RESEARCH LETTER

The impact of azithromycin therapy on the airway microbiota in asthma

INTRODUCTION

There is interest in the use of macrolide antibiotics in asthma. Macrolides have been shown to improve airway hyperresponsiveness (AHR) and measures of airway inflammation.[1] The degree of AHR may relate to the microbiota present in the airways,[2] with a recent study reporting that patients with asthma with a significant improvement in AHR following treatment with clarithromycin had a higher bacterial diversity prior to treatment.[3] To our knowledge, the impact on the asthmatic airway microbiota of an antibiotic has not been reported and we therefore set out to establish if macrolide therapy was associated with a change in airway microbiota in asthma.

METHODS

Five adult patients with moderate/severe asthma (British Thoracic Society step 4–5) (see online supplementary table S1) and no evidence of respiratory infection or bronchiectasis underwent bronchoscopy before and after 6 weeks of daily 250 mg azithromycin therapy. Patients had consented to the study (REC 11/EM/0062). Saline washings of the right upper lobe were obtained following standard procedure, DNA was isolated from the samples (see online supplementary Methods) and the microbiota analysed by using pyrosequencing performed by Molecular Research DNA. Microbiota results were analysed after random resampling of the data[4] and calculation of two diversity indices; richness and Shannon's.

RESULTS

A total of 5223 reads were analysed from five sample pairs (pretreatment and post-treatment). Eighty-nine distinct genera were detected. Bacteria from the genera *Staphylococcus* (10.49%), *Pseudomonas* (9.35%), *Streptococcus* (7.99%) and *Neisseria* (4.75%) were all found to be among the more abundant genera in the pretreatment samples (table 1).

The total abundance of each genus is given as a percentage of the total number of reads within each of the three groups. Parentheses represent the number of samples where the genus was present.

Table 1 Relative abundance of each of the most dominant genera in samples pretreatment and post-treatment

Genus	Total (n=10)	Pretreatment (n=5)	Post-treatment (n=5)
Actinobacillus	2.97 (1)	4.78 (1)	0.00 (0)
Anaerococcus	17.29 (5)	3.89 (3)	39.18 (2)
Fusobacterium	1.82 (4)	2.90 (3)	0.05 (1)
Haemophilus	7.91 (5)	10.74 (3)	3.28 (2)
Neisseria	3.01 (2)	4.75 (1)	0.15 (1)
Prevotella	4.12 (6)	4.54 (4)	3.43 (2)
Pseudomonas	5.80 (2)	9.35 (2)	0.00 (0)
Staphylococcus	8.25 (8)	10.49 (5)	4.59 (3)
Streptococcus	8.08 (6)	7.99 (3)	8.22 (3)
Veillonella	7.39 (6)	4.10 (3)	12.76 (3)
Other	7.37 (3)	7.99 (2)	6.35 (1)

Many genera reduced in abundance after treatment including *Prevotella* (3.43%), *Staphylococcus* (4.59%) and *Haemophilus* (3.28%), with *Pseudomonas* not detected post-treatment. There was an increase in the relative number of *Anaerococcus* (39.18%) observed in two patients after treatment.

Evaluation of richness revealed that the mean number of genera detected in the pretreatment samples was 19.37 genera (SD=5.68, n=5). This was higher than the mean number of genera post-treatment (mean=12.80 genera, SD=3.70, n=5). Equally, the mean Shannon's index in the pretreatment group was 1.62 (SD=0.20, n=5) compared with post-treatment (mean=1.22, SD=0.40, n=5). Non-parametric investigation found near significant differences between the patients pretreatment and post-treatment with richness and Shannon's index (both Kruskal-Wallis $\chi^2=3.15$, p=0.076; figure 1).

CONCLUSION

This is the first study to examine longitudinal changes in airway microbiota following antibiotic treatment in asthma. Azithromycin therapy was associated with decreased bacterial richness in the airways and altered the airway microbiota leading to *Anaerococcus* becoming dominant within the bacterial community in some cases. Importantly, *Pseudomonas*, *Haemophilus* and *Staphylococcus* (three pathogenic genera associated with airway disease) were all reduced. This may explain the clinical improvement observed in asthma[5] and suggests a possible antibiotic as well as immunomodulatory effect of macrolides on AHR. Azithromycin has also been shown to

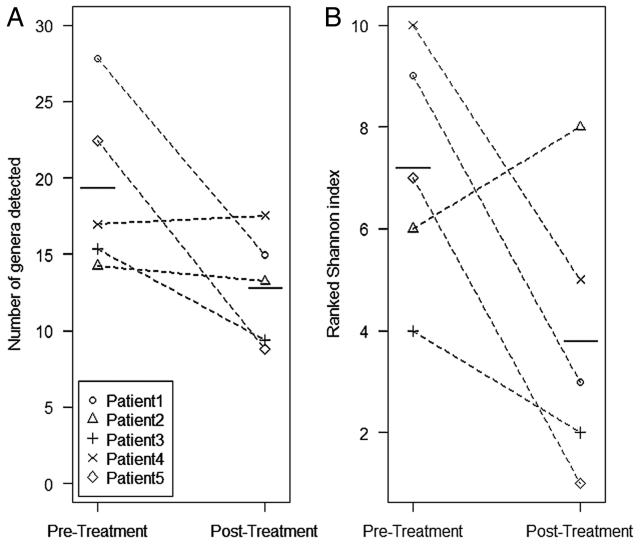

Figure 1 Characteristics of the microbiota in patients prior to and after azithromycin treatment. Each data point represents a single sample with horizontal lines indicating the mean number of genera detected (A) and Shannon's index rank (B). Near significant differences were reported between groups for both measures ($\chi 2=3.15$, p=0.076). Dotted lines are shown to indicate the change in measure for each patient.

decrease mucus secretion, airway neutrophil accumulation as well as specific antibiotic and antipseudomonal activity. This early work indicates that larger studies of the effect of treatments on the airway microbiota and clinical outcomes are now needed.

Mariel Slater,[1] Damian W Rivett,[2] Lisa Williams,[3] Matthew Martin,[3] Tim Harrison,[3] Ian Sayers,[1] Kenneth D Bruce,[4] Dominick Shaw[3]

[1]Department of Therapeutics and Molecular Medicine, University of Nottingham, Nottingham, UK
[2]Department of Ecology and Evolution, Imperial College, London, UK
[3]Department of Respiratory Medicine, University of Nottingham, Nottingham, UK
[4]Institute of Pharmaceutical Science, Kings College London, London, UK

Correspondence to Dr Kenneth Bruce, King's College London, Molecular Microbiology Research Laboratory, Institute of Pharmaceutical Science, 150 Stamford Street, London SE1 9NH, UK; kenneth.bruce@kcl.ac.uk

MS and DWR are joint first authors.

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2013-204517).

Contributors MS was involved in sample acquisition and processing, and contributed to the manuscript. DWR performed sample and statistical analysis, and contributed to the manuscript. LW was involved in sample acquisition and processing, and contributed to the manuscript. MM was involved in study design and contributed to the manuscript. TH was involved in study design and contributed to the manuscript. IS was involved in sample acquisition, study design and contributed to the manuscript. KDB was involved in sample and statistical analysis, study design and contributed to the manuscript. He is responsible for the overall content as guarantor. DS was involved in study design, sample acquisition and processing, and contributed to the manuscript. He is also responsible for the overall content as guarantor.

Funding This work was carried out under a grant from the British Medical Association James Trust award.

Competing interests None

Ethics approval Nottingham Research Ethics Committee 2.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Unpublished data from the study forms the body of Miss Slater's thesis and will be published in due course.

To cite Slater M, Rivett DW, Williams L, *et al*. *Thorax* Published Online First: [*please include* Day Month Year] doi:10.1136/thoraxjnl-2013-204517

Received 15 September 2013
Revised 5 November 2013
Accepted 6 November 2013

Thorax 2013;**0**:1–2.
doi:10.1136/thoraxjnl-2013-204517

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Supplementary Methods

DNA was extracted from the samples using a phenol: chloroform extraction procedure as previously reported (1). The microbiota was analysed using bacterial tag-encoded FLX amplicon pyrosequencing as described in Dowd *et al*. (2). Generation of the sequencing library and was performed by MRDNA (www.mrdnalab.com, Shallowater, TX, USA) along with the pyrosequencing analysis, clean-up and operational taxonomic unit (OTU) identification. Identification was provided using BLASTn database with genus taxonomy given for > 95% sequence identity of the OTU to an appropriate reference sequence. Random resampling was performed to adjust the data for potential biasing caused by differences in the number of reads within a sample. Resampling was achieved by the random selection of a uniform number (n = 426) of reads from each sample. The uniform number corresponded to the smallest sample size. Resampling was repeated 1000 times with diversity indices calculated at each sampling event and the mean values recorded. Comparisons between the diversity measures were achieved using non-parametric analyses due to the small sample sizes. A significance difference was reported if the P-value was below the 0.05 threshold ($P < 0.05$). All statistical analyses and visualisations were performed in the statistical computing environment R (v.3.0.1; R Core Team, 2013). Richness was given by the mean count of the number of unique genera present in each sample after reshuffling. The Shannon's diversity index was calculated using the diversity command in the vegan (v.2.0-7) package.

Gender	**Height (cm)**	**Age (years)**	**Weight (kg)**	**Smoking**	**Treatment**
M	164	51	75	Never	Carbocysteine, Salbutamol, Symbicort, Montelukast
M	177	63	78	Never	Prednisolone, Salbutamol, Seretide
F	165	58	92	Never	Phyllocontin, prednisolone, Montelukast, Symbicort, Terbutaline
F	168	57	102	Never	Salbutamol, Seretide, Montelukast
F	168	18	82	Never	Salbutamol, Seretide, Montelukast

Supplementary Table 1 Patient Demographics

Figure 1: Characteristics of the microbiota in patients prior to and after azithromycin treatment.

Each data point represents a single sample with horizontal lines indicating the mean number of genera detected (a) and Shannon's index rank (b). Near significant differences were reported between groups for both measures ($\chi 2 = 3.15$, $P = 0.076$). Dotted lines are shown to indicate the change in measure for each patient.

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