Clarithromycin Targets Neutrophilic Airway Inflammation in Refractory Asthma

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***Rationale*: Patients with refractory asthma have persistent symptoms despite maximal treatment with inhaled corticosteroids and long-acting bronchodilators. The availability of add-on therapies is limited, and effective add-on therapies that target noneosinophilic airway inflammation are needed. Macrolide antibiotics, such as clarithromycin, have *in vitro* efficacy against IL-8 and neutrophils, key inflammatory mediators in noneosinophilic asthma.**

***Objectives*: To determine the efficacy of clarithromycin in patients with severe refractory asthma and specifically in a subgroup of patients with noneosinophilic asthma.**

***Methods*: Subjects with severe refractory asthma (n = 45) were randomized to receive clarithromycin (500 mg twice daily) or placebo for 8 weeks.**

***Measurements and Main Results*: The primary outcome for this study was sputum IL-8 concentration. Other inflammatory outcomes assessed included sputum neutrophil numbers and concentrations of neutrophil elastase and matrix metalloproteinase (MMP)-9. Clinical outcomes were also assessed, including lung function, airway hyperresponsiveness to hypertonic saline, asthma control, quality of life, and symptoms. Clarithromycin therapy significantly reduced airway concentrations of IL-8 and neutrophil numbers and improved quality-of-life scores compared with placebo. Reductions in neutrophil elastase and MMP-9 concentrations were also observed. These reductions in inflammation were most marked in those with refractory noneosinophilic asthma.**

***Conclusions*: Clarithromycin therapy can modulate IL-8 levels and neutrophil accumulation and activation in the airways of patients with refractory asthma. Macrolide therapy may be an important additional therapy that could be used to reduce noneosinophilic airway inflammation, particularly neutrophilic inflammation, in asthma. Clinical trial registered with the Australian Clinical Trials Registry www.actr.org.au (No. 12605000318684).**

Keywords: refractory asthma; macrolides; induced sputum; IL-8; quality of life

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Effective add-on therapies that target noneosinophilic airway inflammation in severe refractory asthma are needed. Macrolide antibiotics have *in vitro* efficacy against IL-8 and neutrophils, key inflammatory mediators in noneosinophilic asthma.

What This Study Adds to the Field

Clarithromycin therapy can modulate IL-8 levels and neutrophil accumulation and activation in the airways of patients with refractory asthma.

Refractory asthma is a term applied to patients with asthma who require high levels of medication to maintain good disease control or to those who experience persisting symptoms despite high levels of medication (1). Although affecting only a small proportion of all asthma sufferers, patients with refractory asthma have a disproportionate impact on health care costs. Current guidelines suggest a stepwise approach to asthma treatment, adding therapy until asthma control is achieved (2). For those with refractory asthma who are taking high doses of inhaled corticosteroids with long-acting β-agonists, add-on therapies are limited to oral corticosteroids and anti–IgE antibody therapy. These therapies act specifically on eosinophilic inflammation and allergic IgE pathways and offer little or no relief to neutrophilic airway inflammation, a recognized feature of severe asthma (3).

Noneosinophilic forms of asthma are common (4) and are difficult to distinguish from eosinophilic asthma using typical clinical tools such as spirometry and airway challenges (5). Patients with noneosinophilic asthma (NEA) have increased neutrophils and IL-8 levels in the airways (6), and conventional therapies such as inhaled corticosteroids, although very effective in those with eosinophilic inflammation, have limited efficacy when eosinophils are within normal range (7). Thus, there is a need for effective antiinflammatory therapies for patients suffering from NEA.

Macrolide antibiotics, such as clarithromycin, have separate and distinct antibiotic and antiinflammatory actions. Macrolides have been widely used in the treatment of infections caused by bacteria such as *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. There is extensive *in vitro* and growing *in vivo* evidence of antiinflammatory activity of macrolides. Reductions in IL-8 and neutrophil numbers have been observed after macrolide therapy in diffuse panbronchiolitis (8) and chronic airway disease (9). Recent case reports have shown that clarithromycin is an effective add-on therapy in prednisone-dependent asthma (10).

We therefore hypothesized that macrolide antibiotics would be an effective add-on therapy in refractory NEA. Given the limited availability of treatment options for refractory asthma, this study aimed to determine the efficacy of 500 mg clarithromycin twice daily for 8 weeks in patients with refractory asthma, and specifically the effects in a subgroup of patients with NEA.

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Some of the results of this study have been previously reported in the form of abstracts (11, 12).

METHODS

Participants

Nonsmoking adults with symptomatic refractory asthma (n = 79), according to GINA (Global Initiative for Asthma) guidelines (2), with demonstrated airway hyperresponsiveness to hypertonic saline (13) were studied. Participants were recruited from the Ambulatory Care Service of the Department of Respiratory and Sleep Medicine at the John Hunter Hospital (New Lambton, Australia). During the study, participants continued with their baseline medications as prescribed by their physician. At screening, participants were educated about the correct use of their baseline medication devices and any errors rectified. Participants were excluded if they had smoked more than 5 pack-years or if they had any known sensitivity to macrolide antibiotics. Antihistamine therapies were ceased for the duration of the study. The Hunter Area Health Service and University of Newcastle Research Ethics Committees approved this study.

Study Design

A randomized, double-blind, placebo-controlled trial was undertaken. Eligible participants were randomly assigned to receive oral clarithromycin 500 mg twice daily (Klacid; Abbot Australasia, Botany NSW, Australia) or placebo. Randomized participants attended four visits at monthly intervals and treatment was discontinued after 8 weeks. The final study visit was conducted 4 weeks after the end of treatment. Randomization was undertaken by a blinded staff member who took no further part in the study. A random-numbers table was computer generated for treatment allocation using permuted blocks of four. The placebo and active medication were packaged identically by the hospital pharmacy department, which dispensed treatments according to the random-number table. Participants were categorized as having an eosinophilic, a paucigranulocytic, or a neutrophilic inflammatory phenotype at screening, as previously described (5). Randomization was stratified according to those with high (>61%) and low neutrophil proportions at screening. Treatment was assigned randomly for each group separately to ensure equal numbers of subjects with high neutrophil proportions in each of the two treatment groups. A preplanned subgroup analysis of the effect of clarithromycin in participants with NEA (neutrophilic and paucigranulocytic asthma) was undertaken. The study was powered to detect differences in airway IL-8 (α, 5%; power, 80%) based on previous studies in nasal polyposis (14).

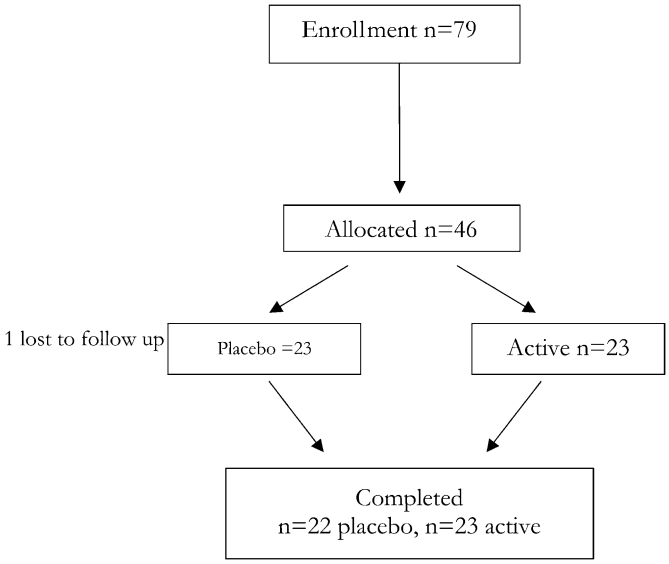

Figure 1. Study profile. Excluded (n = 33). Twenty-three patients declined to participate and 10 did not meet inclusion criteria (4 had no evidence of airway hyperresponsiveness, 2 had previous smoking history of more than 5 pack-years, 2 could not undertake sputum induction due to lung function < 40% predicted, 1 could not produce adequate sputum on two occasions, and 1 commenced doxycycline during run-in).

Assessments

At each visit, participants underwent clinical assessment, spirometry, a combined hypertonic saline challenge, and sputum induction. The asthma control score (15) and quality-of-life score (16) were completed. At screening, smoking history, symptoms, allergy history, and rhinitis symptoms were recorded, and participants undertook a skin allergy test. The presence and severity of wheeze and shortness of breath were assessed using a 5-item symptom score, whereas cough and sputum pro-

TABLE 1. BASELINE CLINICAL CHARACTERISTICS FOR PARTICIPANTS RECEIVING CLARITHROMYCIN AND PLACEBO

	Clarithromycin	Placebo	*P* Value
No.	23	22	
Age, mean yr (range)	60 (27–80)	55 (27–77)	0.199
Female gender, n	13	10	0.329
Clinical			
Age diagnosed with asthma, mean yr (SD)	29 (24)	22 (20)	0.227
Atopy, n (%)	17 (74)	19 (86)	0.252
FEV_1% predicted, mean (SD)	73.6 (15.8)	67.6 (18.8)	0.254
FEV_1/FVC %, mean (SD)	65.9 (17.4)	65.4 (10.7)	0.904
Dose–response slope, median (IQR)*	1.8 (0.6–6.4)	1.0 (0.6–3.2)	0.696
ICS μg equivalents, median (IQR)†	2,000 (1,000–2,000)	2,000 (1,000–2,000)	0.970
Participants receiving			
Combination therapy (ICS and long-acting β_2-agonists), n (%)	19 (83)	18 (82)	0.944
Leukotriene receptor antagonists n (%)	1 (4.3)	1 (5.0)	0.744
Asthma control score, mean (SD)	1.60 (0.63)	1.32 (0.96)	0.255
Quality-of-life questionnaire, total score, mean (SD)	5.5 (0.96)	5.93 (0.98)	0.144
In past 12 months			
Unscheduled visit to medical practitioner	14 (61%)	11 (50%)	0.330
Visit to emergency room	1 (4%)	2 (9%)	0.483
Course of oral corticosteroids	11 (48%)	9 (41%)	0.434

Definition of abbreviations: ICS = inhaled corticosteroids; IQR = interquartile range.

* Dose–response slope: %fall FEV_1/ml 4.5% saline.

† ICS daily dose is calculated a 1 μg of beclomethasone = 1 μg budesonide = 0.5 μg fluticasone.

duction were assessed using a 4-item symptom score. Exhaled nitric oxide was measured using the online NIOX chemiluminescent analyzer (Aerocrine AB, Solna, Sweden) with an expiratory flow rate of 0.05 L/second according to American Thoracic Society guidelines (17). Data for exhaled nitric oxide were not collected for all participants due to the technical problems experienced with the instrument.

Adverse events. The presence of adverse events, including the presence of fever, headache, nausea, vomiting, diarrhea, and skin rashes, was recorded at each study visit and at biweekly intervals between study visits. These data are provided in the online supplement.

The primary outcome was IL-8 levels in sputum supernatant, with secondary outcomes of sputum neutrophil numbers, neutrophil elastase (NE), and matrix metalloproteinase (MMP)-9 levels. Clinical outcomes were reported, including FEV_1% predicted, dose–response slope to hypertonic saline, symptom severity, asthma control score, and asthma quality-of-life questionnaire score.

Sputum induction and saline challenge. Short- and long-acting β-agonists were withheld for their duration of action before testing (12 h for short-acting and 24 h for long-acting). Spirometry and combined bronchial provocation testing and sputum induction with hypertonic saline (4.5%) were performed as previously described (18). A fixed sputum induction protocol of 15 minutes was applied to all participants.

Sputum analysis. Selected sputum (100 μl) was stored in RNA extraction buffer (Qiagen, Hilden, Germany). RNA was prepared and reverse-transcribed to cDNA as described previously (19). The remaining selected sputum was dispersed using dithiothreitol as described (18). A total cell count of leucocytes and viability was performed, supernatant was stored, and cytospins were prepared; and a differential cell count was obtained from 400 nonsquamous cells.

IL-8, NE, and total MMP-9 levels were determined by ELISA (R&D Systems, Minneapolis MN, and Calbiochem, San Diego, CA) (19), and IL-8 gene expression was determined using real-time polymerase chain reaction (20).

Statistical Analysis

Data were analyzed using Stata 9 (Stata Corp., College Station, TX). Descriptive statistics are reported as median and interquartile range

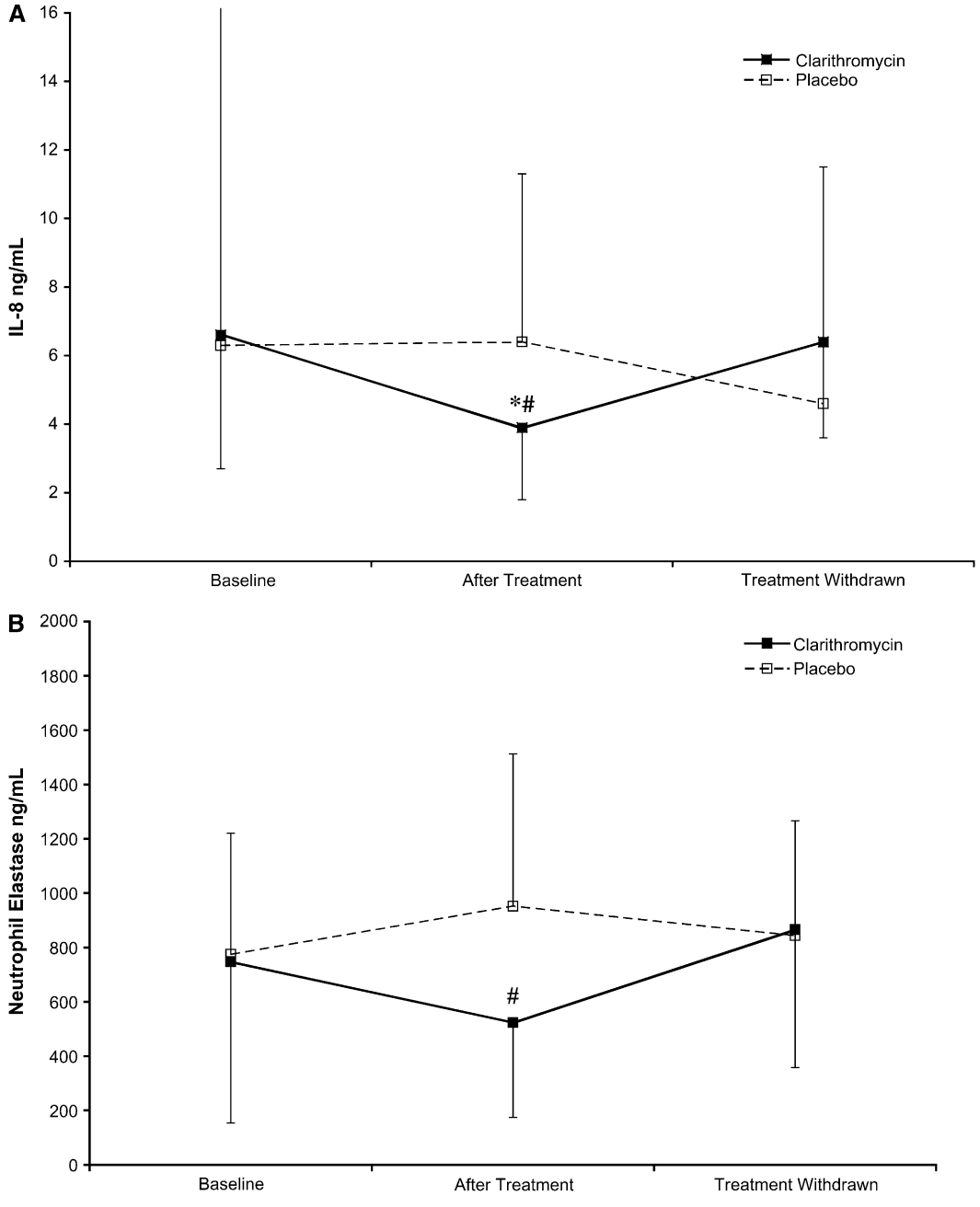

Figure 2. (*A*) Sputum IL-8 concentrations before treatment, after 8 weeks' treatment with clarithromycin and placebo, and after treatment withdrawal. *P = 0.0014 versus before treatment; #P = 0.0018 versus after treatment was withdrawn. (*B*) Sputum NE concentrations before treatment, after 8 weeks' treatment with clarithromycin and placebo, and after treatment withdrawal. #P = 0.0099 versus after treatment was withdrawn.

TABLE 2. BASELINE INFLAMMATORY MARKERS IN PARTICIPANTS RANDOMIZED TO CLARITHROMYCIN OR PLACEBO GROUPS

	Clarithromycin	Placebo	*P* Value
Inflammatory subtype			
Eosinophilic, n (%)	9 (39)	8 (36.5)	
Neutrophilic, n (%)	6 (26)	6 (27)	
Paucigranulocytic, n (%)	8 (35)	8 (36.5)	
Inflammatory			
IL-8, ng/ml, median (IQR)	6.6 (2.7–11.9)	6.3 (3.1–17.3)	0.742
Neutrophils $\times 10^4$/ml, median (IQR)	272 (107–437)	410 (87–734)	0.860
Neutrophil elastase, ng/ml, median (IQR)	747.7 (154.4–1,637)	775.4 (330.3–1,544)	0.860
Total MMP-9, ng/ml, median (IQR)	7,886 (2,280–118,772)	4,366 (2,292–10,142)	0.558

Definition of abbreviations: IQR = interquartile range; MMP = matrix metalloproteinase.

(IQR) unless otherwise indicated. In each of the treatment groups, outcome variables before and after treatment were compared using the Wilcoxon signed rank test. A *P* value of less than 0.05 was considered significant.

RESULTS

Seventy-nine patients were screened for the study and 46 were eligible and allocated to treatment (23 to clarithromycin and 23 to placebo). One participant was randomized but did not complete the first week of treatment and was withdrawn. Analyses were performed on 45 participants (22 male) who completed the study (Figure 1). The duration of sputum induction was not different between the treatment groups at any visit. A sputum sample was collected on 216 of 225 occasions, giving a success rate of 96%. There were no significant differences between treatment groups at baseline with respect to age, gender, atopy, lung function, asthma control score, or daily inhaled corticosteroid dose (Table 1).

The 45 participants who completed the study had a mean age of 58 years and had moderate airflow obstruction with a mean FEV_1% predicted of 71% (SD, 17%). The group had poor asthma control (mean [SD] asthma control score. 1.5 [0.8]) and were receiving high daily maintenance doses of inhaled corticosteroids (median [IQR] dose, 2,000 [1,000–2,000] μg). Thirty-nine (87%) participants were receiving long-acting bronchodilators, the majority (92%) in the form of combination therapies. In the past year, 20 (44%) participants had received a course of oral steroids, 3 had been hospitalized, and a further 10 (22%) had visited their general medical practitioner due to asthma exacerbation. Allergy history and rhinitis symptoms can be found in Table E1 of the online supplement.

Clarithromycin treatment significantly reduced airway IL-8 levels from a median (IQR) of 6.6 (2.7–11.8) ng/ml before treatment to 3.9 (1.8–5.4) ng/ml after treatment (Figure 2A). There was also a significant reduction in neutrophil numbers and reductions in both IL-8 gene expression and neutrophil activation as assessed by concentrations of NE. Total MMP-9 levels were lower after clarithromycin; however, the differences were not significant (Table 3). There was no change in the number or proportion of eosinophils with clarithromycin treatment (data not shown).

Total quality-of-life score significantly improved with clarithromycin treatment (Table 2). Nine (39%) participants had a clinically significant improvement in their score of 0.5 or higher. The number-needed-to-treat (NNT) was 6. Within individual domains, there were significant improvements in both the activities and environmental stimuli domains (Figure 4A). In particular, the greatest improvement was found in the environmental stimuli domain where 15 (65%) participants had an improvement in their score of 0.5 or greater (Figure 4C) (NNT = 3). There was no change in the symptom score or levels of exhaled nitric oxide with clarithromycin treatment (*see* the online supplement).

There was a significant reduction in the proportion of participants reporting wheeze in the clarithromycin group (86% reporting wheeze at visit 2, reduced to 50% at visit 4; $P = 0.043$). There were no changes in any symptoms in the placebo group. There was no change in FEV_1% predicted, dose–response slope to hypertonic saline, or asthma control score after clarithromycin treatment (Table 3).

At the end of clarithromycin treatment, total MMP-9 levels were significantly lower in those who had received clarithromycin compared with placebo. Neutrophil numbers and concentrations

TABLE 3. INFLAMMATORY AND CLINICAL PARAMETERS BEFORE AND AFTER TREATMENT

	Clarithromycin			Placebo		
	Before	After	*P**	Before	After	*P**
IL-8 protein, ng/ml	6.6 (2.7–11.9)	3.9 (1.8–5.4)†	0.0014	6.3 (3.1–17.3)	6.4 (3.7–11.3)	0.931
IL-8 gene expression	41.8 (10.2–68.1)	19.7 (12.6–28.1)	0.0582	23.2 (7.8–87.1)	17.7 (4.9–73.3)	0.679
Neutrophils $\times 10^4$/ml	142.9 (43.2–27.3)	66.7 (23.6– 196.4)	0.0447	132.8 (42.4–364.5)	106.3 (61.4–270.8)	0.420
Neutrophil elastase, ng/ml	747.7 (154.4– 1,637)	524.5 (174.6–774)	0.062	775.4 (330.3– 1,544)	951.5 (390.7– 4,384)	0.852
Total MMP-9, ng/ml	7886 (2,280– 11,772)	3074 (1,806– 7,084)†	0.136	4366 (2,293–10,142)	6724 (3,620– 14,335)	0.501
Total AQLQ score, median (IQR)	5.5 (4.8–6.4)	6.2 (5.4–6.6)	0.0143	6.4 (5.2–6.7)	6.4 (5.7–6.8)	0.745
FEV_1% predicted, mean (SD)	73.6 (15.8)	74.6 (17.1)	0.573	67.6 (18.8)	69 (21)	0.685
Dose–response slope, median (IQR)	1.8 (0.6–6.4)	1 (0.5–4.2)	0.398	1 (0.6–3.2)	1 (0.5–3.3)	0.407
Asthma control score, mean (SD)	1.6 (0.6)	1.3 (0.7)	0.398	1.3 (1.0)	1.2 (0.8)	0.404

Definition of abbreviations: AQLQ = Asthma Quality-of-Life Questionnaire; IQR = interquartile range; MMP = matrix metalloproteinase.
Data are median and interquartile range.
* Before versus after treatment.
† $P < 0.05$ after clarithromycin versus after placebo.

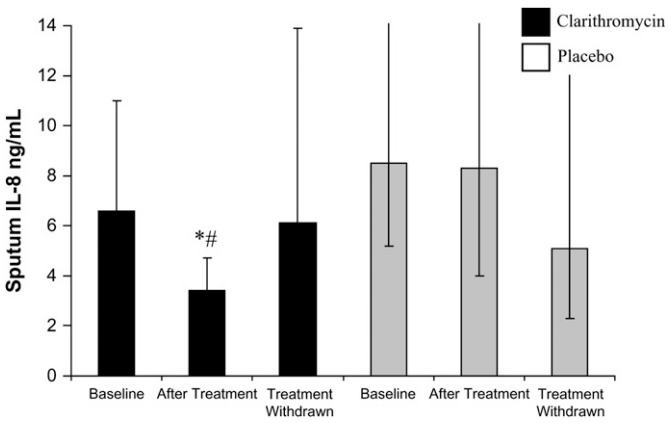

Figure 3. Sputum IL-8 concentrations before treatment, after 8 weeks' treatment with clarithromycin and placebo, and after treatment withdrawal in participants with noneosinophilic asthma. *$P = 0.0046$ versus before treatment; #$P = 0.0096$ after treatment was withdrawn.

of NE were also lower; however, these values were not statistically significant (Table 3).

Participants were reassessed 4 weeks after withdrawal of therapy and a significant increase in the concentration of IL-8 and NE was observed in those participants who had taken clarithromycin (Figures 2A and 2B). Neutrophil numbers were increased and the quality-of-life score deteriorated after clarithromycin was withdrawn, but these changes were not statistically significant. The median (IQR) number of neutrophils had increased to 122.7 (23.6–196) $\times 10^4$ cells/ml after treatment was withdrawn for 4 weeks, which was approaching the pretreatment numbers of 142.9 $\times 10^4$ cells/ml. The median (IQR) total quality-of-life score had reduced from 6.2 (5.4–6.6) at the end of treatment to 5.8 (5.3–6.2). The concentration of total MMP-9 continued to remain low after the withdrawal of clarithromycin. The median (IQR) total MMP-9 concentration after clarithromycin was withdrawn was significantly lower than pretreatment concentrations (median [IQR]: 2,943 [1,900–8,609] vs. 9,111 [2,280–11,772] ng/ml; $P = 0.04$). These changes were not observed in the placebo-treated group.

NEA Subanalysis

Twenty-eight participants had NEA, of which 14 participants each received clarithromycin and placebo. Clarithromycin treatment significantly reduced airway IL-8 protein (Figure 3) and gene expression (Table 4) in subjects with refractory NEA. There was also a reduction in neutrophil numbers and NE, and a significant reduction in total MMP-9 levels after treatment (Table 4). There were no differences in these outcomes after treatment in the participants with eosinophilic asthma (data not shown). A larger and more significant reduction was observed in the total quality-of-life score in participants with NEA. In particular, there were significant improvements in both the activities and environmental stimuli domains (Figure 4B). Six (43%) participants had a clinically significant improvement in their score of 0.5 or higher (NNT = 4) and 10 (71%) participants had a clinically significant improvement in the environmental stimuli domain specifically (Figure 4C). There was no change in FEV_1% predicted, dose response to hypertonic saline, or asthma control scores after clarithromycin treatment (Table 4).

At the end of treatment, levels of NE and MMP-9 were significantly lower in those who had received clarithromycin compared with placebo. Neutrophil numbers were also lower but these values were not significant (Table 4).

DISCUSSION

We report a double-blind, randomized, placebo-controlled trial of clarithromycin in severe refractory asthma and show that 8 weeks of clarithromycin therapy significantly reduces airway IL-8 and neutrophil numbers and improves quality of life. Concentrations of MMP-9 and NE were also reduced in those receiving clarithromycin, suggesting an overall down-regulation of neutrophil activation and mediator release. The antiinflammatory effect of clarithromycin treatment was most marked in those with refractory NEA and provides an opportunity to further investigate the potential of clarithromycin as an add-on therapy for refractory disease.

Sputum IL-8 levels were significantly reduced after 8 weeks of clarithromycin therapy and returned to pretreatment levels when therapy was withdrawn. IL-8 is a potent neutrophil chemoattractant and activator of neutrophils and is produced by a number of airway inflammatory cells, including neutrophils (21). We have previously shown that levels of IL-8 are elevated in NEA (6), and in asthma are correlated with airflow obstruction (5), suggesting IL-8 may play a key inflammatory role in refractory NEA.

IL-8 suppression with macrolide therapy has been reported in a number of other airway conditions, including bronchiectasis (22), bronchiolitis (23), and chronic sinusitis (14, 24). In chronic obstructive pulmonary disease (COPD), the evidence is conflicting with only two randomized controlled trials of clarithromycin treatment. In one study, there were significant reductions in sputum IL-8 levels (25), and in the other, no changes in sputum IL-8 levels were observed after 3 months of therapy with

TABLE 4. INFLAMMATORY PARAMETERS AND ASTHMA QUALITY-OF-LIFE TOTAL SCORE BEFORE AND AFTER TREATMENT FOR PARTICIPANTS WITH NONEOSINOPHILIC ASTHMA

	Clarithromycin			Placebo		
	Before	After	*P**	Before	After	*P**
IL-8 protein, ng/ml	6.6 (2.1–11.0)	3.4 (1.9–4.7)†	0.0046	8.5 (5.2–28.9)	8.3 (4.0–31.4)	0.917
IL-8 gene expression	40.2 (8.9–69.9)	14.3 (3.6–22.5)	0.0229	12.6 (7.7–104.3)	15 (3.1–142)	0.790
Neutrophils $\times 10^4$/ml	110.7 (32.8–496.8)	70.7 (23.6–131)	0.0843	254.5 (60.9–503.8)	107.7 (70.1–281.2)	0.2132
Neutrophil elastase, ng/ml	698.4 (150.5–1,637)	491 (174.6–729.9)†	0.0597	1,202 (545–5,880)	1,032 (551.3–7,088)	0.463
Total MMP-9, ng/ml	9,111 (2,280–11,772)	3,183 (1,806–5,115)†	0.0166	6,455 (3,459–14,871)	9,826 (3,415–15,844)	0.959
Total AQLQ score, median (IQR)	5.5 (4.7–6.3)	6.2 (5.6–6.6)	0.0202	6.0 (5.0–6.6)	6.1 (4.8–6.8)	0.900

Definition of abbreviations: AQLQ = Asthma Quality-of-Life Questionnaire; IQR = interquartile range; MMP = matrix metalloproteinase.
Data are median and interquartile range.
* Before versus after treatment.
† $P < 0.05$ after clarithromycin versus after placebo.

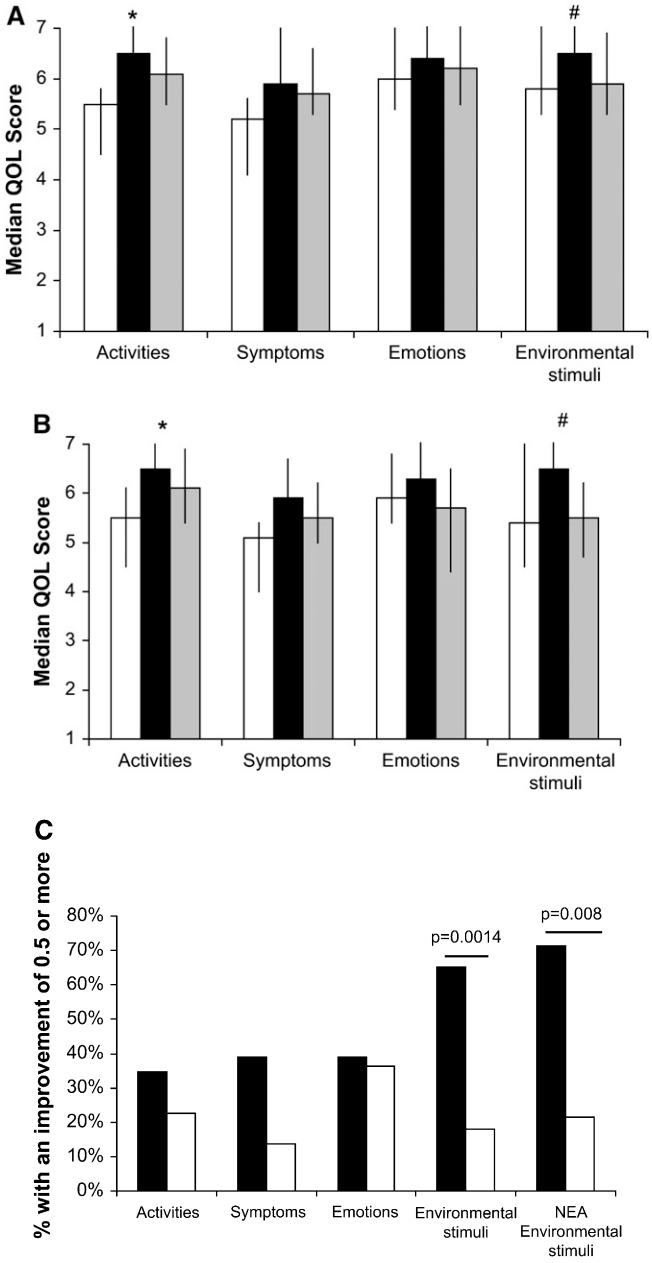

Figure 4. (*A*) Quality-of-life (QOL) score domains before treatment, after 8 weeks' treatment with clarithromycin and placebo, and after treatment withdrawal. *$P = 0.02$; #$P = 0.0025$ versus before treatment. (*B*) QOL score domains before treatment, after 8 weeks' treatment with clarithromycin and placebo, and after treatment withdrawal in patients with NEA. *$P = 0.01$; #$P = 0.003$ versus before treatment. (*A, B*) *open bars*, baseline; *solid bars*, after treatment; *shaded bars*, treatment withdrawn. (*C*) Proportion of participants (%) with a clinically important improvement in quality of life by domain. NEA = noneosinophilic asthma. *Solid bars*, clarithromycin; *open bars*, placebo.

clarithromycin (26). A key difference between the two study designs is the dosing regime; in the first study, treatment was given twice daily and in the latter, once daily. It is possible that a single dose of clarithromycin may not be sufficient to induce and maintain the antiinflammatory actions of this treatment in the airways. Clarithromycin and other macrolides also inhibit IL-8 release from a number of other inflammatory cells, including monocytes (27), nasal epithelial cells (24), bronchial epithelial cells (28), and eosinophils (29).

Treatment with clarithromycin significantly reduced the number of neutrophils in the airway lumen as well as the concentration of NE in the sputum supernatant, suggesting a reduction in both neutrophil accumulation and activation. Sputum IL-8 gene expression was reduced 2-fold and airway concentrations of MMP-9 reduced by 2.5-fold with clarithromycin therapy, consistent with a reduction in neutrophil activation. Reductions in neutrophils and NE have been reported in patients with chronic airway disease (diffuse panbronchiolitis and bronchiectasis) (9), and neutrophil proportions have been shown to be reduced in both bronchiectasis (22) and COPD (26). Resolution of neutrophilic inflammation in the airways is achieved by the removal of apoptotic neutrophils by alveolar macrophages (efferocytosis). One mechanism whereby clarithromycin may reduce neutrophil numbers is via improvements in phagocytosis of apoptotic cells. Alveolar macrophages treated with macrolide antibiotics have an increased phagocytic capacity for apoptotic neutrophils (30) and epithelial cells (31).

Participants on clarithromycin therapy had a significant improvement in the Juniper asthma quality-of-life score, with better results in both the environmental stimuli and physical activity domains. More than 60% of participants had a clinically significant improvement in their environmental stimuli domain of 0.5 or greater. This domain asks the participant about his or her symptoms in the past month due to exposure to dusts, smoke, and other particulates. Such a marked improvement in this domain suggests that the immunologic response to these triggers may be reduced with clarithromycin therapy. Improvements in St. George's Respiratory Questionnaire scores have been observed when macrolides have been used to treat acute exacerbations of chronic bronchitis (32) and in the symptom score only for stable COPD (33).

An improvement in the environmental stimuli domain may be explained by the IL-8–modifying effects of clarithromycin. By reducing the amount of IL-8 available, neutrophil activation responses are also modified, and symptoms that result from typical innate immune or nonallergic stimuli, such as particulates, smoke, and dust, may also diminish. We have previously demonstrated high levels of endotoxin and innate immune activation in the airways of patients with neutrophilic asthma and bronchiectasis (34), and macrolide therapy appears to be an effective neutrophil modifier in both diseases.

Fewer participants reported wheeze after clarithromycin therapy, with a significant reduction of more than 30%. We observed no changes in airway hyperresponsiveness to hypertonic saline in the participants receiving clarithromycin. Although other studies have shown improvements in hyperresponsiveness after macrolide therapy (35–38), these have largely been on mild well-controlled asthma, which did not use hypertonic saline as the stimulus. Airway responsiveness to hypertonic saline may be a better marker of eosinophilic airway inflammation, and therefore relatively nonresponsive to therapies targeting noneosinophilic mechanisms in asthma.

When patients with noneosinophilic refractory asthma were analyzed separately, 8 weeks of clarithromycin therapy significantly reduced IL-8 protein and gene expression. There was also a statistically significant reduction in sputum MMP-9 levels in the airways after clarithromycin therapy for those with NEA. Quality of life was also better with more than 75% of participants with refractory NEA having a clinically significant improvement in the environmental stimuli domain. There was a similar reduction in the frequency of participants who reported experienced wheezing in the previous month.

This study was powered to detect differences in sputum IL-8 concentrations with clarithromycin treatment and not to investigate clinical markers of disease, such as airway hyperresponsiveness,

asthma control, or quality of life. Despite this, we found evidence of improved quality of life with clarithromycin treatment in refractory asthma, specifically for those with NEA. It is possible that the other clinical markers chosen (e.g., presence of symptoms and asthma control score) may not be responsive measures of clinical improvement in NEA. Future larger studies are required to closely examine the effects of macrolide antibiotics on clinical asthma outcomes and should consider other clinical outcomes that may be more responsive to treatment, such as symptoms visual analog scale.

The precise triggers of neutrophilic inflammation in NEA are unknown but may include colonization with typical or atypical bacterial pathogens. Both chlamydia and mycoplasma species have been found in the airways of patients with chronic asthma (39) with an increased frequency compared with healthy control subjects (40). Chlamydia pneumonia infection results in neutrophil accumulation and the bacteria are able to replicate within neutrophils, indicating that it may be an important trigger of neutrophilic inflammation (41). When patients with asthma and chlamydia or mycoplasma infection are treated with macrolide antibiotics, small improvements in lung function have been observed (42, 43). No study, however, has investigated alternations in airway IL-8 or neutrophil levels, and further research is required to examine atypical bacterial infection and macrolide therapy in patients with NEA.

In conclusion, we have studied the antiinflammatory effect of clarithromycin in severe refractory asthma and provide data demonstrating significant reductions in levels of IL-8 and numbers of neutrophils in the airway lumen combined with a significant improvement in quality-of-life score and a reduction in self-reported wheeze. This approach confirms the potential of clarithromycin therapy in the treatment of airway inflammation, and more specifically in noneosinophilic refractory asthma where there are no proven treatment options.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

1. Wenzel SE, Fahy JV, Irvin C, Peters SP, Spector S, Szefler SJ. Proceedings of the ATS workshop on refractory asthma. *Am J Respir Crit Care Med* 2000;162:2341–2351.
2. Global Initiative for Asthma. Global strategy for asthma management and prevention [Internet]. Bethesda (MD): National Heart, Lung, and Blood Institute: 2006 [accessed Jul 2007]. Available from: http://www.ginasthma.org
3. Wenzel SE, Schwartz LB, Largmack EL, Halliday JL, Trudeau JB, Gibbs RL, Chu HW. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999; 160:1001–1008.
4. Douwes J, Gibson P, Pekkanen J, Pearce N. Non-eosinophilic asthma: importance and possible mechanisms. *Thorax* 2002;57:643–648.
5. Simpson JL, Scott R, Boyle M, Gibson P. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;11:54–61.
6. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma. *Chest* 2001;119:1329–1336.
7. Berry MA, Morgan A, Shaw DE, Parker D, Green RH, Brightling CE, Bradding P, Wardlaw AJ, Pavord ID. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007;62:1043–1049.
8. Sakito O, Kadota J, Kohno S, Abe K, Shirai R, Hara K. Interleukin 1 beta, tumor necrosis factor alpha, and interleukin 8 in bronchoalveolar lavage fluid of patients with diffuse panbronchiolitis: a potential mechanism of macrolide therapy. *Respiration* 1996;63:42–48.
9. Oishi K, Sonoda F, Kobayashi S, Iwagaki A, Nagatake T, Matsushim K, Matsumoto K. Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases. *Infect Immun* 1994;62:4145–4152.
10. Garey KW, Gotfried MH, Khan IJ, Varma S, Danziger LH. Long-term clarithromycin decreases prednisone requirements in elderly patients with prednisone dependent asthma. *Chest* 2000;118:1826–1827.
11. Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Anti-inflammatory effects of clarithromycin in refractory non-eosinophilic asthma [abstract]. *Am J Respir Crit Care Med* 2007;175:A483.
12. Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Anti-inflammatory effects of clarithromycin in refractory non-eosinophilic asthma [abstract]. *Respirology* 2007;12:A11.
13. Smith CM, Anderson SD. Inhalation provocation tests using nonisotonic aerosols. *J Allergy Clin Immunol* 1989;84:781–790.
14. Yamada T, Fujieda S, Mori S, Yamamoto H, Saito H. Macrolide treatment decreased the size of nasal polyps and IL-8 levels in nasal lavage. *Am J Rhinol* 2000;14:143–148.
15. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902–907.
16. Juniper EF, Guyatt GH, Ferrie PJ, Griffith LE. Measuring quality of life in asthma. *Am Rev Respir Dis* 1993;147:832–838.
17. American Thoracic Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;172:912–930.
18. Gibson PG, Wlodarczyk JW, Hensley MJ, Gleeson M, Henry RL, Cripps AR, Clancy RL. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998;158:36–41.
19. Simpson JL, Scott RJ, Boyle MJ, Gibson PG. Differential proteolytic enzyme activity in eosinophilic and neutrophilic asthma. *Am J Respir Crit Care Med* 2005;172:559–565.
20. Grissell TV, Powell H, Shafren DR, Boyle MJ, Hensley MJ, Jones PD, Whitehead BF, Gibson PG. Interleukin-10 gene expression in acute virus-induced asthma. *Am J Respir Crit Care Med* 2005;172:433–439.
21. Baggiolini M, Walz A, Kunkel SL. Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. *J Clin Invest* 1989;84:1045–1049.
22. Yalcin A, Kiper N, Ozcelik U, Dogru D, Firat P, Shain A, Ariyurek M, Mocan G, Gurcan N, Gocmen A. Effects of clarithromycin on inflammatory parameters and clinical conditions in children with bronchiectasis. *J Clin Pharm Ther* 2006;31:49–55.
23. Tahan F, Ozcan A, Koc N. Clarithromycin in the treatment of RSV bronchiolitis: a double blind, randomised, placebo-controlled trial. *Eur Respir J* 2007;29:91–97.
24. Fujita K, Shimizu T, Majima Y, Sakakura Y. Effects of macrolides on interleukin-8 secretion from human nasal epithelial cells. *Eur Arch Otorhinolaryngol* 2000;257:199–204.
25. Basyigit I, Yildiz F, Ozkara SK, Yildirim E, Boyaci H, Ilgazli A. The effect of clarithromycin on inflammatory markers in chronic obstructive pulmonary disease: preliminary data. *Ann Pharmacother* 2004;38: 1400–1405.
26. Banerjee D, Honeybourne D, Khair OA. The effect of oral clarithromcin on bronchial airway inflammation in moderate to severe stable COPD. *Treat Respir Med* 2004;3:59–65.
27. Kikuchi T, Hagiwara K, Honday Y, Gomi K, Kobayashi T, Takahashi H, Tokue U, Watanabe A, Nukiwa T. Clarithromycin suppresses lipopolysaccharide-induced interleukin-8 production by human monocytes through AP-1 and NF-kappa-B transcription factors. *J Antimicrob Chemother* 2002;49:745–755.
28. Abe S, Nakamura H, Inoue S, Takeda H, Saito H, Kato S, Mukaida N, Matsushima K, Tomoike H. Interleukin-8 gene repression by clarithromycin is mediated by the activator protein-1 binding site in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2000;22: 51–60.
29. Kohyama T, Takizawa H, Kawasaki S, Akiyama N, Sato M, Ito K. Fourteen-member macrolides inhibit interleukin-8 release by human eosinophils from atopic donors. *Antimicrob Agents Chemother* 1999;43: 235–240.
30. Yamaryo T, Oishi K, Yoshimine H, Tsuchiashi Y, Matsushima K, Nagatake T. Fourteen-member macrolides promote the phosphatidylserine receptor-dependent phagocytosis of apoptotic neutrophils by alveolar macrophages. *Antimicrob Agents Chemother* 2003;47:48–53.
31. Hodge S, Hodge G, Brozyna S, Jersmann H, Holmes M, Reynolds PN. Azithromycin increases phagocytosis of apoptotic bronchial epithelial cells by alveolar macrophages. *Eur Respir J* 2006;28:486–495.

32. Milstone A, Patsimas J, Farzan D, Castaldo R, Singh H, Feurer I, Harnett J, Luke DR; PROPeR Use (Patient Reported Outcomes, Productivity and Resource Utilisation of acute exacerbated chronic bronchitis) Study Group. Prospective observational study of patient-reported outcomes for azithromycin versus usual care in the treatment of bacterial acute exacerbation of chronic bronchitis. *Clin Ther* 2005; 27:926–939.
33. Banerjee D, Khair OA, Honeybourne D. Impact of sputum bacteria on airway inflammation and health status in clinical stable COPD. *Eur Respir J* 2004;23:685–691.
34. Simpson JL, Grissell TV, Douwes J, Scott RJ, Boyle MJ, Gibson PG. Innate immune activation in neutrophilic asthma and bronchiectasis. *Thorax* 2007;62:211–218.
35. Miyatake H, Taki F, Taniguchi H, Suzuki R, Takagi K, Satake T. Erythromycin reduces the severity of bronchial hyperresponsiveness in asthma. *Chest* 1991;99:670–673.
36. Amayasu H, Yoshida S, Ebana S, Yamamoto Y, Nishikawa T, Shoij T, Nakagawa H, Hasegawa H, Nakabayashi M, Ishizaki Y. Clarithromycin suppresses bronchial hyperresponsiveness associated with eosinophilic inflammation in patients with asthma. *Ann Allergy Asthma Immunol* 2000;84:594–598.
37. Kostadima E, Tsiodras S, Alexopoulos EI, Kaditis AG, Mavrou I, Georgatou N, Papamichalopoulos A. Clarithromycin reduces the severity of bronchial hyperresponsiveness in patients with asthma. *Eur Respir J* 2004;23:714–717.
38. Kamoi H, Kurihara N, Fujiwara H, Hirata K, Takeda T. The macrolide antibacterial roxithromycin reduces bronchial hyperresponsivenss and superoxide anion production by polymorphonuclear leukocytes in patients with asthma. *J Asthma* 1995;32:191–197.
39. Martin RJ, Kraft M, Chu HW, Berns EA, Cassell GH. A link between chronic asthma and chronic infection. *J Allergy Clin Immunol* 2001; 107:595–601.
40. Biscione GL, Corne J, Chauhan AJ, Johnston SL. Increased frequency of detrection of Chlamydophilia pneumoniae in asthma. *Eur Respir J* 2005;24:745–749.
41. van Zandbergen G, Gieffers J, Kothe H, Rupp J, Bollinger A, Aga E, Klinger M, Brade H, Dalhoff K, Maass M, *et al.* Chlamydia pneumoniae multiply in neutrophil granulocytes and delay their spontaneous apoptosis. *J Immunol* 2004;172:1768–1776.
42. Kraft M, Cassell GH, Pak J, Martin RJ. Mycoplasma pneumoniae and Chlamydia pneumoniae in asthma: effect of clarithromycin. *Chest* 2002; 121:1782–1788.
43. Black PN, Blasi F, Jenkins CR, Scicchitano R, Mills GD, Rubinfeld AR, Ruffin RE, Mullins PR, Dangain J, Cooper BC, *et al.* Trial of roxithromycin in subjects with asthma and serological evidence of infection with *Chlamydia pneumoniae*. *Am J Respir Crit Care Med* 2001;164:536–541.