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Atorvastatin in combination with inhaled beclometasone modulates inflammatory sputum mediators in smokers with asthma

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ABSTRACT

Background: Statins have pleiotropic immunomodulatory effects that may be beneficial in the treatment of asthma. We previously reported that treatment with atorvastatin improved asthma symptoms in smokers with asthma in the absence of a change in the concentration of a selection of sputum inflammatory mediators.
Objective: To determine the effects of atorvastatin alone and in combination with inhaled corticosteroid on a range of sputum cytokines, chemokines and growth factors implicated in the pathogenesis of asthma, and their association with asthma control questionnaire (ACQ) and/or asthma quality of life questionnaire (AQLQ) scores.
Methods: Sputum samples were analysed from a sub-group of 39 smokers with mild to moderate asthma recruited to a randomised controlled trial comparing atorvastatin (40 mg/day) versus placebo for four weeks, followed by inhaled beclometasone (400 μg/day) for a further four weeks. Induced sputum supernatant fluid was analysed (Luminex or biochemical analyses) for concentrations of 35 mediators.
Results: Sputum mediator concentrations were not reduced by inhaled beclometasone alone. Atorvastatin significantly reduced sputum concentrations of CCL7, IL-12p70, sCD40L, FGF-2, CCL4, TGF-α and MMP-8 compared with placebo and, when combined with inhaled beclometasone, reduced sputum concentrations of MMP-8, IL-1β, IL-10, MMP-9, sCD40L, FGF-2, IL-7, G-CSF and CCL7 compared to ICS alone. Improvements in ACQ and/or AQLQ scores with atorvastatin and ICS were associated with decreases in G-CSF, IL-7, CCL2 and CXCL8.
Conclusion: Short-term treatment with atorvastatin alone or in combination with inhaled beclometasone reduces several sputum cytokines, chemokines and growth factors concentrations unresponsive to inhaled corticosteroids alone, in smokers with asthma.

1. Introduction

In addition to reducing cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, statins have pleiotropic immunomodulatory effects [1] that may be beneficial in the treatment of chronic inflammatory diseases [1–3]. Pre-clinical *in vitro* and *in vivo* studies, including experimental models of allergic [4,5] and tobacco-smoke-induced lung inflammation [6,7] have shown that statins reduce components of airway inflammation potentially relevant to the pathogenesis of asthma and smoke-induced airway diseases. In addition, statins might restore corticosteroid sensitivity in asthma [8,9]. Taken together,

Abbreviations: ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; ATS, American Thoracic Society; BMI, body mass index; CCL, chemokine (C-C motif) ligand; CXCL, chemokine (C-X-C motif) ligand; EGF, epidermal growth factor; FGF, fibroblast growth factor; G-CSF, granulocyte-colony stimulating factor; GRO, growth related oncogene; ICAM, intercellular adhesion molecule; ICS, inhaled corticosteroid; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein; LPS, lipopolysaccharide; MDA, malondialdehyde; MDC, macrophage-derived chemokine; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; OPG, osteoprotegerin; PAI-1, plasminogen activator inhibitor-1; sCD40L, soluble CD40 ligand; TGF-α, transforming growth factor alpha; TNFα, tumour necrosis factor-alpha; VEGF, vascular endothelial growth factor.

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these findings suggest that statin treatment may have anti-inflammatory effects in people with asthma who smoke. Smokers with asthma have poor symptom control, accelerated decline in lung function and an attenuated therapeutic response to corticosteroids compared to never smokers with asthma [10–14]. Optimal strategies for managing smokers with asthma remain to be established. In addition to smoking cessation, there is an unmet need for alternative or additional drugs for treating smokers with asthma [15].

In a randomised controlled study of short-term treatment with atorvastatin in smokers with mild to moderate asthma, improvements in asthma control questionnaire (ACQ) and asthma quality of life questionnaire (AQLQ) scores were found in the absence of a decrease in the concentration of a selection of sputum and serum acute inflammatory mediators [16]. It is possible that the range of sputum mediators measured in this study [16] may have failed to detect more long-term anti-inflammatory effects of atorvastatin. In the present study we selected sputum mediators that included cytokines, chemokines, and growth factors implicated in the pathogenesis of asthma and that would be potential targets for anti-inflammatory therapies for asthma. The aim of the present study was to test the hypothesis that atorvastatin alone and in combination with inhaled corticosteroid (ICS) suppressed the concentration of a range of cytokines, chemokines and growth factors in sputum samples collected during the previous clinical trial [16].

2. Methods

2.1. Subjects and study design

Cigarette smokers with chronic asthma symptoms of more than one year duration, aged 18–60 years were recruited to a randomised controlled trial comparing atorvastatin (40 mg per day) versus placebo for four weeks, followed by inhaled beclometasone (Clenil® Modulite® 200 μg twice daily) added to both treatment arms for a further four weeks [16] (Fig. 1). Current smokers were defined as smoking ≥5 cigarettes per day and with a history of five pack years or more. Patients were excluded if they were already taking a statin or medications known to interact with statins such as antifungal agents, macrolide antibiotics, cyclosporin, gemfibrozil, verapamil and amiodarone. All subjects demonstrated reversibility in FEV_1 following albuterol of ≥12% and ≥200 ml or $PC_{20} \leq 8$ mg/ml, or ≥20% variability in peak expiratory flow (PEF) [17]. At baseline, 4 and 8 week visits, electronic PEF data were downloaded and spirometry performed. At these visits patients completed a validated asthma control questionnaire (ACQ) and asthma quality of life questionnaire (AQLQ); induced sputum was performed. Sputum samples were analysed from a sub-group of 39 smokers with asthma recruited to the trial in which there was sufficient supernatant fluid for mediator analyses. The West Glasgow Research Ethics Committee approved the study and all patients gave written informed consent.

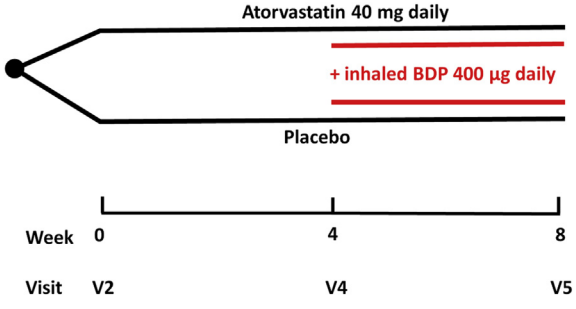

Fig. 1. Study design of randomised controlled trial comparing atorvastatin (40 mg per day) versus placebo for 4 weeks, followed by inhaled beclometasone (BDP: 400 μg per day) for a further 4 weeks. Baseline samples were taken at V2, +/− atorvastatin at V4, and ICS and atorvastatin + ICS at V5. Abbreviation: V = visit number.

Table 1
Baseline demographic, clinical and inflammatory characteristics.

	Smokers with asthma included in current study, n = 39		Smokers with asthma included in RCT of atorvastatin [16], n = 68
	Placebo (n = 24)	Atorvastatin (n = 15)	
Age (years)	43.5 (10.7)	37.9 (7.8)	41.6 (10.0)
Gender: male, n (percent)	12 (50.0%)	5 (33.3%)	30 (44.1%)
Asthma duration (years)	26.5 (16.0)	25.0 (11.0)	19.8 (11.9)
BMI kg/m [2]	25.6 (5.4)	23.4 (5.2)	25.5 (4.9)
Pack years smoked	21.8 (12.7)	24.9 (11.0)	26.0 (18.1)
Atopic, n (percent)	15 (62.5%)	7 (46.7%)	39 (60.9%)
ACQ score	2.0 (0.7)	2.0 (1.0)	1.9 (0.9)
AQLQ score	5.1 (1.2)	4.9 (1.4)	5.2 (1.0)
FEV_1 (% predicted) pre-bronchodilator	78.1 (17.1)	77.1 (22.0)	81.3 (19.2)
FEV_1 BD response	15.1 (11.9)	16.3 (13.6)	14.9 (12.6)
PEF (L/min) pre-bronchodilator	377.9 (79.0)	349.5 (98.2)	348.9 (116.4)
Sputum eosinophils (% of total) median (IQR)	0.46 (0.1, 1.7)	0.73 (0.2, 1.6)	0.49 (0.1, 1.7)
Sputum neutrophils (% of total) median (IQR)	32.3 (22.2, 48.2)	30.7 (22.8, 54.1)	30.7 (17.3, 52.0)
Sputum macrophages (% of total) median (IQR)	46.9 (39.4, 58.5)	38.7 (32.7, 53.9)	45.1 (31.8, 58.7)

Data presented as mean (SD) except where indicated.
Abbreviations: ACQ; asthma control questionnaire, AQLQ; asthma quality of life questionnaire, BD; bronchodilator, BMI, body mass index, FEV_1; forced expiratory volume in 1 s, IQR; interquartile range, RCT; randomised controlled trial.

2.2. Measurements

Sputum induction was performed as previously described [18], using a low concentration of DTT (0.003%) to disperse cells without undue effects on mediator measurements. Supernatant fluid was analysed for a range of mediators including IL-1α, IL-1β, IL-1RA, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p70, IL-13, IL-17A, TNFα, IFN-β, IFN-γ, sCD40L, CCL2 (MCP1), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL7 (MCP3), CCL11 (eotaxin), CCL22 (MDC), CXCL1 (GROα), CXCL8 (IL-8), CXCL10 (IP-10), CX3CL1 (fractalkine), VEGF, TGF-α, G-CSF, EGF, FGF-2, MDA, PAI-1, OPG, MMP-8 and MMP-9 (Luminex multiplex analysis, or biochemical analysis; further details in online supplementary data) and calculated concentrations of analytes were corrected to the total protein concentration in the supernatant fluid.

PEF was recorded using Piko-1 electronic peak flow meters (nSpire Hertford UK) and ACQ [19] and AQLQ [20] results were also recorded. Spirometry was performed to American Thoracic Society guidelines [21]. Exhaled carbon monoxide levels were measured by a hand held Smokerlyser (Bedford scientific, UK). Airway hyperresponsiveness was measured by Cockcroft's methacholine challenge test with concentrations from 0.03 mg/ml to 16 mg/ml [22].

2.3. Statistical analysis

Baseline characteristics were described by number and percentage of patients for categorical variables, and mean (SD) or median (inter-quartile range) for normally distributed or skewed continuous variables respectively. The statistical assessment was by non-parametric methods (Kruskal-Wallis and Wilcoxon Mann-Whitney tests). Correlation analysis was by Spearman's rho with