

Original article

Simvastatin does not exhibit therapeutic anti-inflammatory effects in asthma

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Background: Statins lower cholesterol and also exhibit anti-inflammatory properties. *In vitro* and animal studies have suggested they may be useful for the treatment of a number of inflammatory conditions.

Objective: To evaluate the *in vivo* therapeutic potential of simvastatin as an anti-inflammatory agent in patients with asthma.

Methods: Potential signal from treatment effect was optimized by withdrawing all anti-inflammatory treatment for the duration of the study. Participants received 1 month of daily simvastatin and 1 month of daily placebo in a randomized, double-blind crossover trial. A total of 16 patients completed per protocol. Asthmatic inflammation was evaluated by measuring exhaled tidal nitric oxide, alveolar nitric oxide, sputum and peripheral eosinophil count, methacholine hyperresponsiveness, salivary eosinophilic cationic protein, and C-reactive protein. Measurements of dynamic and static lung volumes and of cholesterol were also made.

Results: After initial withdrawal of usual asthma medication, there was a 1.43 geometric mean fold increase (ie, 43% difference) in fraction of exhaled nitric oxide (95% CI, 1.15 to 1.78; $P = .004$). Compared with placebo, simvastatin led to a 0.86 geometric mean fold decrease (95% CI, 0.7 to 1.04; $P = .15$) in exhaled nitric oxide (ie, a 14% difference), and a -0.18 doubling dilution shift (95% CI, -1.90 to 1.55 ; $P = 1.0$) in methacholine hyperresponsiveness. There were no significant differences in other inflammatory outcomes, lung volumes, or airway resistance between simvastatin and placebo. Treatment with simvastatin led to a significant reduction ($P < .005$) of total and low-density lipoprotein cholesterol.

Conclusion: There is no evidence to suggest simvastatin has anti-inflammatory activity in patients with asthma.

Clinical implications: Simvastatin is not useful for the treatment of asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Asthma, simvastatin, nitric oxide, inflammation, methacholine challenge, sputum eosinophils, clinical trial

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Abbreviations used

CRP: C-reactive protein
ECP: Eosinophilic cationic protein
FENO: Fraction of exhaled nitric oxide
HDL: High-density lipoprotein
ICS: Inhaled corticosteroid
LDL: Low-density lipoprotein

Statins are a widely prescribed class of medication indicated for primary and secondary prevention of cardiovascular disease that effectively reduce cholesterol by inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase and consequently improve morbidity and mortality.^{1,2} A number of large trials with statins have noted greater reductions in cardiovascular risk than would be anticipated by cholesterol lowering alone, in association with reduced levels of circulating C-reactive protein (CRP).^{3,4} Statins are now recognized as atypical anti-inflammatory agents possessing a variety of immunomodulating effects in addition to their conventional lipid-lowering properties.^{5,6} The precise mechanism by which statins exert this anti-inflammatory effect is unclear, although a number of possibilities exist.⁶ The mevalonate metabolic pathway is reversibly inhibited by statins and is known to be integral to cell membrane lipid raft formation.⁷ Lipid rafts are essential for immune system autocrine signaling, MHC expression, and inflammatory cell interactions including antigen presentation.⁷⁻⁹ In addition, the mevalonate pathway is also responsible for synthesis of prenylated proteins, which are important for immune cell maturation and function including production of inflammatory messengers.^{6,10} Furthermore, statins have been shown to prevent effective immune progenitor cell adhesion and migration,¹¹ inhibit inducible MHC expression,^{12,13} reduce circulating levels of TNF- α ,¹⁴ and directly inhibit T-lymphocyte differentiation and function.¹⁵ Statins also effectively inhibit dendritic cell antigen presentation *in vitro*, possibly by a combination of these effects.¹⁶ Recently it has been postulated that statins may have a role in inflammatory lung disease, with investigators demonstrating that in a rat model of chronic obstructive pulmonary disease, simvastatin suppressed matrix metalloproteinase 9 induction and reduced pulmonary inflammation and vascular hypertension.¹⁷

Together these data suggest components of the asthmatic inflammatory cascade could be amenable to treatment with statins. In particular, antigen presentation, T-cell maturation, inflammatory cytokine expression, and intracellular adhesion molecule 1/leukocyte function-associated antigen-dependent cell trafficking are all important aspects of asthmatic inflammation that have been successfully attenuated with statin treatment in previous experimental models.⁶

The theoretical potential for asthma pharmacotherapy with statins is supported further by recent data from animal and *in vitro* studies. In a murine model of asthma, simvastatin given immediately before ovalbumin challenge in sensitized mice led to significant inhibition of the inflammatory response with reduction of bronchoalveolar lavage proinflammatory cytokine levels and eosinophils, and reduced peribronchial infiltration with inflammatory cells.¹⁸ In addition, an *ex vivo* experiment on peripheral eosinophils derived from 10 patients with asthma showed that simvastatin induced apoptosis in a reversible, dose-dependent manner.¹⁹ Furthermore, statins have also been shown to inhibit IgE-mediated mast cell histamine release from human lung mast cells *in vitro*.²⁰ Despite the potential therapeutic role for statins in asthma, no studies have been published to date examining this hypothesis. We have performed the first randomized controlled trial investigating the potential utility of simvastatin as an anti-inflammatory agent in asthma.

METHODS

The Tayside committee for medical research ethics approved the protocol before commencement of the trial, and written informed consent was obtained from each participant. The trial was conducted between June 2005 and May 2006 in Dundee, Scotland. Additional methodological data are available in this article's [Online Repository](http://www.jacionline.org) at www.jacionline.org.

Participant selection

Patients with mild to moderate asthma with documented normal liver function and an FEV₁ $\geq$ 60% of predicted on a regular maintenance dose of inhaled corticosteroids of $\leq$ 2000 μ g daily of beclomethasone or equivalent were invited to participate. Patients were excluded if they were pregnant, were smokers, were already receiving or were known to be allergic to statins, or had an exacerbation of their asthma requiring treatment with oral corticosteroids during the preceding 3 months.

Randomized treatment periods

All anti-inflammatory asthma medication including inhaled corticosteroids (ICSs), leukotriene receptor antagonists, theophylline, and antihistamines were tapered, then stopped completely for the duration of the study in a manner previously described elsewhere.²¹ Briefly, all second-line anti-inflammatory agents including theophylline, leukotriene receptor antagonists, and anti-histamines were stopped. Thereafter, the participants' dose of ICS was halved on a weekly basis and when $\leq$ 400 μ g per day beclomethasone dipropionate stopped completely. Participants were begun on salmeterol 50 μ g twice daily at the beginning of this step-down period, and this was continued for the duration of the study to palliate symptoms. Short-acting rescue β_2 -receptor agonists were allowed throughout. Once all anti-inflammatory therapy had been completely stopped, participants underwent a 1-week blind run-in period during which they

received nonrandomized placebo (1 tablet daily) before baseline assessment. After this run-in period, patients were required to have a fraction of exhaled nitric oxide (FE_{NO}) $\geq$ 20 ppb and a methacholine PC₁₀ $\leq$ 2 mg/mL. They were then randomized to sequential double-blind treatment blocks of simvastatin 20 mg daily for 2 weeks, then simvastatin 40 mg daily for 2 weeks, or placebo once daily for 4 weeks (Fig 1) in a crossover fashion, so that all participants received both placebo control and active simvastatin treatment. Previous studies in healthy volunteers have shown that 14 days of statin treatment is sufficient to mediate effects on the immune system.¹³ Medication was administered once daily in the evening.

Participant safety

All participants were evaluated at 14-day intervals to ensure their spirometry had not deteriorated beyond a predefined safety threshold of FEV₁ $<$ 50%. Participants withheld their long-acting β -agonist (LABA) for 48 hours before each of these visits. In addition, at each safety visit, participants were assessed for signs of any adverse drug reaction including myositis or rhabdomyolysis. This involved examination by a physician and sampling of blood for creatinine kinase, liver function tests, and renal function tests. Only a physician independent of the conduct of the trial viewed the results. Furthermore, each participant was supplied with a contact number via which they could seek advice from one of the investigators 24 hours a day should any problems arise.

Treatment evaluation

Participants were assessed for treatment response at baseline after placebo run-in and after each 4-week randomized treatment block. Salmeterol was withheld for 48 hours and all short-acting β_2 -receptor agonists for 6 hours before each assessment. On each occasion, the following were undertaken: quantification of FE_{NO}²²; derivation of compartmental nitric oxide levels (alveolar fraction and bronchial flux) using a linear regression model²³; abbreviated bronchial challenge to evaluate methacholine PC₁₀^{24,25}; induced sputum eosinophil count²⁶; spirometry²⁷; whole-body plethysmography²⁸; salivary eosinophilic cationic protein (ECP) estimation²⁹; peripheral blood eosinophil count; serum CRP; and total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol levels.

Statistical methods

SPSS version 12 (SPSS Inc, Chicago, Ill) was used to perform the statistical analysis. The study was designed with 90% power to detect a 25% reduction in FE_{NO} with 12 patients completed per protocol; all other endpoints were considered secondary. FE_{NO} was chosen as the primary outcome measure because of the previously documented steep dose-response relationship observed with conventional asthma pharmacologic agents, thus maximizing the possibility of detecting any anti-inflammatory effect. Data were initially assessed for normality using distribution plots; any nonnormally distributed data were log-transformed before analysis. Data were evaluated by an overall ANOVA with treatment, subject, and sequence as cofactors, followed by Bonferroni-corrected pairwise comparisons with a 2-tailed α -error set at 0.05.

RESULTS

Twenty-six participants were recruited, of whom 2 were withdrawn after the step-down period at the visit before placebo run-in, and a further 4 dropped out during the placebo run-in phase. Twenty participants were randomized, and 16 completed per protocol (Table I). Of the

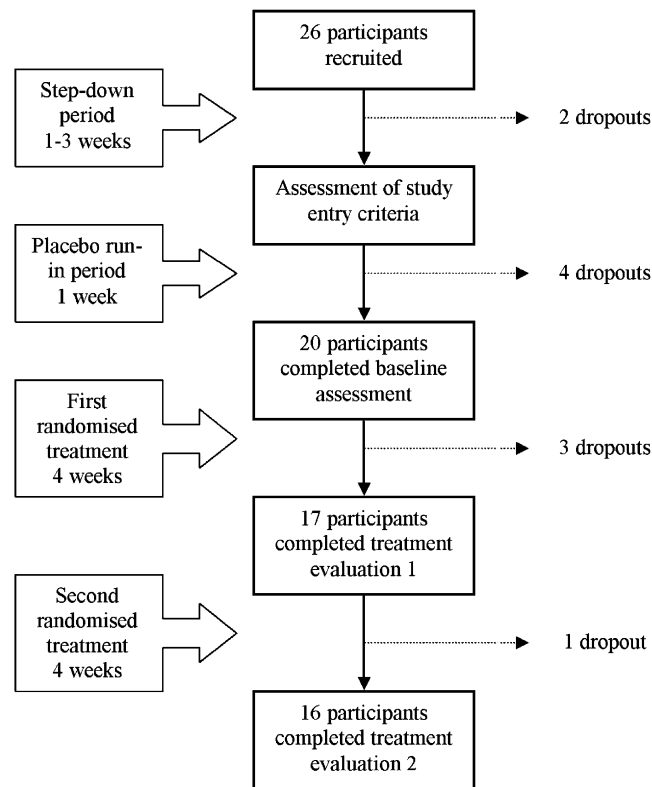


FIG 1. Study design. All participants underwent initial screening followed by a step-down period of between 1 and 3 weeks depending on the starting dose of ICS. Thereafter, all participants underwent a 1-week nonrandomized placebo run-in and were required to have elevated levels of inflammatory indices (exhaled nitric oxide ≥ 20 ppb, methacholine $PC_{10} \leq 2$ mg/mL) before entering the randomized crossover phase of the trial. All participants received randomized treatments of simvastatin (20 mg per day for 2 weeks, then 40 mg per day for 2 weeks) and placebo in a double-blind crossover fashion. Reasons for participant dropout included worsening symptoms (5 participants), personal reasons (4 participants), and deterioration in pulmonary function (1 participant).

participants who were recruited but did not complete per protocol, 6 were withdrawn because of deterioration in their asthma control, and 4 failed to comply fully with the protocol or withdrew for personal reasons (see this article's [Table E1](#) in the Online Repository at www.jacionline.org). There was no significant difference in percentage predicted FEV₁ ($P = .49$), dose of ICS ($P = .50$), FE_{NO} ($P = .35$), or methacholine PC_{10} ($P = .69$) between participants who completed the study per protocol and those who did not complete the study ([Table I](#) and [Table E1](#) in the Online Repository at www.jacionline.org). There was no evidence of any sequence or period effect for any inflammatory outcome measure (see this article's [Table E2](#) in the Online Repository at www.jacionline.org).

After withdrawal of usual asthma medication (ie, comparing values before step-down with those before placebo run-in), there was an average 1.43-fold change (ie, a 43% increase) in FE_{NO} (95% CI, 1.15 to 1.78; $P = .004$; [Table I](#)).

There was a 1.08 geometric mean fold difference (ie, an 8% change) in exhaled nitric oxide levels (95% CI, 0.87 to 1.33; $P = 1$) after 1 month of randomized treatment with

placebo, compared with the baseline value after 1 week of nonrandomized placebo run-in. Four weeks of treatment with simvastatin failed to attenuate any aspect of the asthmatic inflammatory cascade. Specifically, there was no difference in FE_{NO} between randomized treatments with either simvastatin and placebo (geometric mean fold change of 0.86 [ie, a 14% reduction]; 95% CI, 0.70 to 1.04; $P = .15$) or simvastatin and baseline (geometric mean fold change of 0.92 [ie, an 8% reduction]; 95% CI, 0.70 to 1.14; $P = .92$; [Table II](#) and [Fig 2](#)). The uncorrected P value without Bonferroni adjustment for the difference in FE_{NO} between simvastatin and placebo was .07, and between simvastatin and baseline was .22. Examining the airway in a compartmental fashion by specifically evaluating the alveolar contribution to total FE_{NO} and bronchial flux revealed similar consistent results, with no treatment effect observed with either outcome ([Table II](#) and [Fig 2](#)). Alternative methods of estimating asthmatic airway inflammation also showed no significant demonstrable therapeutic effect with simvastatin. Airway hyperresponsiveness was unchanged by simvastatin treatment with a -0.18 (95% CI, -1.90 to 1.55) doubling-dilution

TABLE I. Participant demographics

Patient no.	Sex	Age (y)	GINA severity	SPT	FEV ₁ (% predicted)	FEF ₂₅₋₇₅ (% predicted)	Daily dose ICS (beclometasone dipropionate equivalent)	Methacholine PC ₁₀ (mg/mL)	FE _{NO} at trial enrollment (ppb)	FE _{NO} after step-down (ppb)
2	M	53	III	NA	84	72	400	0.32	67.2	92.7
3	M	58	III	D, C	72	49	2000	0.08	15.7	34.0
5	F	37	II	GP, HDM	127	124	200	0.39	10.7	20.0
6	M	56	II	GP, HDM	92	81	0	1.68	28.9	23.0
8	M	30	II	HDM, D, C	68	39	400	0.15	19.7	82.2
9	F	38	II	GP, WP	97	56	0	0.06	38.5	46.5
10	F	48	II	HDM	80	81	200	1.68	16.4	20.7
11	F	34	II	GP, WP, HDM, D, C	97	71	400	0.22	18.2	32.8
13	M	56	II	NA	86	68	400	2.00	12.0	20.1
16	F	53	III	TP, WP	76	47	2000	0.29	23.8	25.4
18	M	37	II	GP, WP	80	79	2000	0.14	34.0	35.3
19	F	31	III	GP, HDM, D, C	72	36	0	0.13	51.3	48.7
20	F	46	II	GP, D, C	81	49	0	0.21	34.9	34.9
22	F	47	II	GP, HDM, C	95	60	0	0.11	37.2	39.8
23	F	19	II	TP, WP, GP, HDM, C, D	98	115	1000	0.08	34.3	81.1
24	F	40	III	GP, HDM, D, C	76	66	400	0.08	86.4	115.8
Mean (SEM)		42.7 (2.8)			86.3 (3.7)	68.3 (6.1)	400 (0-850)*	0.24 (0.18-0.31)†	28.0 (24.1-32.5)†	40.0 (34.3-46.6)†

D, Dog; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of forced vital capacity; GP, grass pollen; HDM, house dust mite; NA, nonatopic; SPT, skin prick test; TP, tree pollen; WP, weed pollen.

The values reported are after withdrawal of usual asthma maintenance therapy (ie, after the step-down period). The dose of ICS is that prescribed at the time of trial enrollment. Global Initiative for Asthma (GINA) classification was determined by their FEV₁ and symptoms after withdrawal of usual maintenance therapy (ie, after the step-down period), and their usual prescribed dose of ICS. Exhaled nitric oxide levels (FE_{NO}) before and after withdrawal of anti-inflammatory medication (ie, at trial enrollment and after step-down) are shown for comparison. All participants were required to have a methacholine PC₁₀ ≤ 2 mg/mL and an FE_{NO} ≥ 20 ppb at baseline to be included in the trial.

*Median (interquartile range).

†Geometric mean (SE of geometric mean).

difference in methacholine PC₁₀ compared with placebo, and a 0.02 (95% CI, −1.55 to 1.88) doubling-dilution shift from baseline, as was sputum eosinophil count, with a 0.31% (95% CI, −5.08 to 5.69) difference between treatments (Table II, and see this article's Figs E1 and E2 in the Online Repository at www.jacionline.org). There was also no difference between simvastatin and placebo on downstream markers of systemic inflammation including CRP (mean fold difference, 0.97; 95% CI, 0.32 to 2.93), salivary ECP (geometric fold difference, 1.34; 95% CI, 0.51 to 3.52), or absolute peripheral eosinophil count ($0.03 \times 10^9/L$; 95% CI, −0.05 to $0.10 \times 10^9/L$; Table II, and see this article's Figs E3 and E4 in the Online Repository at www.jacionline.org). Furthermore, conventional indices of airway caliber and lung volumes were also unaffected by treatment, with no detectible improvement in any parameter (Table III and Fig 3). Four weeks of simvastatin did significantly reduce total

cholesterol compared with placebo (absolute reduction, −0.93 mmol/L; 95% CI, −1.57 to −0.30; $P < .005$) and LDL cholesterol (absolute reduction, −1.10 mmol/L; 95% CI, −1.72 to −0.48; $P = .001$; Fig 4).

DISCUSSION

Trial design and main results

This study is the first to investigate the use of statins as a potential anti-inflammatory agent in asthma. We have done so by using a comprehensive raft of airway specific and systemic inflammatory biomarkers, as well as standard measures of bronchial caliber and lung volume. Airway caliber is often well preserved in patients with mild to moderate asthma and can be dissociated from underlying disease activity³⁰; as such, it is a relatively insensitive tool by which to gauge treatment effect. Therefore, evaluation of a range of direct and indirect

TABLE II. Inflammatory outcome measures as a shift from placebo baseline after 4 weeks of treatment with placebo and after 4 weeks of treatment with placebo*

	Shift from baseline with treatment					
	Placebo		Simvastatin		Difference between placebo and placebo	
	Mean	95% CI (P)	Mean	95% CI (P)	Mean	95% CI (P)
Exhaled nitric oxide†	1.08	0.87 to 1.33 (1)	0.92	0.7 to 1.14 (.92)	0.86	0.70 to 1.04 (.15)
Alveolar nitric oxide, ppb	0.58	−0.96 to 2.12 (.94)	−0.16	−2.6 to 2.26 (1)	−0.74	−3.5 to 2.0 (1)
Bronchial flux, nL/s	1.03	−0.35 to 2.40 (.18)	0.05	−0.61 to 0.72 (1)	−0.97	−2.32 to 0.37 (.21)
Methacholine PC ₁₀ ‡	0.193	−0.98 to 1.36 (1)	0.02	−1.55 to 1.58 (1)	−0.18	−1.90 to 1.55 (1)
Sputum eosinophil count, %	1.05	−3.99 to 6.09 (1)	1.36	−3.68 to 6.40 (1)	0.31	−5.08 to 5.69 (1)
Peripheral eosinophil count × 10 ⁹ /L	0.02	−0.06 to 0.09 (1)	0.04	−0.04 to 0.13 (1)	0.03	−0.05 to 0.10 (1)
Salivary ECP†	1.21	0.30 to 4.75 (1)	1.61	0.47 to 5.55 (.85)	1.34	0.51 to 3.52 (1)
High-sensitivity CRP assay†	0.85	0.48 to 1.51 (1)	0.82	0.29 to 2.42 (1)	0.97	0.32 to 2.93 (1)

*Outcomes are expressed as absolute change relative to placebo baseline (shift from baseline with treatment), and as the difference in values after 4 weeks of treatment with placebo compared with 4 weeks of treatment with simvastatin (difference between placebo and simvastatin). Data requiring transformation before analysis are expressed as either †geometric mean fold change (ie, the proportion between values) or ‡doubling-dilution shift in methacholine hyperresponsiveness. All *P* values are Bonferroni-corrected.

markers of asthmatic inflammation was deliberately incorporated into the trial design to ensure detection of any potential therapeutic effect across the inflammatory process. Specifically, quantification of sputum eosinophils provided a direct measure of inflammatory cellular infiltration; methacholine challenge assessed secondary airway hyperresponsiveness; combined FE_{NO} and alveolar NO fraction allowed characterization of anatomical components of the airway; and ECP, CRP, and peripheral eosinophil count evaluated downstream signaling. By differentiating alveolar from tidal nitric oxide, we ensured that if any anti-inflammatory effect were confined to the small airways, it would not have been masked by sampling only the total exhaled fraction. Furthermore, conventional anti-inflammatory treatments were discontinued for the duration of the trial, and participants were selected a priori for evidence of ongoing asthmatic airway inflammation to maximize detection of any therapeutic signal that might be present, while simultaneously eliminating potential confounding from other prescribed medications. We are therefore confident that had simvastatin treatment led to any significant attenuation of the inflammatory process, it would have been detectable using this model. We also ensured that any effect of salmeterol was washed out for 48 hours before each visit.

It is interesting to note that there was no reduction in CRP, which has been a consistent feature of cardiovascular trials using statins.^{3,4} Because CRP is a downstream marker produced by hepatocytes in response to IL-6 immune cell signaling from a distant site, this is perhaps unsurprising given the lack of therapeutic influence on the primary inflammatory process occurring in the airway.³¹ In addition to this, no effect was seen on dynamic and static lung volume indices or on airway resistance. This may be considered predictable given the absence of any therapeutic modulation of the underlying inflammatory pathology. However, this also reassuringly demonstrates a consistent lack of effect regardless of the modality used to measure disease activity. Importantly,

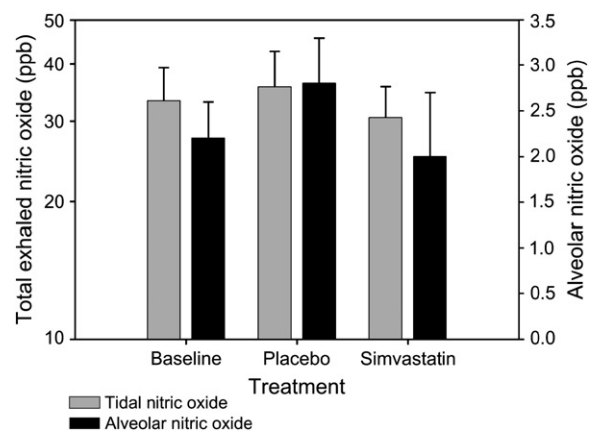


FIG 2. Exhaled and alveolar nitric oxide. Exhaled nitric oxide was measured with a mouth flow rate of 50 mL/s and is depicted as the geometric mean and SE of the geometric mean on a log₁₀ scale. Alveolar nitric oxide was normally distributed and is plotted on a linear scale.

the anticipated reduction in total cholesterol and LDL cholesterol was clearly demonstrated. This indicates that the dose and duration of treatment used were sufficient for simvastatin to exert a therapeutic metabolic effect, although not as an anti-inflammatory agent.

Varying biological action of different statins

The pleiotropic effects of statins are independent of their lipid-lowering ability, with each compound in the class exhibiting different effects on inflammatory cells.³² These differential actions are in part related to the relative lipophilicity of individual agents with statins such as simvastatin and atorvastatin having the greatest anti-inflammatory potential.³³ This is explained by their preferential ability to enter a variety of somatic cells, in contrast with hydrophilic compounds such as pravastatin that are reliant on active uptake by hepatocytes to mediate their metabolic activity.³⁴ In addition, it is paramount to

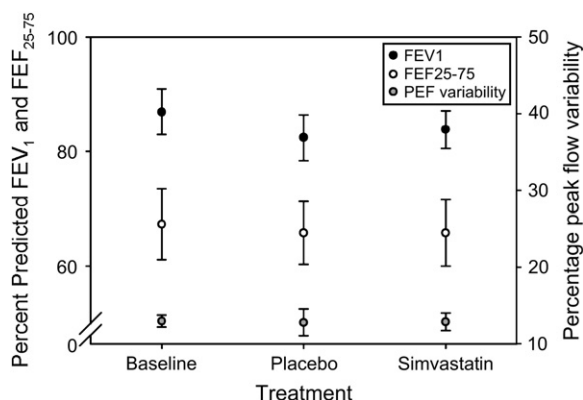
TABLE III. Spirometric lung volume and airway resistance indices as change from placebo baseline after 4 weeks of treatment with placebo and simvastatin*

	Shift from baseline with treatment					
	Placebo		Simvastatin		Difference	
	Mean	95% CI (P)	Mean	95% CI (P)	Mean	95% CI (P)
FEV ₁	-4.5	-8.5 to -0.5 (.03)	-3.1	-7.7 to 1.6 (.29)	1.4	-2.0 to 4.9 (.82)
FEF ₂₅₋₇₅	-1.5	-14.5 to 11.5 (1)	-1.5	-14.0 to 11.0 (1)	0.0	-15.3 to 15.3 (1)
PEF	-4.1	-11.4 to 3.2 (.45)	-2.9	-10.0 to 4.2 (.87)	1.3	-5.7 to 8.2 (1)
PEF variability	-0.3	-3.7 to 3.2 (1)	-0.15	-2.3 to 2.0 (1)	0.13	-3.5 to 3.7 (1)
Residual volume	-4.5	-31.4 to 22.4 (1)	-18.2	-58.9 to 22.5 (.71)	-13.7	-43.1 to 15.8 (.67)
Total lung capacity	1.4	-8.5 to 11.3 (1)	-3.7	-14.2 to 6.8 (1)	-5.1	-14.2 to 4.0 (.43)
Vital capacity	8.8	-6.4 to 23.9 (.41)	2.75	-2.3 to 7.8 (.47)	-6.0	-21.4 to 9.4 (.91)
Specific airways resistance	19.8	-6.3 to 45.8 (.17)	2.3	-25.1 to 29.7 (1)	-17.5	-38.1 to 3.2 (.11)

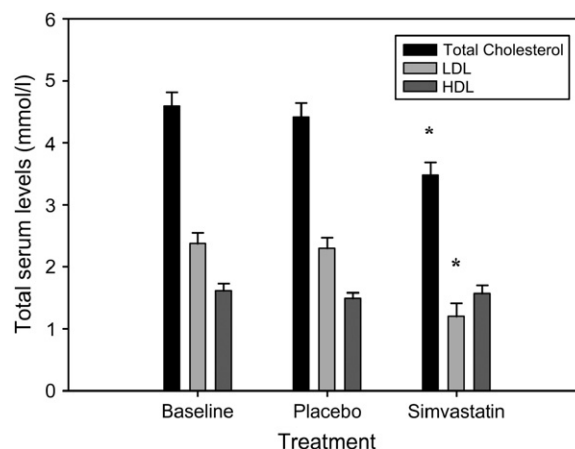
FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of forced vital capacity.

*All values expressed as change in percentage of predicted value except peak expiratory flow (PEF) variability, which is change in percentage variability.

All P values are Bonferroni-corrected.

**FIG 3.** Spirometry at baseline and after treatment with placebo and simvastatin. All values expressed as percentage of predicted value except peak expiratory flow (PEF) variability, which is percentage variability. FEF₂₅₋₇₅, Forced expiratory flow at 25% to 75% of forced vital capacity.

note that different statins can exhibit contrasting effects on immune function. A study involving 27 healthy volunteers showed that 14 days of treatment with 40 mg simvastatin led to increased T-cell expression of HLA-DR and MHC38, suggesting activation of these cells, whereas treatment with 20 mg atorvastatin for the same duration downregulated the same markers.¹³ Moreover, simvastatin caused suppression of superantigen-mediated T-cell activation, but atorvastatin did the opposite and sensitized these cells, clearly indicating that important within-class differences exist between the drugs. This trial also demonstrated that 14 days of treatment with simvastatin was sufficient time to induce relevant immunologic changes. These observations, together with the data demonstrating that simvastatin can lead to a reduction in allergic airway inflammation in ovalbumin-sensitized mice, suggested that simvastatin had the greatest theoretical potential as a therapeutic agent in asthma, and that the dose used (40 mg/d) was theoretically the most promising.¹⁸ In addition, because the pleiotropic effects are dependent on the

**FIG 4.** Total, LDL and HDL cholesterol at baseline and after treatment with placebo and simvastatin. *Significant difference between baseline and placebo; $P < .05$.

individual drug used, there was less evidence to suggest a therapeutic effect in asthmatic inflammation for another drug in the same class, such as atorvastatin or pravastatin.³²

Interpretation of findings

The lack of response seen in this trial may have a number of causes. Subtle metabolic differences may mean demonstrable anti-inflammatory properties seen in animal and *in vitro* models do not directly translate into human beings. However, it is more likely that the beneficial effect of reduced antigen presentation, attenuation of inflammatory cell chemotaxis, and alteration of cytokine excretion attributable to statins is offset by simultaneous modulation of T-cell subset differentiation. Much of the data from both laboratory and clinical trials suggest that statins may cause preferential switch from a T_H1-driven to T_H2-driven immune response.⁶ In a Lewis rat model of myocarditis, administration of atorvastatin reversed production of IFN- γ and IL-2 and promoted IL-4 and

IL-10 production with an associated improvement in myocardial function, suggesting a class switch from a T_H1 to a predominantly T_H2 immune profile.³⁵ Similarly, Youssef et al³⁶ showed that administration of atorvastatin in a murine model of multiple sclerosis induced preferential development of T_H0 into T_H2 cells, and suppressed production of T_H1 -associated cytokines including TNF- α , IFN- γ , and IL-2. This pattern has been repeated in other animal models of disease, including uveitis and arthritis,⁶ and may explain the encouraging trial results previously reported in conditions such as multiple sclerosis, rheumatoid arthritis, and organ transplant rejection that are primarily T_H1 -mediated.^{14,37,38} Supporting this hypothesis, there is a single published case report of atorvastatin-induced refractory nasal polyposis associated with asthma that the authors speculate was driven by T_H1 / T_H2 deregulation directly attributable to statin therapy.³⁹ There is undoubtedly a contributory T_H1 component to asthma, which may be particularly important in severe asthma, as was eloquently highlighted by Berry et al,⁴⁰ who demonstrated increased levels of TNF- α in the peripheral monocytes of patients with refractory disease, and also disease attenuation after treatment with the soluble TNF- α receptor etanercept. However, this role is probably minor compared with the predominant contribution from the T_H2 subset, particularly in mild to moderate disease.⁴¹

Limitations of the study

It is important to note that FE_{NO} may be influenced by a host of biological cofactors and therefore may not specifically represent asthmatic airway inflammation, nor necessarily be the best tool by which to gauge treatment effect.⁴² In addition, we designed the study to detect a 25% reduction in FE_{NO} , which was taken to represent a clinically relevant effect and was less than the 43% change observed after withdrawal of conventional anti-inflammatory medication our population (Table I). Although post hoc calculations revealed that there was 96% and 98% power to detect a 25% reduction in FE_{NO} and a 1 doubling-dilution shift in methacholine PC_{10} , respectively, we concede that this study design would not be adequate to detect smaller changes in inflammatory indices. Although Bonferroni adjustment of P values does minimize the chance of Type I error, the converse is that Type II error may be introduced, although analysis of the primary outcome (FE_{NO}) without this correction also failed to reach statistical significance. The 14% mean difference in FE_{NO} between the randomized treatments should be interpreted in the context of the mean 43% increase after inhaled steroid withdrawal and the 8% difference in FE_{NO} when comparing the post run-in baseline and randomized placebo values. It is also important to appreciate that although no anti-inflammatory effect was evident after treatment with simvastatin, the pleiotrophic effects of individual statins differ, so it is conceivable that using another type of statin (eg, Atorvastatin or Pravastatin) may have produced different results, and our results cannot be generalized to the drug class as a whole.

CONCLUSION

We are aware of at least 2 other ongoing prospective trials investigating the use of statins in asthma (both using atorvastatin), although significantly these differ from our design by using the trial drug as an add-on to conventional therapy, and have not withdrawn ICSs to optimize the inflammatory signal.⁴³ Although we await the results of these ongoing studies with interest, the current evidence does not support a therapeutic role for the use of statins in asthma.

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METHODS

Randomization and blinding

A clinical trials pharmacist independent of the study blinded medication off site (Western Infirmary, Glasgow, United Kingdom). Tablets were encapsulated in gelatin and randomized by a computer-generated number table. Unblinding took place after the final participant had completed per protocol.

Exhaled fractional and alveolar nitric oxide

An online chemiluminescence analyser (Niox; Aerocrine AB, Solna, Sweden) was used to quantify exhaled nitric oxide. This was done at mouth flow rates of 50 mL/s, 100 mL/s, and 200 mL/s with a technically acceptable maneuver defined as one in which a sustained plateau of at least 8 seconds was observed.^{E1} The arithmetic mean of 3 maneuvers at each flow rate was reported. Alveolar nitric oxide and bronchial flux were derived by using the 2-compartment model described by Tsoukias and George^{E2} using the assumption that above an exhalation rate of 50 mL/s, nitric oxide output becomes a linear function of flow rate.

Methacholine challenge

An abbreviated methacholine challenge protocol was used whereby the concentration of methacholine causing a 10% fall in FEV₁ (PC₁₀) was calculated in place of the conventional 20% fall. Our group has previously demonstrated that this abbreviated method is equally as valid as the conventional technique, but has the added advantage of exposing the patient to less methacholine, and causing less bronchoconstriction.^{E3} This was particularly important for the current trial, because participants were withdrawn from all anti-inflammatory medication for the duration of the trial, leading to a significant chance of deterioration in control.

Eosinophilic cationic protein

After mouth rinsing with tap water, a minimum of 1 mL saliva was collected. Specimens were stored at -20°C and analyzed as a single

batch using a radioimmunoassay technique to quantify ECP levels (UniCAP; Sweden Diagnostics, Milton Keynes, United Kingdom). The intra-assay coefficient of variation was 1%.

High-sensitivity CRP assay

ELISA was used to determine high-sensitivity CRP assay levels (Kalon Biological Ltd, Guilford, United Kingdom). The interassay coefficient of variation was 11%.

Sputum induction and analysis

The technique described by Pin et al^{E4} was used to obtain and process sputum samples. Briefly, all participants were administered 200 µg salbutamol via a pressured metered dose inhaler, and then inhaled incremental concentrations of saline (3%, 4%, and 5%) for 10 minutes each via an ultrasonic nebulizer (Sonix 2000; Clement Clark International, Harlow, United Kingdom). After each nebulization, participants were instructed to rinse their mouths to prevent salivary contamination, and then encouraged to expectorate. A sputum cyto-spin was performed within 30 minutes, and a modified Giemsa stain was applied. Cell counts were performed by a single blind observer.

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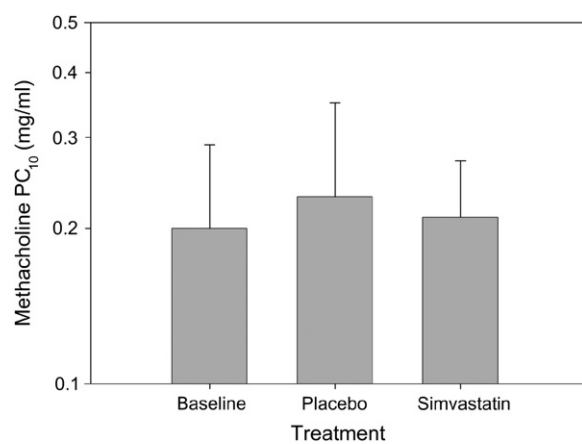


FIG E1. Geometric mean (+SEM) methacholine PC₁₀ at baseline and after 1 month of treatment with placebo and simvastatin.

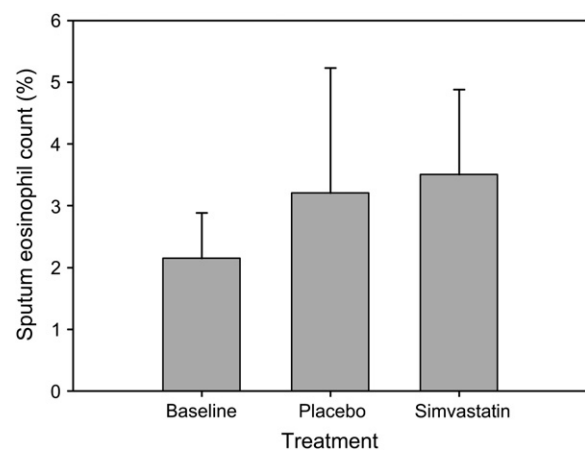


FIG E2. Mean (+SEM) sputum eosinophil count baseline and after 1 month of treatment with placebo and simvastatin.

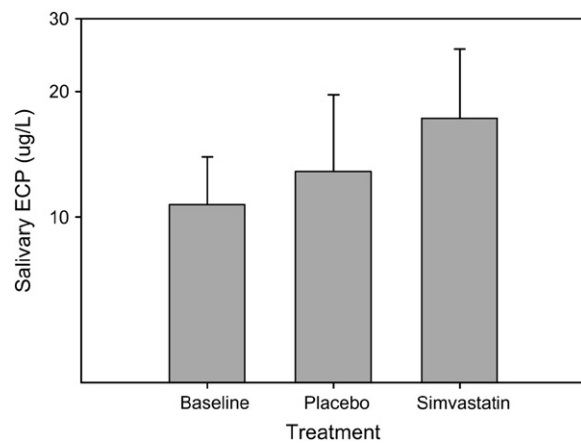


FIG E3. Geometric mean (+SEM) salivary ECP at baseline and after 1 month of treatment with placebo and simvastatin.

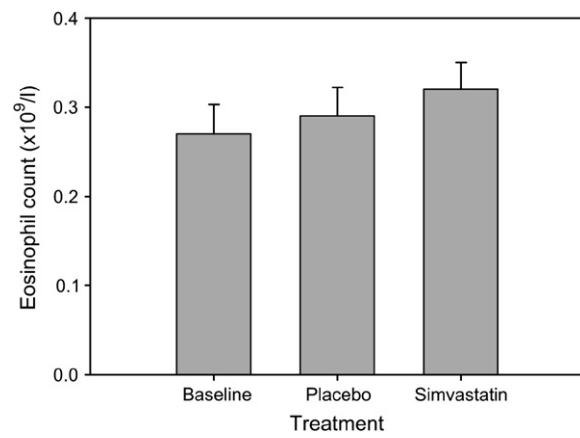


FIG E4. Mean (+SEM) absolute peripheral eosinophil count at baseline and after 1 month of treatment with placebo and simvastatin.

TABLE E1. Demographic details of withdrawn participants*

Patient no.	Sex	Age (y)	GINA severity	SPT	FEV ₁ (% predicted)	FEF ₂₅₋₇₅ (% predicted)	Daily dose ICS (beclometasone dipropionate equivalent)	Methacholine PC ₁₀ (mg/mL)	Exhaled nitric oxide (ppb)
1	F	50	II	WP	98	72	400	0.06	35.9
4	F	67	I	NA	95	99	400	0.50	20.1
7	F	41	I	GP, HDM, C	93	61	0	0.28	22.7
12	M	61	III	HDM	77	69	250	0.04	51.9
14	M	54	I	GP, TP, WP, HDM, D, C	86	44	800	0.03	46.7
15	F	55	I	GP, HDM	95	46	1000	0.85	78.0
17	M	67	III	GP	72	46	800	1.55	17.2
21	F	61	II	NA	101	75	200	0.16	23.3
25	F	36	I	GP, WP, D, C	96	75	400	0.23	32.8
26	M	49	II	GP, D, C	86	54	500	0.14	33.3
Mean (SEM)		54.1 (3.3)			89.9 (3.0)	64.1 (5.5)	400 (237.5-800)†	0.19 (0.13-0.29)‡	32.6 (28.1-37.8)‡

F, Female; M, male; D, dog; FEF₂₅₋₇₅, forced expiratory flow at 25% to 75% of forced vital capacity; GP, grass pollen; HDM, house dust mite; NA, nonatopic; SPT, skin prick test; TP, tree pollen; WP, weed pollen.

*The values reported are after withdrawal of usual asthma maintenance therapy (ie, after the step-down period). The dose of ICS is that prescribed at the time of trial enrollment. Global Initiative for Asthma (GINA) classification was determined by their FEV₁ and symptoms after withdrawal of usual maintenance therapy (ie, after the step-down period), and their usual prescribed dose of ICS.

†Median (interquartile range).

‡Geometric mean (SE of geometric mean).

TABLE E2. Period analysis for selected outcome measures

	Period 1	Period 2	Difference	
	Mean (SEM)	Mean (SEM)	Mean	95% CI (P)
Exhaled nitric oxide (ppb)*	33.9 (28.9 to 39.8)	34.5 (29.4 to 40.5)	0.98†	0.82 to 1.16 (.815)
Methacholine PC ₁₀ (mg/mL)*	0.23 (0.15 to 0.37)	0.24 (0.17 to 0.33)	−0.14‡	−1.38 to 1.09 (.818)
High-sensitivity CRP assay (mg/L)*	0.88 (0.64 to 1.23)	0.80 (0.64 to 1.00)	1.11†	0.49 to 2.51 (.793)
FEV ₁ (% predicted)	84.0 (3.67)	82.1 (3.65)	1.94	−0.83 to 4.7 (.157)

*Geometric mean.
†Geometric fold change.
‡Doubling-dilution shift.