature of inflammatory airways disease, suggesting a role for leukocyte infiltration and activation. Stable CF is associated with predominantly neutrophilic airway inflammation and the degree of sputum neutrophilia is more pronounced during pulmonary exacerbations (Fahy JV, 1995). Neutrophil activation has been linked to pH reduction in animal models of

pleural sepsis (Sahn SA, 1983) and the EBC pH in COPD is inversely correlated with induced sputum neutrophil counts (Kostikas K, 2002). Finally, buffering of airway lining fluid in CF might be impaired by an inflammation-dependent reduction in epithelial glutaminase expression and NH_3 generation, as has been described in acute asthma (Hunt JF, 1995).

The mechanism underlying the low FE_{NO} in CF, despite the presence of chronic airways infection and inflammation, is uncertain. Firstly, inhaled corticosteroid treatment (which was being taken by 9 of the 18 CF patients in contrast to none of the asthmatic or control subjects) and must be considered as a possible confounding factor. FE_{NO} measurements did not differ significantly between those CF patients who were receiving inhaled corticosteroids and those who were not (mean 5 vs. 3 ppb), although the possibility of a type II error cannot be excluded. Previous studies of the effect of corticosteroids on FE_{NO} in CF have produced conflicting findings (Balfour-Lynne IM, 1996; Linnane SJ, 2001). Secondly, the excess secretions and mucus in CF airways may inhibit the diffusion of gaseous NO into the airway lumen. This would be consistent with the positive correlations demonstrated between FE_{NO} and spirometric measurements in stable CF, as more advanced lung disease is presumably associated with increased retention of mucus. It is also in keeping with the elevated EBC levels of NO_2 and NO_2/NO_3 in CF demonstrated here and in other studies of NO metabolites in EBC (Tate S, 2002; Ho LP, 1998) or sputum (Linnane SJ, 1998; Grasemann H, 1998). Finally, there may be a primary defect in NO production in CF. Expression of iNOS is decreased in CF airway epithelium CF (Kelley TJ, 1998; Meng Q-H, 1998) and the presence of a low FE_{NO} in infants with newly diagnosed disease (Elphick HE, 2001) suggests that this is not simply

a consequence of inflammation. Moreover, experiments in human tracheal epithelial cells in vitro and in CFTR-deficient mice have shown that loss of functional CFTR results in reduced epithelial iNOS expression and NO generation (Steagall WK, 2000)

Theoretical concerns have been raised about whether EBC measurements are truly reflective of the lower airways in terms of pH and concentrations of nonvolatile solutes. Firstly, fluid formation involves the dilution of aerosolized droplets by condensed exhaled water vapour and the degree of dilution, as estimated from condensate electrolyte concentrations, can be both substantial and highly variable (Effros RM, 2002). Currently, there is no readily available dilutional marker that can be used to calculate absolute concentrations of nonvolatile solutes in lower respiratory tract lining-fluid. Despite this, their relative concentrations in EBC may serve to provide information about the presence and activity of disease processes. This would be consistent with recommendations for interpreting solute concentrations in BAL fluid, (Haslam PL, 1999) where a reliable dilution marker is also lacking. A second concern has been that exhaled NH_3, derived mainly from the mouth, might influence condensate pH independently of processes in the lower airways (Effros RM, 2002). Against this, the pH of undiluted tracheal secretions obtained during bronchoscopy is almost identical to same-subject EBC collections (Hunt JF, 2000) and, at least in the absence of respiratory disease, similar observations have been made in EBC collected from isolated lower airways (Vaughan J, 2003).

In conclusion, mild stable atopic asthma is associated with a normal EBC pH and an elevated FE_{NO} in contrast to CF where the EBC pH is low, particularly during acute

exacerbations, but FE_{NO} is reduced. These findings demonstrate a dissociation between EBC pH and FE_{NO} in inflammatory airways disease.

They also draw attention to the complexity of NO metabolism, where multiple pathways of NO synthesis and clearance are likely to have variable relevance in different circumstances.

CHAPTER FOUR

EFFECT OF SALBUTAMOL ON SMOKING RELATED COUGH

4.1 INTRODUCTION

Chronic cigarette smoking leads to a dose related cough and smokers cough is typically productive (Janson C, 1991; Gerrard JW, 1980; Cullinan P, 1992). It could be argued that cough is one of the symptoms which drives the patient to quit and that any therapy aimed at treating the symptoms of smoking could encourage patients to continue the habit. However smoking cessation is associated with a number of symptoms including craving, depression and continued or heightened cough (Ward MM, 2001). If cigarettes diminished cough or inhibited the cough reflex in smokers there would be a positive feedback mechanism encouraging continued smoking. The presence of cough and the need for a cigarette to inhibit or "break" the cough may cause failure of attempted smoking cessation.

Non specific anti tussive therapy is at best only partially effective (Parvez L, 1996).

A more successful approach in the treatment of cough is to target the mechanisms causing cough. Smoking is associated with airway inflammation manifested as an increase in macrophages and cells expressing interleukin and adhesion molecule receptors. The consequence of this inflammation is an increase in non-specific bronchial hyperresponsiveness, even in subjects with normal lung function (Gerrard JW, 1980).

This study hypothesised that targeting therapy at this consequence of inflammation could lead to an effective antitussive in smoking related cough. Beta agonists have a marked bronchoprotective effects on non-specific bronchial hyperresponsiveness but have little or no effect on the cough reflex in health. Salbutamol has no effect on induced cough in normal subjects, but does alter cough response in asthmatics (Pounsford JC, 1985).

A study was performed to determine the antitussive effects of salbutamol in smoking related cough.

4.2 METHODS

A two way cross over study was performed to determine the efficacy of salbutamol via inhaler versus placebo on natural (i.e. on waking, before contact with triggers) and evoked (following the first cigarette) cough in habituated smokers.

The primary efficacy endpoint was a reduction in cough frequency after waking on treatment day one over the following periods: 0-10 min, 10-20 min, 20-40 min, and 40 min- 1 hour. The secondary efficacy endpoints were a reduction in cough frequency on treatment days 2-5, nocturnal disturbance due to cough, change in cough symptoms. (severity, chest tightness, expectoration volume, global symptom score), lung function recorded by peak flow measurements and cough threshold elevation measured as a response to citric acid challenge.

71 subjects were screened following recruitment by external advertisement. 44 healthy male and female smokers aged 18 to 65 years with chronic troublesome cough were randomised. Baseline spirometry was required to demonstrate FEV_1 >80% predicted. Subjects had to smoke 15 cigarettes a day with a greater than 5 pack year smoking history. Morning cough was required on all five days of screening diary record card assessment and persistent daily morning cough for a minimum of three months prior to entry onto the study.

The initial screening to assess suitability for the study involved salbutamol reversibility (< 12% with 400 microgram via MDI and spacer) and a methacholine challenge (Provocative dose causing a fall in FEV1 of 20% (PD20) <0.5mg) to detect undiagnosed asthma (Crapo RO, 2000). Subjects attended two treatment periods of five days separated by a washout period of 2-7 days.

They were admitted to the Clinical Trials Unit at Castle Hill Hospital on the night prior to treatment day one, were randomised and told to continue smoking as normal until midnight when they abstained from cigarettes.

On waking the next morning overnight abstinence was confirmed by the use of carbon monoxide monitoring (Smokealyser) (Middleton ET, 2000). Peak flow was recorded and then subjects received either 400 micrograms of salbutamol or placebo via a MDI and spacer. Cough frequency was recorded from 0-10 minutes and 10-20 minutes using a voice activated analogue tape recorder. Coughs following a cigarette at 20 minutes were observed at 20-40 minutes and 40-60 minutes.

1 hour after medication subjects underwent a citric acid cough challenge (Morice A, 2001). Sensitivity of the cough reflex was expressed as C2 measurements (The dose of citric acid causing at least 2 coughs per inhalation). Subjects were then allowed to smoke as they wished with the number and time of cigarettes recorded. Cough challenge was then repeated at 2 and 4 hours. They then received 200 micrograms of salbutamol or placebo at midday.

Patients then went home with their diary card and allotted medication of salbutamol or matched placebo via MDI and spacer. Medication schedule on discharge was Day1: 1 evening dose, Day 2: First dose in the morning on waking, second dose at mid-day and third dose in the evening. Days 3, 4 and 5: medication taken as required but no more than 3 doses in 24 hours.

Procedures in treatment period 2 mirrored those in treatment 1. Subjects were asked to smoke with the same frequency and at the same points as they did during study day 1 in the Clinical Trials Unit. Following discharge with a diary card subjects were allowed to

smoke as they wished. At the end of the treatment they were asked to record any treatment preference.

4.3 STATISTICAL ANALYSIS

Results are expressed as arithmetic mean with 95% confidence intervals (CI) for cough frequency and geometric mean for citric acid cough challenge C2. Statistical comparison was by paired t test. Hypotheses tests were 2 sided and of 5% significance level. The results were analysed on an intention to treat basis.

4.4 RESULTS

31 males and 13 females of age range 20-61 years were recruited. Mean FEV_1 was 4.1 l (Range 2.8-6) and mean pack years 20.4 (Range 5 to 100). At screening mean salbutamol reversibility was 5 % and mean PD10 was 0.67 mg.

Total cough frequency was reduced from 6 to 4.5 in the first 20 minutes after salbutamol inhalation ($P < 0.05$, CI of difference 0.02 and 2.9). There was no statistically significant reduction in total cough frequency at the other time intervals. (Figure 4.1 and Tables 4.1 & 4.2). On the placebo day cough frequency 20 minutes post cigarette was significantly less than in the 20 minutes pre cigarette: 6 versus 3.9 coughs ($P = 0.02$, CI 0.8 - 3.4). There was no significant difference in cough frequency pre and post cigarette in salbutamol subjects. $P = 0.2$ and CI –0.4 and 1.8. (Figure 4.2).

Citric acid cough challenge was significantly increased by salbutamol in the first hour. $P = 0.01$, CI of the mean difference 17.5 - 196.7. Subsequent cough challenge was not significantly different (Figure 4.3).

No statistically significant difference between placebo and salbutamol was found for mean nocturnal disturbance, cough severity, chest tightness and morning peak flow. There was a small but significant increase in evening peak flow in the salbutamol arm of the study. Placebo mean evening peak flow was 545.5 compared to 554.8 for salbutamol ($p< 0.01$, CI 2.5 - 16.1).

TABLE 4.1

COUGH FREQUENCY IN FIRST HOUR FOLLOWING MEDICATION

TIME	**Cough frequency post placebo**	**Cough frequency post salbutamol**	**P VALUE (95% confidence)**
0-20 MIN	6	4.5	0.05 (0.02, 2.9)
20-40 MIN	3.9	3.9	0.918 (-0.9, 0.8)
40-60 MIN	2.5	2.6	0.830 (-0.8, 0.9)

* Cigarette given at 20 minutes

TABLE 4.2

COUGH FREQUENCY DAY 2 TO 5

TIME	Cough frequency post placebo	Cough frequency post salbutamol	P VALUE (95% confidence)
Day2 Waking-12pm	10.3	10.8	0.676 (-1.8, 2.8)
Day2 12pm-10pm	12.6	11.8	0.546 (-3.8, 2.1)
Day3 Waking-12pm	12.0	12.0	0.728 (-4.1, 2.9)
Day3 12pm-10pm	13.0	13.1	0.991 (-2.9, 2.9)
Day4 Waking-12pm	11.3	12.8	0.161 (-0.6, 3.4)
Day4 12pm-10pm	11.9	12.7	0.583 (-1.7, 3.0)
Day5 Waking-12pm	11.3	10.5	0.738 (-3.7, 2.6)
Day5 12pm-10pm	13.0	12.8	0.760 (-2.9, 2.1)

FIGURE 4.1

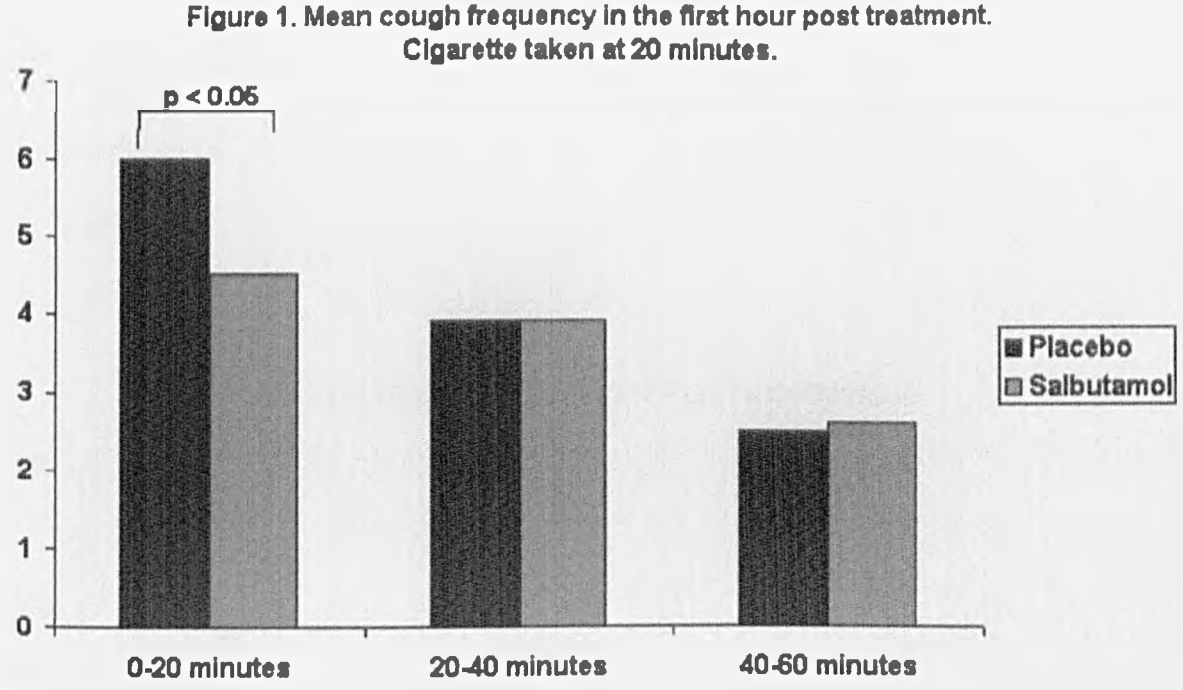


FIGURE 4.2

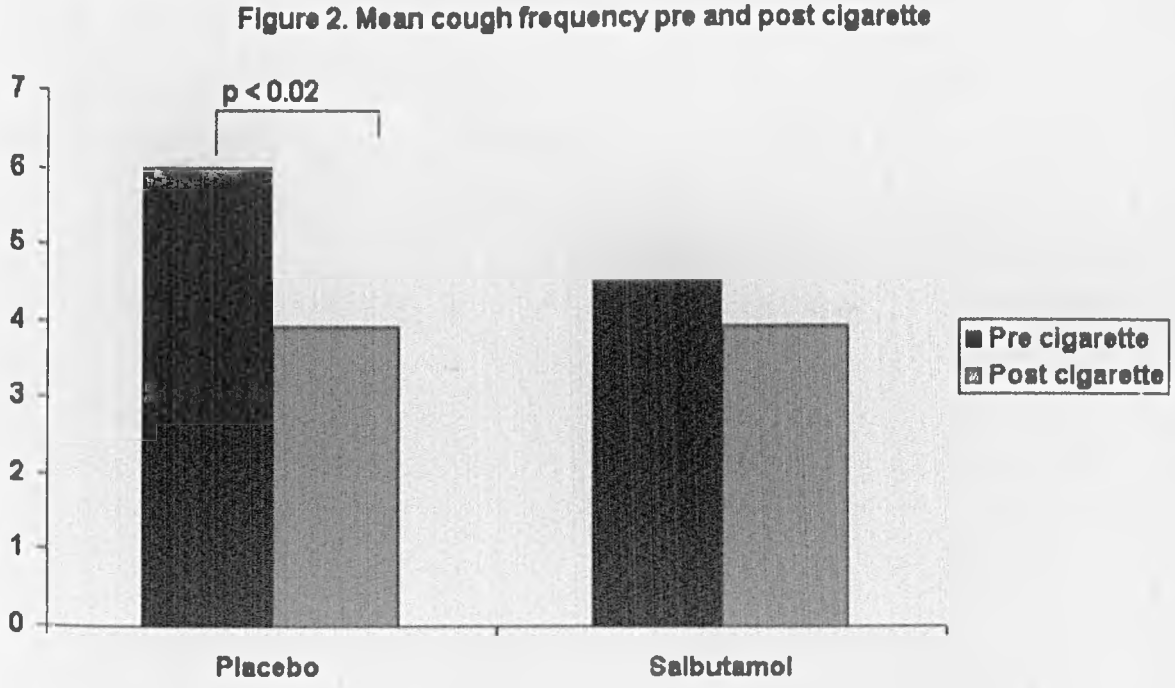
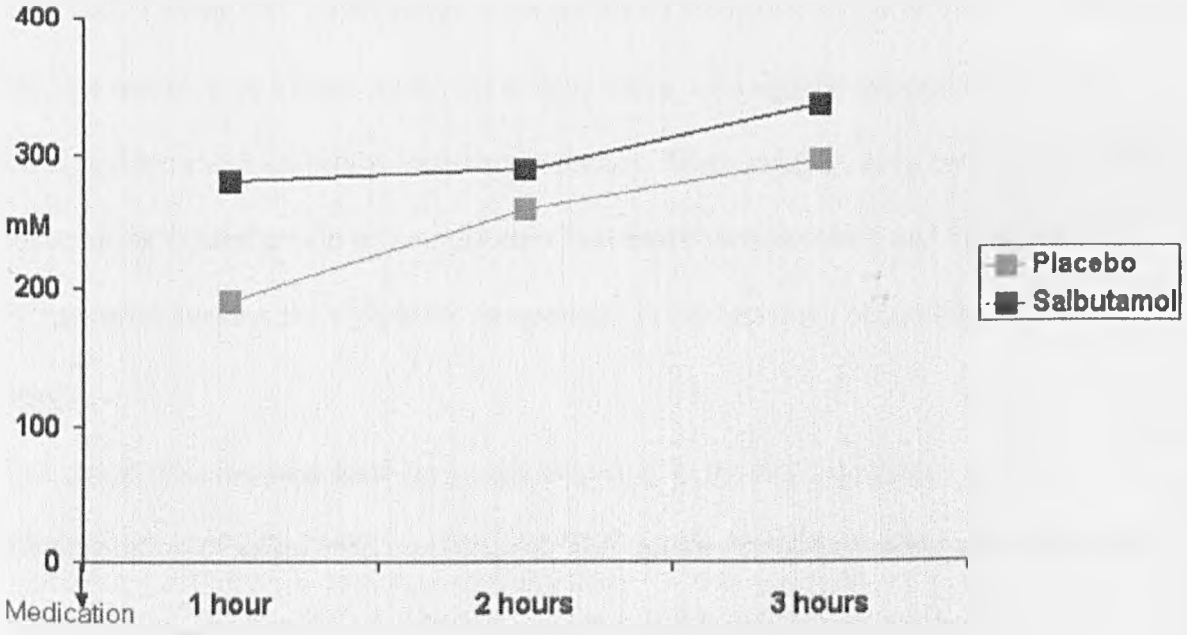


FIGURE 4.3

Figure 3. Citric acid cough challenge post treatment.



4.5 DISCUSSION

Cough is a troublesome symptom in smokers and in those attempting smoking cessation. Salbutamol appears to have antitussive properties in asthma due to bronchodilation and reduced bronchial hyperresponsiveness. This study investigated the hypothesis that salbutamol may have a role in the treatment of smoking related cough. The cough associated with smoking is multifactorial, related to bronchoconstriction, reduced cough threshold, (Wong CH, 1999) airway inflammation (Turato G, 1995; Wright JL, 1988) and the fact smoke is an irritant fume. As a short acting beta agonist salbutamol has marked bronchodilator and bronchoprotective properties. Were salbutamol to have a major effect on smoking related cough this would infer that bronchoconstriction and bronchial hyperresponsiveness are significant components in the aetiology of smoking related cough.

In a group of habituated smokers cough frequency in the first 20 minutes after administration of salbutamol was reduced. This demonstrated that salbutamol reduced cough before the stimulus of a cigarette and β agonists could therefore have a role in smoking cessation. In contrast, after the cigarette salbutamol had no effect on cough frequency. Cough frequency was reduced in the placebo group following the cigarette to levels similar to those seen post salbutamol. Since there was not a longer abstention period for comparison it cannot be presumed that this reduction in cough frequency is solely due to the effect of cigarette smoking. It does suggest smokers have a heightened cough frequency early in the morning and the findings are consistent with the theory that smokers "need" a cigarette to reduce cough. Both smoking and salbutamol may reduce

cough to basal levels and so β agonists may have little additional antitussive effect on cough in those who continue to smoke.

The cough challenge data is consistent with the observations of cough frequency. C2 concentration for citric acid was increased in the first hour after taking salbutamol. Again the effect was short lived with no significant effect seen on subsequent challenges. Previous studies have demonstrated the association of improvement in cough challenge with diminished cough in disease (O'Connell F, 1994). The finding of a reduction in cough sensitivity at a time when there was no difference in cough frequency suggests cough challenge may detect small differences in cough reflex sensitivity which are below the level which impact on cough frequency.

The subjects did not have a history of asthma and did not bronchodilate in response to salbutamol to the conventional levels required to support a diagnosis of asthma. Bronchodilation whilst highly predictive of asthma does not exclude the diagnosis. The best diagnostic test for exclusion of asthma is bronchial challenge. Subjects had a negative methacholine challenge as defined by PD20 > 0.5mg and a mean PD10 of 0.67 mg. Smokers have been shown to have an increase in non specific bronchial reactivity which is intermediate between asthma and normal (Gerrard JW, 1980). These findings suggest the study subjects do not have asthma but have an increase in bronchial reactivity, which is responsive to salbutamol. This is supported by the small but significant increase in FEV1 seen post salbutamol at screening and the increase in evening PEFR with salbutamol treatment.

In asthma the effect of salbutamol on cough challenge has been studied (Pounsford JC, 1985). Cough response and respiratory resistance fell in asthmatic subjects whereas only

respiratory resistance fell in normal subjects. The authors suggested that salbutamol in decreasing airway tone resets the irritant receptors or alters the threshold of airway smooth muscle receptors. The mechanism for bronchial hyperresponsiveness in asthma is unknown but is related to inflammation. Smoking leads to airway inflammation and inflammatory mediators may play a part in the cough associated with smoking. The effect of smoking on airway inflammation would seem to be the same in current smokers and in ex smokers with symptoms of chronic bronchitis (Turato G, 1995). Both these groups have an increase in some inflammatory cells and markers compared to non smokers and this inflammation may persist after smoking cessation.

The mechanism leading to inflammation in smoking may be via free radical production and consequent oxidative stress. Lipid peroxidation occurs in oxidative stress (Rokach J 1997) and causes an increase in Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) levels. This may be a local marker of airway inflammation. Pratico et al showed that the levels of urinary $PGF_{2\alpha}$ III isomer is raised in patients with COPD and levels increase with exacerbations (Practico D, 1998). It has also been shown that smokers show an increase in F_2-isoprostane in blood and urine (Morrow JD, 1995). The effect of $PGF_{2\alpha}$ on capsaicin induced cough and its modulation by beta $_2$ adrenergic and anticholinergic drugs has been studied (Nichol G, 1990). Capsaicin induced cough increased after inhalation of $PGF_{2\alpha}$. Salbutamol reduced cough after inhalation of $PGF_{2\alpha}$ and after the augmentation of capsaicin induced cough by $PGF_{2\alpha}$. Hence salbutamol's activity in reducing the cough associated with smoking cessation is by acting as a functional antagonist of inflammatory mediators such as $PGF_{2\alpha}$.

Alternatively nicotine could have a direct pharmacological effect on airway inflammation. Wang et al have recently shown that macrophages possess a nicotinic receptor consisting

of a monomer of the α7 subunit which on binding acetylcholine released by the vagus nerve inhibits cytokine release (Wang H, 2003). Nicotine may mimic acetylcholine at this receptor and reduce airway inflammation in smokers by inhibiting macrophage cytokine production. However such a mechanism may be more important in the long term effects of smoking on cough rather than short term antitussive activity.

This study used chronic cough in cigarette smokers to test salbutamol as an antitussive agent. The cough associated with cigarette smoking is multifactorial and mimics many of the features present in patients with both acute and chronic cough syndromes. Unlike acute cough due to respiratory tract infection the time course of the smoking cough model is relatively constant as demonstrated by the reproducibility of the reported cough over the five day observation period. In a chronic cough model the effect of treatment depends on the underlying pathology causing cough and results may be generalised to other disease processes. Smoking related cough with its associated airway inflammation may be a useful model for testing the effectiveness of antitussive therapies.

It has been demonstrated that cough frequency is reduced in smokers following a cigarette. The reduction in cough frequency after salbutamol and before a cigarette suggests that β agonists may be a useful treatment for the cough associated with abstaining from cigarettes. However the effect is small and of relatively short duration. It is therefore unlikely to be clinically useful. Other more potent and longer acting agents may prove more effective.

CHAPTER 5

ADRENAL AXIS SUPPRESSION UNRELATED TO THE DYNAMICS OF DOSING WITH BECLOMETHASONE MONOPROPIONATE

5.1 INTRODUCTION

Inhaled corticosteroids (ICS) are the cornerstone of asthma treatment. It is well known that long-term oral steroids have systemic side effects such hypothalamic-pituitary - adrenal (HPA) axis suppression, cataracts and osteoporosis (Kwong, 1987; Urban, 1988). ICS have local activity and high first pass metabolism, which minimises systemic side effects but at high doses ICS can also give rise to systemic complications because of absorption from the lung and partial clearance at first pass (Pederson S, 1997; Lipworth, 1999).

Although the side effects of ICS appears to be dose related the delivery system also has a contributory effect. In an editorial Dekhuijzen and Honour speculated on the mechanisms behind the apparent lack of HPA suppression caused by chlorofluorocarbon (CFC) free Beclomethasone dipropionate (BDP) (Dekhuijzen, 2000). This method of drug delivery produces particles with a smaller median mass aerodynamic diameter, which not only results in greater lung deposition but also a more rapid absorption of drug. Thus peak blood levels occur more rapidly compared to dose equivalent CFC BDP. One hypothesis was that a rapid achievement of peak circulating exogenous corticosteroid levels, as occurs with the rapid absorption of CFC free BDP, led to less HPA axis suppression. The aim of this study was to establish whether a rapid rate of change in exogenous steroid caused less HPA axis suppression than a slower elevation in plasma steroid level. The steroid used was intravenous 17-Beclomethasone monopropionate (17-BMP) the active metabolite of beclomethasone dipropionate. If the mechanism of suppression of the HPA axis caused by the different pharmacokinetic profiles of ICS could be elucidated

then it would allow drug delivery that minimised side effects without compromising the beneficial effects of inhaled steroids.

5.2 HYPOTHESIS

That the rate of steroid absorption is an important determinant of the degree of adrenal suppression.

5.3 METHODS

8 healthy adult males, mean age 34 were recruited to a randomised double blind placebo controlled trial. Ethical approval was obtained from the local ethics committee and patient consent taken prior to screening. Sensitivity to adrenocortical suppression was demonstrated by a >30% suppression of early morning cortisol following 1 mg dexamethasone. Randomised subjects then attended in the evening on 2 occasions receiving 500 micrograms of intravenous 17-BMP supplied as a 5-ml ampoule containing 500 micrograms of 17-BMP in a vehicle of propylene glycol and ethanol (BCM Specials, Nottingham). The infusion was given for either 15 minutes or 2 hours alongside appropriate saline placebo. Overnight urinary cortisol creatinine (C/C) ratio was measured before and after the infusion and an 8 am serum cortisol was measured following the infusion. Cortisol was measured using the DPC (Llanberis, UK) Immulite 2000 immunoassay analyser. Creatinine was measured using the Beckman Coulter (High Wycombe, UK) LX20 PRO analyser.

The study was powered (80%) at 0.05 significance for a 20% difference in cortisol creatinine ratio (C/C).

5.4 RESULTS

Mean C/C pre and post 15 minute infusion was 5.97 and 3.22 (p=0.005). (See Fig 5.1)

Mean C/C pre and post 2 hour infusion was 6.31 and 4.15 (p= 0.004). (See Fig 5.2)

Thus the difference in C/C associated with the 15 minute and 2 hour infusion was 2.74 (SD 1.9) and 2.16 (SD 1.5) (p = NS)

The coefficient of variance for the pre infusion C/C was 21%.

Mean 8 am cortisol for the 15 minute and 2 hour infusion was 425 nmol/l and 400 nmol/l respectively. (p=NS).

FIGURE 5.1

Pre and post infusion overnight urinary cortisol creatinine ratios following 15 minute infusion of 17-BMP. Pre = ■ Post = ●

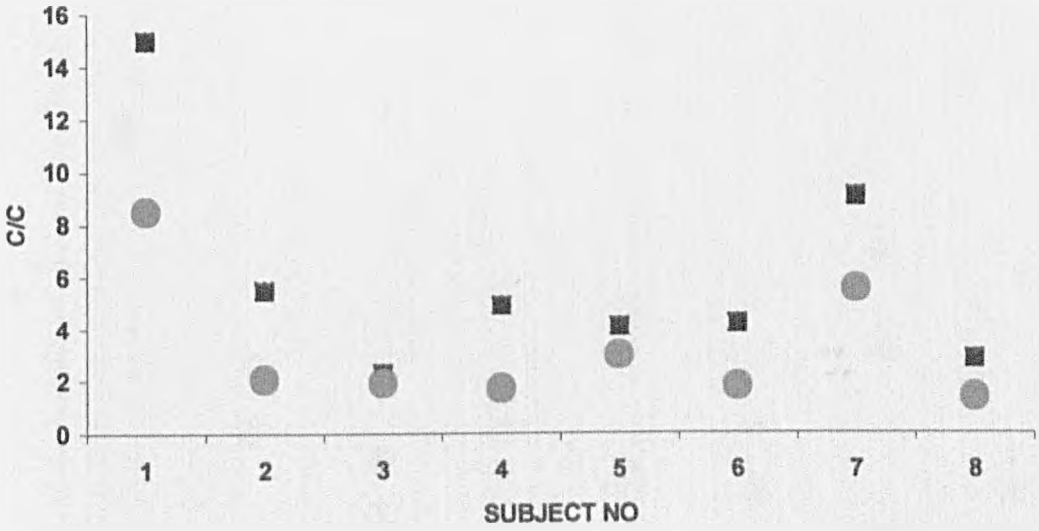


FIGURE 5.2

Pre and post infusion overnight urinary cortisol creatinine ratios following 2 hour infusion of 17-BMP. Pre = ■ Post = ●

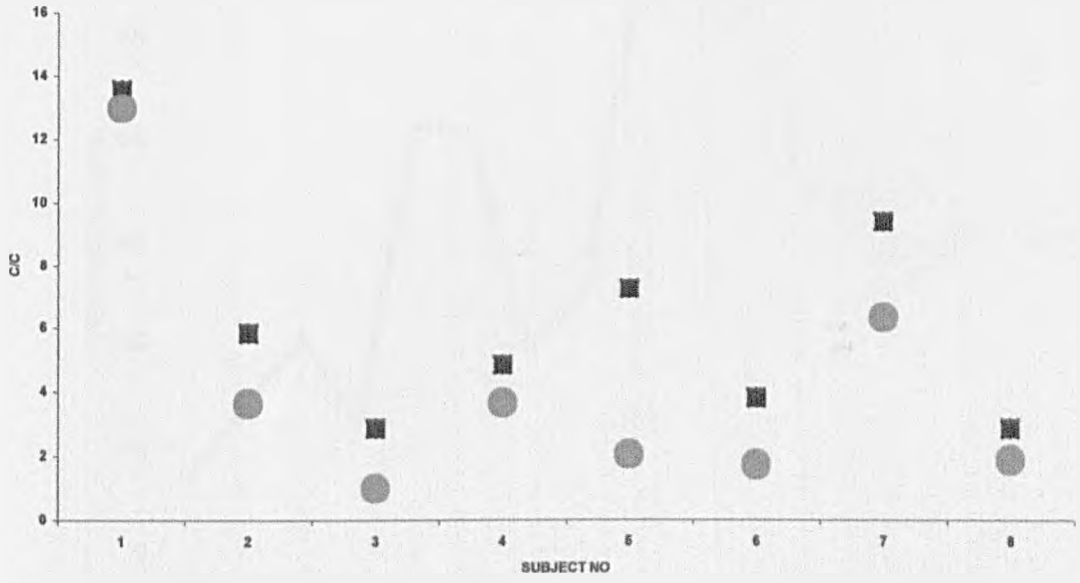
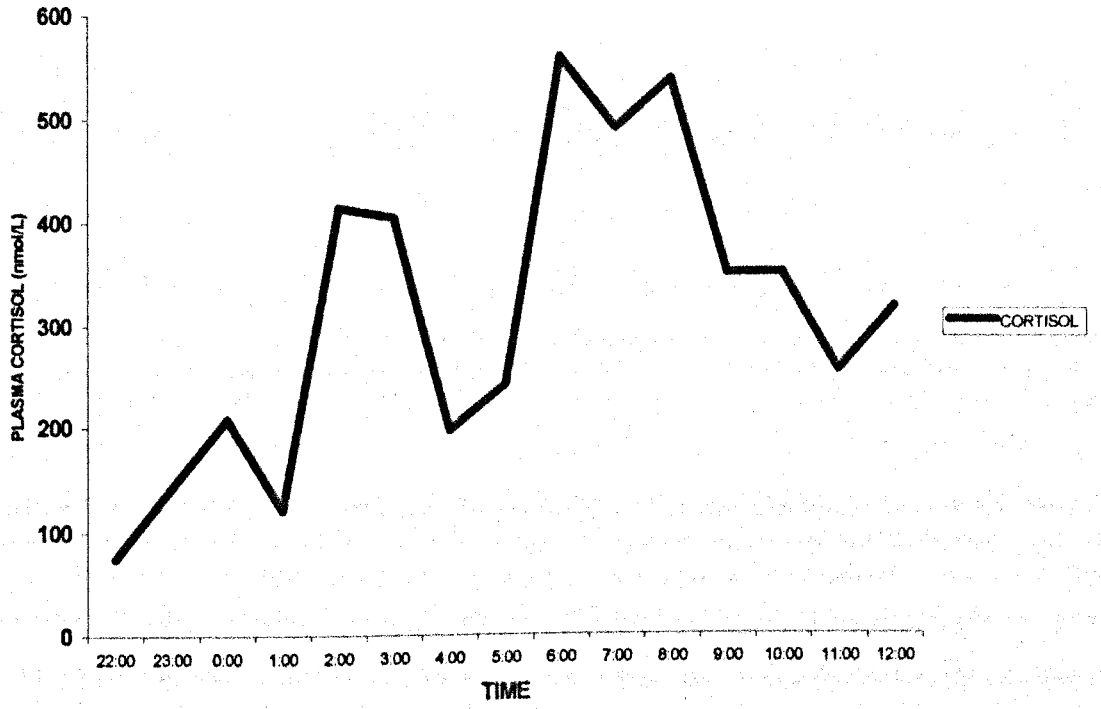


FIGURE 5.3

Overnight plasma cortisol profile in a subject during the pilot study

15 minute sampling



5.5 DISCUSSION

To my knowledge this is the first use of intravenous 17-BMP to mimic the pharmacokinetics of inhaled BDP. BDP is metabolised in the lung to 17- BMP, 21-BMP and beclomethasone. 17-BMP has the highest affinity to glucocorticoid receptors and it is known to circulate at greater concentrations in the serum compared to other metabolic break down products (Rohdewald, 1985; Wurthwein, 1990; Anderson, 1984). Daley-Yates and colleagues showed that the main active metabolite of inhaled BDP is 17-BMP and that following an inhaled dose of BDP the bioavailability 17-BMP is high (Mean percentage 62%) (Daley-Yates, 2001). Hence by infusing 17-BMP at two different rates the study sought to mimic rapid and slow bioavailability of 17-BMP. The use of the active metabolite allowed us to avoid any individual variation in the conversion of parent drug to 17-BMP.

The different rates of infusion of intravenous 17-BMP were chosen to reflect the pharmacokinetics of systemic corticosteroid bioavailability when inhaled from CFC free and conventional inhalers. The infusion times reproduced the peak blood concentrations achieved after inhalation of CFC and HFA BDP. They are based on the work of Seale et al who showed that the serum concentrations of beclomethasone dipropionate (BDP) plus metabolites peaked at 15 minutes and 2 hours following single inhaled doses of HFA-BDP and CFC-BDP respectively (Seale, 1998).

The dose of 17-BMP was chosen to reflect the concentration achieved following high dose inhaled steroids and the level of urinary C/C suppression achieved was similar to that shown following 2000 micrograms of inhaled beclomethasone (McIntyre, 1995).

Because the study is small an accurate measurement of adrenal suppression was required. There is controversy regarding the best method of assessing adrenal suppression. Assessing HPA axis suppression in heathly adults is a different scenario to the assessment of pathological, long term, adrenal suppression when stimulation tests are used.

Methods of assessment of basal adrenocortical secretory activity commonly used include 24 hour urine collection, which is associated with a risk of poor compliance, and overnight assessment of plasma cortisol. In studies using plasma cortisol the area under the curve (AUC) is generally assessed to establish the degree of suppression (Nelson, 2002; Grahnen, 1994). This method however requires frequent blood sampling because of the pulsatile secretion of the adrenal hormones. Figure 5.3 shows the short term variability of overnight cortisol on a study subject during the pilot study. Fifty-three blood samples were taken. Overnight plasma cortisol estimation is therefore time consuming, expensive and labour intensive.

Since this study is of a crossover design and aimed to investigate the relative degree of adrenal suppression, the assessment of a population normal range of cortisol secretion at baseline was not required. The primary endpoint was therefore overnight urinary cortisol creatinine ratios as it is claimed that within individuals it is a sensitive and reproducible index of change in cortisol secretion (Lipworth, 1999; McIntyre, 1995; Kong, 1999; Lipworth, 1998). Others have previously demonstrated that overnight and timed morning urinary C/C ratios are as sensitive as 24 hour urinary free cortisol excretion (McIntyre, 1995; Kong, 1999).

This study shows that individual C/C ratios were reproducible and a highly significant degree of adrenal suppression could be demonstrated at dosing consistent with clinical usage. It suggests that in correctly designed studies this highly practical method of assessing adrenal suppression has advantages over other methods for gauging the relative potential of inhaled steroids to cause systemic side effects. It demonstrated that differences in rates of infusion of 17-BMP had little effect on the degree of adrenal suppression in normal male subjects. The 1/2 life of 17-BMP formed following an intravenous dose of BDP is 2.7 hours and it has extensive tissue distribution (Daley-Yates, 2001). Thus although these results suggest that time to peak concentrations is not the determinant of adrenal suppression, the suggestion that an infusion time greater than 2 hours may be required to show a significant difference in HPA axis suppression cannot be excluded. In conclusion the differences in absorption rate of ICS seem unlikely to explain any observed differences in HPA axis suppression.

CHAPTER 6

OPTIMIZING NEBULISER THERAPY IN CYSTIC FIBROSIS PATIENTS USING PLANAR LUNG SCINTIGRAPHY

6.1 INTRODUCTION

Lung disease in cystic fibrosis (CF) is associated with recurrent respiratory infections, excessive mucus secretion and bronchial obstruction. These complications have been shown to respond to nebulised medication, particularly nebulised antibiotics and mucolytics (Mukhopadhyay, 1996; Ziebach, 2001). Types of nebuliser used by cystic fibrosis patients vary and recent advances in nebuliser technology are claimed to have been beneficial for this group of patients.

Nebulisers enable direct delivery of a wide range of soluble drugs to the lungs, permitting administration of drugs inactive by the oral route. They ensure a rapid therapeutic response, allow smaller doses of medication to be used, and thus minimise systemic side effects compared to oral treatment (British National Formulary 2005; Lipworth, 1995). Nebulisers are designed to produce a fine aerosol mist with particle diameter typically 1-5μm, which is sufficiently small to be deposited in peripheral airways. Unfortunately they are extremely inefficient and 88 to 93% wastage has been demonstrated (Clay, 1987). Individual ability to use a particular nebuliser also varies (Kastelik J, 2002).

The Pari nebuliser combines continuous nebulisation with an intermittent increase during inspiration. This occurs via a valve that allows air to be drawn into the nebuliser during inhalation. The valve closes on expiration and although this reduces drug loss, a significant amount of drug (at least 50%) is still wasted during exhalation (O'Callaghan, 1997).

The Prodose is a breath actuated jet nebuliser utilising adaptive aerosol delivery (AAD) technology, which continuously monitors the patients breathing pattern. It produces aerosol in the first half of the inspiration cycle when inhalation velocity is maximum depositing a greater portion of aerosol in the peripheral airways, and as a result, it is thought to reduce atmospheric wastage to that exhaled by the patient (Denyer, 2004). The Prodose monitors the amount of aerosol delivered in each breath and terminates when the preset dose has been delivered.

Scintigraphy is a non-invasive test that can be used to demonstrate aerosol deposition within the lung. The hypothesis of this study was that the technique could be employed to compare two or more nebulisers for an individual patient. The mapping and quantification of the percentage of aerosol deposited within the lungs would allow for a more thorough assessment of which nebuliser best suits an individual. The study also aimed to assess the reproducibility of the technique with a view to routine implementation within a busy department.

In severe CF lung disease treatment with mucolytic, antibiotics and bronchodilators have an approximate daily cost of £100(British National Formulary 2005). Cost effective drug delivery therefore has important consequences. These factors prompted a focus on CF patients.

6.2 METHODS

CF patients with a diagnosis confirmed by gene analysis or sweat test were recruited. Patients were excluded from the study if they were clinically unstable. With the exception of bronchodilators, routine daily medication was administered on the study day. All patients provided informed written consent in accordance with local research ethics committee guidelines. The administration of radioactive aerosol was approved by the Administration of Radioactive Substances Advisory Committee (A.R.S.A.C) and administered by trained nuclear medicine technicians.

Scintigraphy was used to compare lung deposition from two different nebulisers namely Pari LC Plus using Turbo Boy compressor (Pari Medical Ltd, Surrey, UK) and the Prodose Nebuliser (Profile Therapeutics, Bognor Regis, UK) utilising adaptive aerosol delivery technology (AAD).

Each volunteer attended the nuclear medicine department on three separate occasions with a minimum of 72 hours between visits. Instructions as to the correct use of the nebuliser, using tidal breathing techniques, were given. On each visit, FEV1 and FVC measurements were taken before and 20 minutes after nebulised inhalation of 5mg salbutamol in 3ml saline. The nebuliser used on the first two visits was assigned randomly whilst the nebuliser on the third visit was assigned so that 50% of patients used Pari LC Plus twice and 50% used Prodose twice. This allowed the repeatability of lung aerosol deposition to be assessed.

A syringe containing approximately 150MBq of Diethylenetriaminenepentaacetate labelled technetium-99m (Tc^{99m}-DTPA) was prepared and the exact activity and reference time recorded. After nebulisation of salbutamol, the nebuliser was washed out and the Tc99m-DTPA solution transferred into the nebuliser chamber. The residual syringe activity was measured and recorded along with the time of measurement.

These measurements were used to accurately determine the activity in the nebuliser chamber at the start of inhalation. For radiation protection purposes the nebuliser was placed in a lead shielded stand, removing the need to hold the nebuliser directly. Patients were provided with nose clips and an apron. The solution was nebulised over a 6min period (Pari LC Plus) or until shut-off using Prodose. The radioactive nebulisation start time was recorded and used to correct lung images for radioactive decay between the start of inhalation and the time of imaging.

Radiation Protection

To minimise radioactive atmospheric contamination, an exhalation filter was fitted to the Pari nebuliser. It was not possible to fit such a filter to the Prodose, however the room in which nebulisation was performed was monitored before and after nebuliser use and no significant radioactive contamination was found.

Gamma Camera & Imaging Protocol

The patients were imaged immediately after radioactive nebulisation using a Philips Forte dual headed gamma camera. A low energy general purpose parallel hole collimator was used and an energy window of 140keV±10% was set. Static anterior and posterior images of the lungs were taken simultaneously for 240s, using the full field of view and a 256x256 pixel matrix. The nebuliser chamber was imaged for 10s and the mouthpiece for 30s, using a single detector.

Image Processing

The Image J freeware software package (National Institutes of Health, USA), with an in-house developed software plug-in, was used to process the gamma camera scintigraphic images. Images were transferred to Image J as DICOM part10 metafiles. The software uses previously recorded details of syringe activity to calculate activity of Tc99m-DTPA in the nebuliser at the time nebulisation was started. Regions of interest (ROI) were drawn around the right and left lungs on the anterior and posterior images. Lateral background regions were also drawn for each lung. From these ROIs, the background corrected counts within each lung on the anterior and posterior images were obtained. The software uses these values to calculate the geometric mean counts for each lung. This value was decay corrected to the start time of nebulisation, the counts were converted into Becquerels using the previously determined camera sensitivity and expressed within each ROI as a percentage of original nebuliser chamber activity. No attenuation correction was performed. The stomach and central thoracic region were also outlined and processed in the same way.

Similar calculations were performed for the percentage of activity that remained un-nebulised in the chamber, and deposited in the mouthpiece and filter (where applicable). These calculations were obtained from a single image rather than a geometric mean. Another member of the research team, who was blinded to the results of the first operator, repeated the processing several days later. This enabled assessment of inter-operator variability.

Statistical analysis

Results were expressed as a percentage of the total activity administered. Bland Altman analysis was used to assess nebuliser repeatability and inter operator variability.
The Wilcoxon matched-paired signed rank test was used to assess the significance of the difference between the two nebulisers, and p values < 0.05 were considered significant.

6.3 RESULTS

10 CF patients (7 male), mean age 25.7yrs (range 18-47) took part in the study. Mean increase in FEV1 was 3.20% predicted for Pari compared to 3.6% with Prodose. Mean lung deposition was 1.47%±0.46% for Pari compared with 2.58%±0.78% for Prodose. The individual percentage of aerosol deposited in the lungs, difference between nebulisers and change in FEV1 are tabulated in Table 6.1. The average percentage of activity detected elsewhere is summarised in Table 6.2. The Pari nebulised 42% of the solution available compared to 13.37% for the Prodose.

Figure 6.1 illustrates the typical heterogeneous distribution of aerosol in images (normalised to the same point) from each nebuliser. Although the mean difference between Pari and Prodose was 1.1% individuals differed markedly in relative deposition from the two nebulisers. Thus patient 3 had no difference whereas patient 1 had three-fold difference. (see figure 6.2).

Inter operator variability and reproducibility

The repeatability of aerosol lung deposition of those returning to use the same nebuliser on a subsequent visit was analysed. The mean difference between repeat measurements of nebuliser deposition was 0.26±0.33% (p=0.049)

The mean inter-operator differences were 0.02%±0.06 for the Pari (p= 0.148) and -0.028%±0.076 for the Prodose (p=0.38). Greater difficulty is experienced in the

contouring procedure when outlining the lungs in those patients using the Prodose nebuliser as deposition in the regions of the stomach and central airway need to be excluded from lung ROI's (see figure 6.1).

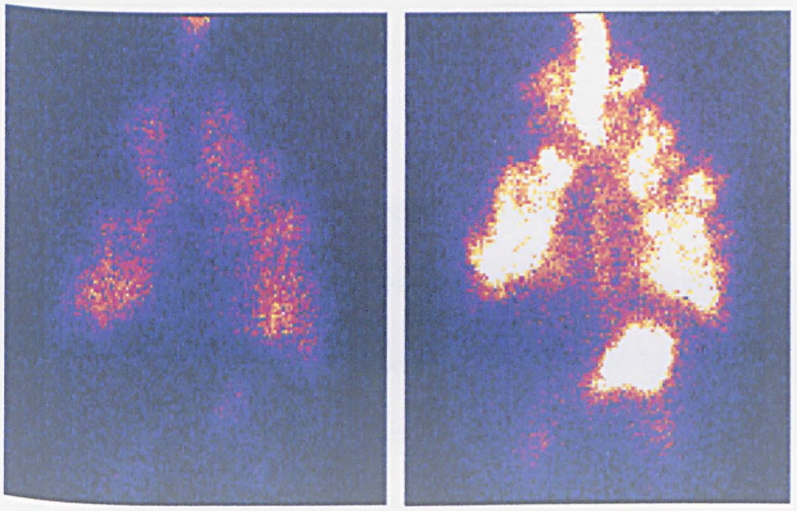
	Pari LC Plus %				Prodose %			
Patient	FEV1 * Increase	Right Lung % Dep.	Left Lung % Dep.	Total % Dep.	FEV1 Increase	Right Lung % Dep.	Left Lung % Dep.	Total % Dep.
1	1	0.30	0.44	0.75	2	0.99	1.24	2.23
2	2	0.94	0.89	1.83	10	1.85	1.72	3.57
3	6	1.43	0.16	1.59	10	1.33	0.26	1.59
4	1	0.83	0.63	1.46	3	1.2	0.85	2.05
5	9	1.17	1.15	2.32	-3	2.11	1.54	3.65
6	-1	0.61	0.49	1.10	2	1.51	1.00	2.51
7	3	0.78	0.90	1.68	1	0.87	0.97	1.83
8	5	0.79	0.68	1.47	1	1.747	1.779	3.53
9	2	0.35	0.53	0.88	5	0.73	1.14	1.86
10	4	0.85	0.72	1.57	5	1.38	1.06	2.44

Table 6.1 Percentage of aerosol delivered to the lungs as a percentage of original activity.

* FEV1 percent predicted.

	Pari LC Plus %	Pro-dose %	Significance (p)
Av. Total lung deposition	1.47±0.46	2.53±0.78	≤0.002
Av. Activity Un-nebulised	57.95±0.07	86.63±0.80	≤0.002
Av. Activity Mouthpiece/filter	39.66±0.08	4.90±0.72	≤0.002
Av. Activity in Stomach	0.016±0.013	0.18±0.10	≤0.002
Av. Activity in Central Thorax	0.041±0.032	0.32±0.26	≤0.002

Table 6.2 Mean deposition as a percentage of original activity



A B

Figure 6.1 Normalised lung scintigraphic images for a patient using

A: Pari and B: Prodose.

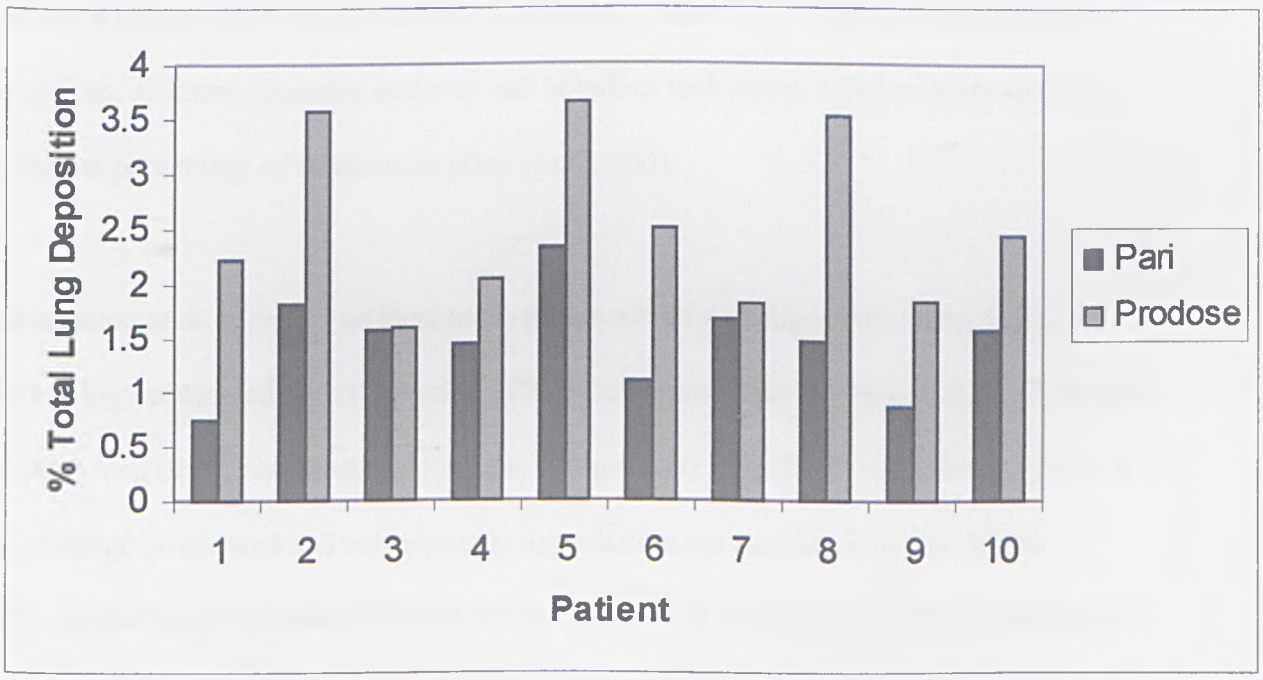


Figure 6.2 Total lung deposition from Pari LC Plus and Prodose

6.4 DISCUSSION

Lung ventilation scintigraphy is an established nuclear imaging technique commonly used to detect pulmonary emboli and requiring small amounts of radiation. These radiation doses are significantly smaller than received by natural background over a one year period in the UK, or commonly requested scans such as chest CT (Hart D et al. 2002). It is therefore a safe and widely available technique.

The lung ventilation scintigraphy was adapted using specially written software (available to download free from our web site http://www.hull.ac.uk/CFNEB). It provided both a pictorial and quantifiable indication of nebulised drug deposition, allowing assessment of the relative lung deposition of two nebulisers commonly used in the treatment of patients with CF. There are many different types of nebuliser on the market each claiming to deliver a large percentage of aerosol to the lung. Patients with significant respiratory problems, differing diseases, anatomy and nebuliser techniques will inevitably receive a different percentage of medication (Boe et al, 2000).

Devadason *et al.* reported an increase in peripheral airway deposition using AAD technology compared with the Pari LC Plus (Devadason, 2001). This study demonstrated greater lung deposition from the Prodose compared to Pari (Table 6.2). Similarly the percentage swallowed and subsequently deposited in the stomach was also higher.
The greater lung deposition with the Prodose infers the respirable output is considerably greater whereas the Pari LC Plus had greater percentage deposited on the mouthpiece and filter. These results are comparable with known differences in nebuliser design.

The reproducibility of the technique was high with no significant inter-operator differences. Although a significant difference in deposition was noted between repeat study days, the difference observed was small in comparison to total lung deposition. Thus this technique can demonstrate clinically significant differences even when performed by different operators.

Salbutamol was inhaled before the scan in order to provide a uniform baseline of post bronchodilator FEV1 on each study day. The FEV1 increases in study subjects were small indicating little baseline bronchoconstriction. However in routine practice predosing with salbutamol is important since up to 50% of patients with CF demonstrate bronchial lability (Holzer, 1981; Eggleston, 1988).

I acknowledge the limitations of the study in that Tc99m-DTPA was used as an indirect marker for inhaled drug rather than direct labelling of the drug itself. It is generally accepted that gamma scintigraphy provides an accurate assessment of whole lung deposition and that lung deposition data can be correlated to clinical response to inhaled asthma drugs (Newman, 1998). Nebulised antibiotics and mucolytics have different physical properties (viscosity and density) and clinical effects compared to bronchodilators. Hence a crossover comparison and direct correlation is not possible. The chief utility of this technique however, rests in its ability to compare deposition of different nebulisers.

In this analysis no attempt was made to correct for attenuation. This would have been desirable since it may have allowed calculation of absolute as opposed to relative lung deposition. However, for a crossover comparison of nebulisers no correction for attenuation is required. A number of techniques for attenuation correction have been suggested, but these can be difficult to perform and may in practice degrade inter-study repeatability.

This test could potentially be used to ensure adequate drug delivery in a wide variety of respiratory disorders. For example, in order to maximise deposition of expensive, disease modifying drugs such as nebulised iloprost and nebulised insulin it is imperative the correct nebuliser is chosen. Any patient who is been considered for nebuliser therapy for which a number of nebuliser options are available may be assessed in this way.

In conclusion this study demonstrates the development of a standard operating procedure for utilising a common nuclear medicine technique in the individualisation of nebuliser treatment. Planar scintigraphy reliably demonstrated the probable percentage of medication received

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 INTRODUCTION

This thesis has concentrated on the diagnosis and treatment of conditions associated with airway inflammation. The studies describe methods of non-invasive diagnosis and disease monitoring, treatment of symptoms, treatment side effect assessment and individualisation of nebuliser therapy.

As outlined in Chapter One the mechanisms producing airway inflammation differ depending on the disease process and whilst COPD, asthma and CF have different aetiologies they share similar symptoms and treatments.

Exhaled NO and EBC are proving to be useful in assisting with diagnosis and disease monitoring. These techniques also enable further understanding of the molecular aetiology and consequences of airway inflammation.

Chapter Three showed that mild stable atopic asthma is associated with a normal EBC pH and an elevated FE_{NO}. In CF the EBC pH is low, particularly during acute exacerbations, but FE_{NO} is reduced. The findings demonstrated dissociation between EBC pH and FE_{NO} in inflammatory airways disease. The chapter also described the complexity of NO metabolism, where multiple pathways of NO synthesis and clearance are likely to have variable relevance in different diseases.

The various therapies available for COPD, asthma and CF are discussed in Chapter One, giving background on the treatments used in Chapters Four, Five and Six. In view of the overlap of symptoms and treatment used some extrapolation between diseases with regards to the treatments discussed can be made.

Chapter Four described the use of chronic cough in cigarette smokers to test salbutamol as an antitussive agent. The cough associated with cigarette smoking is multifactorial and associated with airway inflammation. It therefore mimics those inflammatory airway conditions that present with both acute and chronic cough.

The study showed that cough frequency is reduced in smokers following a cigarette. The reduction in cough frequency after salbutamol and before a cigarette suggests that β agonists may be a useful treatment for the cough associated with abstaining from cigarettes. Although the effect of salbutamol is of relatively short duration and unlikely to be clinically useful, other more potent and longer acting agents may prove more effective.

Steroids are an extremely useful treatment for inflammatory airway disorders, particularly in eosinophilic inflammation. With high doses side effect profile becomes important. Chapter Five addresses one mechanism that may explain the differing degrees of HPA axis suppression caused by different forms of inhaled steroids. Using intravenous 17-beclomethasone monopropionate, the study assessed whether the rate of steroid absorption determines the degree of adrenal suppression. It also highlighted the best method for assessing HPA axis suppression.

Individual C/C ratios were reproducible and a significant degree of adrenal suppression could be demonstrated at dosing consistent with clinical usage. Overnight urinary C/C is therefore a highly practical method of assessing adrenal suppression and has significant advantages over other methods for gauging the relative potential of inhaled steroids to cause systemic side effects. The study demonstrated that differences in rates of infusion of 17-BMP had little effect on the degree of adrenal suppression in normal male subjects.

Nebulised treatment is utilised widely in respiratory medicine and as revealed in Chapter Six deposition can be demonstrated using scintigraphy. The study demonstrated the development of a standard operating procedure for utilising a common nuclear medicine technique in the individualisation of nebuliser treatment. Scintigraphy reliably demonstrates the probable percentage of medication received

The results suggest that this test could potentially be used to ensure adequate drug delivery in a wide variety of respiratory disorders. Thus any patient who is been considered for nebuliser therapy for which a number of options are available may be assessed in this way.

7.2 FUTURE RESEARCH

7.2.1 HOW SHOULD COPD AND COPD TREATMENT RESPONSE BE MONITORED?

It is known that higher FE_{NO} is found with severe disease and exacerbations and EBC pH is lower in COPD (Kostikas, 2002; Maziak, 1998) Chapter three shows that NO and EBC pH can potentially be utilised in disease diagnosis and monitoring of treatment. Current monitoring methods used in COPD studies include spirometry, quality of life measurements, dyspnoea scores and exacerbation rate (Vincken, 2002; Donohue, 2002). Short-term spirometric changes are probably of doubtful clinical significance. Long term change in spirometry is linked to mortality but requires prolonged monitoring. Exacerbations are an important determinant of mortality and morbidity in COPD (Groenewegen, 2003). They also affect quality of life, more frequent exacerbations being associated with reduced exercise capacity, greater dyspnoea and decline in health status (Hodgev, 2004; Spencer, 2001). These methods of monitoring are however subjective and retrospective in nature.

NO and EBC pH are potentially objective prospective measures that could be utilised in defining populations at risk of exacerbations

Smoking cessation has been shown to slow down the rate of decline in FEV_1 in COPD patients and biopsy studies have aimed to analyse airway inflammation following smoking cessation (Willemse, 2005). The non-invasive aspect of exhaled NO and EBC

measurement enables rapid repeated assessment, which could be exploited in the monitoring of airway inflammation in ex smokers.

Thus monitoring pH, NO and EBC inflammatory markers both before and after smoking cessation may give information on underlying inflammatory mechanisms.

As chapter four highlighted smokers can have a troublesome cough, which may also be present in COPD patients with chronic bronchitis. Smokers have been shown to have an increase in non-specific bronchial reactivity, which is intermediate between asthma and normal (Gerrard JW, 1980). The mechanism for bronchial hyper-responsiveness in asthma is unknown but is related to inflammation. Smoking leads to airway inflammation and inflammatory mediators may play a part in the cough associated with smoking.

I suggest that the cough associated with smoking and COPD is due in part to bronchial hyper-responsiveness and that functional antagonism is one of the mechanisms by which B2 agonists exert their therapeutic effect.

Long acting B2 agonists are more effective and convenient when compared to short acting bronchodilators. They can be used either alone or in combination with inhaled steroids. Combination treatment has been shown to lead to a larger increase in FEV1, reduced dyspnoea and reduced exacerbations in those with severe disease when compared to inhaled steroids alone (Calverley, 2003). There is also a theory that long acting B2 agonists have anti-inflammatory properties and a synergistic mechanism of action when combined with corticosteroids (Ottonello, 1996; Faurschou, 1996; Barnes, 2002; Jeffery, 2002).

A cough recorder based on voice recognition and digital processing is currently being developed and will be advantageous for a study analysing the effect of long acting B2 agonists and combination treatment on COPD. It will allow accurate, objective assessment of one of the major symptoms associated with COPD.

The investigation of the effect of these treatments on surrogate markers of airway inflammation in smokers and COPD patients would potentially give useful information as to the mechanisms of action of treatments. These markers could also be used as a surrogate measurement of clinical response to treatment and possibly utilised in early drug development. They may allow for smaller initial studies prior to larger randomised control trials and could be utilised to identify subgroups that have a particular profile, for example a steroid responsive subgroup.

7.2.2 CAN EBC AND FE_{NO} BE UTILISED IN MONITORING ASTHMA EXACERBATIONS AND RESPONSE TO TREATMENT?

British thoracic society guidelines recommend symptom assessment, lung function and short acting βagonist use to assess asthma control (British thoracic society, 2003). The use of lung function as an objective measure of respiratory health is problematic as it correlates poorly with subjective measures of asthma (Mortimer, 2001; Fink, 2001). In addition, these characteristics do not reflect the degree of underlying airway inflammation which drives the disease process. Dyspnoea perception can be positively or negatively correlated or unrelated to airway inflammation in mild asthma. In severe asthmatics with recurrent exacerbations there is a negative correlation between perception and inflammation (Veen, 1998; Roisman, 1995; Salome, 2002).

Both airway hyper-responsiveness and sputum eosinophilia are correlated with poor asthma control and are objective measures that have been used to manage asthma treatment. Green et al showed that treatment aiming to normalise sputum eosinophils leads to a reduction in asthma exacerbations without an increase in anti-inflammatory treatment (Green, 2002). A management strategy aimed at improving airway hyper-responsiveness again lead to a reduction in exacerbations and a reduction in the subepithelial reticular layer (Sont, 1999). There is therefore a definite need for objective measures of airway inflammation to enable effective therapeutic management that ensures a reduction in airway inflammation. These measures could then be correlated with more subjective measures

It has previously been shown that in asthma, FE_{NO} falls in response to inhaled steroids and can be used to adjust the dose of inhaled steroid given to an individual (Smith AD,2005). This study used FE_{NO} to guide dose titration and demonstrated a 40 % reduction in the dose of inhaled steroids could be achieved without deterioration in asthma control or increased exacerbation rate.

Measuring FE_{NO} along with EBC inflammatory markers could therefore enable tailoring of treatment on an individual basis.

In future portable devices eg R tube ™ will allow ease of collection and greater application of these surrogate markers of inflammation both in research and clinical practice.

The effect of combination inhalers on exhaled NO and EBC pH, inflammatory markers has not been demonstrated and analysis may provide information on the mechanism for the increased efficacy of this treatment.

Long acting B2 agonists may have a role in cough variant asthma via the mechanisms described above. As described in chapter four this could be demonstrated using methacholine challenge, cough challenge and cough recording.

7.2.3 WILL EBC ASSIST IN EXACERBATION RECOGNITION AND THE ASSESSMENT OF TREATMENT RESPONSE IN CF?

As shown in chapter three, EBC pH is lower in CF compared to controls and falls further with exacerbation. This observation could potentially be used to assist diagnosis, management of exacerbations and also monitor treatment response. An early indication of a response is vital in CF and objective evidence of improvement or decline will allow adjustment of treatment.

Correlation of EBC inflammatory markers to infections with viruses or different bacteria could give further information on underlying inflammatory responses.

The evidence for the role of inhaled corticosteroids in CF is minimal but again the studies use respiratory function to assess response (Romano, 1994; Bisgaard, 1997). Reduced bronchial hyper-reactivity is seen following a 6-week course of inhaled budesonide and correlation of this to reduced exhaled breath inflammatory markers would be supportive. The effect of long acting B2 agonists steroid combinations has yet to be studied. The effect on inflammatory activity and pseudomonas infection may allow assessment of the synergist activity of this treatment.

A study showing the effect of a combination treatment to symptoms, bronchial hyperesponsiveness and EBC pH/inflammatory markers would again correlate clinical response and inflammatory mechanisms

7.2.4 WHAT IS THE BEST METHOD OF MONITORING ADRENAL SUPPRESSION IN COPD PATIENTS ON INHALED STEROIDS?

The systemic side effects of different doses of inhaled steroids will vary in individuals. High dose inhaled steroids can result in systemic absorption and increased risk of the side effects associated with oral steroid use. Doses above 1.5 mg/day can lead to significant adrenal suppression and is associated with a reduction in bone density. Long-term high dose inhaled steroids increases the risk of ocular complications and skin bruising (Lipworth, 1999).

Adrenocortical function is assessed via basal adrenocortical activity tests or dynamic tests. Basal activity tends to be assessed with single morning test or urinary cortisol excretion and the short synacthen test is an example of a dynamic test. Many of the multicentre studies assessing inhaled steroids use a single morning cortisol measurement as a screening for adrenal suppression (Barnes, 1993; Lundback, 1993). This is extremely insensitive given the marked diurnal variation of cortisol levels in humans and studies utilising this marker of basal activity have unsurprisingly found minimal suppression. Urinary cortisol creatinine ratio has greater sensitivity and overnight collection is as sensitive as 24 hour.

Many of the studies of inhaled corticosteroid in COPD use doses well above the dose response curve (Calverley, 2003; Burge, 2000) and this increases the risk of systemic absorption and subsequent side effects.

The utilisation of overnight urinary C/C in optimising the dose of inhaled steroids whilst minimising side effects in COPD patients has not been investigated. Individual variation in the degree of suppression with different doses of inhaled steroids could be screened by using overnight C/C. Lipworth et al suggest that this should be repeated if a low value is obtained and if the repeat is low a dynamic test should be performed (Lipworth, 1997)

The relationship of the degree of cortisol suppression, as demonstrated by overnight C/C, to long- term steroid side effects such as osteoporosis, diabetes and cataracts could also be assessed. This would however require long term follow up and is complicated by an increased risk of these illnesses in the elderly.

7.2.5 HOW CAN SCINTIGRAPHY BE UTILISED TO GUIDE NEBULISER CHOICE?

In the future scintigraphy could be used to ensure adequate deposition of expensive drugs such as iloprost. Three hundred 20 microgram vials costs £4244 and the recommended dosing is 2.5 to 5 micrograms 6 to 9 times daily. With improved deposition the required dose could potentially be halved saving approximately £3500 per year. Thus, optimising nebuliser delivery could be very cost effective as lower doses can be given when using a more efficient nebuliser.

Dnase is expensive but has a fixed vial dose so adjustment of dose would be problematic. Maximum lung deposition is still however a priority and scintigraphy would assess for this.

Alpha 1 antitrypsin can also be nebulised and has been used in treatment of CF. It is given via an ultrasonic nebuliser and the amount of deposition is dependent on the vibration intensity and the degree of inhalation (Flament, 1999). This could be assessed with scintigraphy.

Insulin can now be administered via inhalation and inhalers have been developed for this purpose (Quattrin, 2004; Quattrin, 2004). Nebulisation may increase lung deposition and by using radiolabelled insulin, scintigraphy could identify the most appropriate device.

7.3 CONCLUSION

Although this thesis highlights the diversity of conditions associated with airway inflammation, it demonstrates the similar ways they can be assessed and treated. Genetics and environmental idiosyncrasies complicate prescribing and the individual response as well as the risk benefit ratio of treatments should be considered. Subjective assessment of improvement should be coupled with objective measures to ensure adequate treatment. Treatment should be tailored to the individual patient and objective monitoring of the response to and side effects of therapies enables maximum effect with minimal risk.

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ABBREVIATIONS

AAD	ADAPTIVE AEROSOL DELIVERY
APS	AIRWAY PROVOCATION SYSTEM
ARSAC	ADMINISTRATION OF RADIOACTIVE SUBSTANCES ADVISORY COMMITTEE
AUC	AREA UNDER THE CURVE
BAL	BRONCHOALVEOLAR LAVAGE
BMP	BECLOMETHASONE MONOPROPIONATE
BDP	BECLOMETHASONE DIPROPIONATE
C2	CITRIC ACID CONCENTRATION CAUSING 2 COUGHS PER INHALATION
C/C	CORTISOL CREATININE RATIO
CF	CYSTIC FIBROSIS
CFC	CHLOROFLOUROCARBONS
CO2	CARBON DIOXIDE
c AMP	CYCLIC ADENOSINE MONOPHOSPHATE
c GMP	CYCLIC GUANOSINE MONOPHOSPHATE
CFTR	CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR
COPD	CHRONIC OBSTRUCTIVE PULMONARY DISEASE
CF	CYSTIC FIBROSIS
DTPA	DIETHYLENETRIAMININEPENTAACETATE
EBC	EXPIRED BREATH CONDENSATE
FENO	FRACTIONAL EXHALED CONCENTRATION OF NITRIC OXIDE
FEV_1	FORCED EXPIRATORY VOLUME IN 1 SECOND

FVC	FORCED VITAL CAPACITY
GM-CSF	GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR
HCO3	BICARBONATE
HPA	HYPOTHALAMIC-PITUITARY-ADRENAL
ICS	INHALED CORTICOSTEROIDS
IgE	IMMUNOGLOBULIN E
IL	INTERLEUKIN
L-NAME	N^G-NITRO-L-ARGININE METHYL ESTER
L-NMMA	N^G-MONOMETHYL-L-ARGININE
LT	LEUKOTRIENE
MDI	METERED DOSE INHALER
NH3	AMMONIA
NO	NITRIC OXIDE
NOS	NITRIC OXIDE SYNTHASE
e NOS	ENDOTHELIAL NOS
i NOS	INDUCIBLE NOS
n NOS	NEURONAL NOS
NO_2	NITRITE
NO_3	NITRATE
PAF	PLATELET ACTIVATING FACTOR
PD20	PROVOCATIVE DOSE CAUSING A FALL IN FEV1 OF 20%
PEFR	PEAK EXPIRATORY FLOW RATE
PG	PROSTAGLANDIN

PPB	PARTS PER BILLION
rh DNase	RECOMBINANT DNase
ROI	REGION OF INTEREST
TNF	TUMOUR NECROSIS FACTOR