Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial

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Summary

Background The macrolide antibiotic azithromycin has anti-inflammatory properties potentially beneficial in cystic fibrosis. Since findings of open pilot studies seemed to show clinical benefit, we undertook a formal trial.

Method 41 children with cystic fibrosis, aged 8–18 years, and with a median forced expiratory volume in 1 s (FEV_1) of 61% (range 33–80%) participated in a 15-month randomised double-blind, placebo-controlled crossover trial. They received either azithromycin (bodyweight ⩽40 kg: 250 mg daily, >40 kg: 500 mg daily) or placebo for 6 months. After 2 months of washout, the treatments were crossed over. The primary outcome was median relative difference in FEV_1 between azithromycin and placebo treatment periods. Sputum cultures, sputum interleukin 8 and neutrophil elastase, exercise testing, quality of life, antibiotic use, and pulmonary exacerbation rates were secondary outcome measures. Side-effects were assessed by pure tone audiometry and liver function tests. Analysis was by intention-to-treat.

Findings Median relative difference in FEV_1 between azithromycin and placebo was 5·4% (95% CI 0·8–10·5). 13 of 41 patients improved by more than 13% and five of 41 deteriorated by more than 13% (p=0·059). Forced vital capacity and mid-expiratory flow did not significantly change overall. 17 of 41 patients had 24 fewer oral antibiotic courses when on azithromycin than when taking placebo, and five had six extra courses (p=0·005). Sputum bacterial densities, inflammatory markers, exercise tolerance, and subjective well-being did not change. There were no noticeable side-effects.

Interpretation A 4–6-month trial of azithromycin is justified in children with cystic fibrosis who do not respond to conventional treatment. The mechanism of action remains unknown.

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Introduction

Cystic fibrosis is the commonest recessively-inherited disease in white people, and affects all exocrine glands, the most clinically important being the lungs, pancreas, liver, and vas deferens. Since 95% of patients ultimately die of respiratory failure, a major aim in management of this disease is the treatment of endobronchial infection and inflammation. A narrow range of organisms, including *Staphylococcus aureus* and mucoid *Pseudomonas aeruginosa*, cause such infections in these patients. *P aeruginosa* is almost exclusively seen in patients with cystic fibrosis. Whether inflammation precedes infection or is exaggerated in response to it is uncertain.[1,2] However, there is no doubt that the influx of large numbers of neutrophils into the airway, with the release of mediators such as neutrophil elastase, itself causes damage to the lungs in addition to the damage caused by infecting micro-organisms.

Diffuse panbronchiolitis is a disorder that affects, in particular, middle-aged Japanese men.[3] It has many features in common with cystic fibrosis, including a high rate of respiratory infection with mucoid *P aeruginosa*. The 10-year mortality of 90% for this disorder was by chance found to be reduced to only 10% by the administration of erythromycin, a macrolide antibiotic not normally regarded as having any activity against pseudomonas.[4]

This finding led to much speculation about the mechanism of action and the potential of macrolide antibiotics in treatment of cystic fibrosis. Many mechanisms have been proposed, both in vitro and in vivo, including effects on neutrophil function,[5] interleukin-8 production,[6] sputum rheology,[7] effects on the alginate biofilm produced by *P aeruginosa*,[8] and even direct anti-pseudomonal activity.[9] All these suggestions have been reviewed but the mechanism is still unknown.[10]

We previously reported an open-label so-called rescue pilot study of long-term azithromycin, a macrolide antibiotic similar to erythromycin but with longer action.[11] Our findings showed a sufficiently positive response in seven children with cystic fibrosis to merit a formal trial. We aimed to determine whether there was an overall clinical response without side-effects, and whether the response was an antibacterial or anti-inflammatory effect.

Methods

Patients

The study was undertaken at two paediatric cystic fibrosis centres, the Royal Brompton Hospital, London, and Queen Mary's Children's Hospital, Sutton, UK. One consultant had a role in both clinics and identical management protocols were used. Ethics approval for the trial was obtained from each centre and written informed consent was obtained from every parent and child.

Entry criteria were a diagnosis of cystic fibrosis based on characteristic symptoms and signs, plus a positive sweat test, or two defined cystic fibrosis gene mutations; age older than 8·0 years, ability to undergo spirometry and swallow

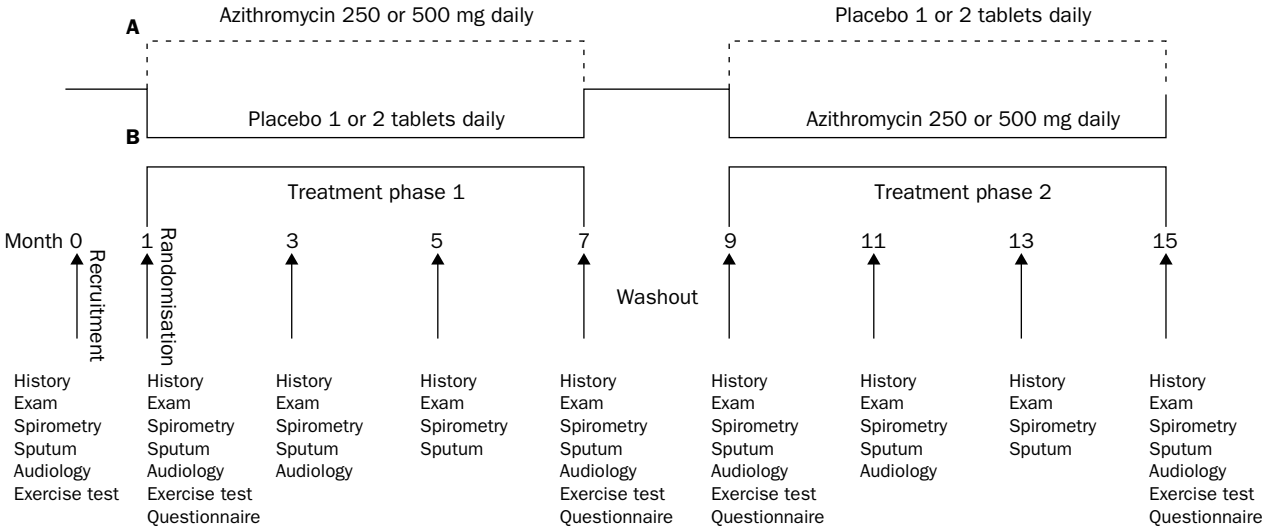

Figure 1: **Study protocol**

tablets, high likelihood of attending all visits; and a forced expiratory volume in 1s (FEV_1) of less than 80% predicted, corrected for sex, age, and height. Previous chronic infection with *P aeruginosa* was not a specific entry criterion.

Exclusion criteria were cystic-fibrosis-related liver disease, abnormal clotting indices, or liver function tests greater than three times the laboratory upper limit; history of deafness in the patient or first degree relative; a previous culture of *Burkholderia cepacia*; previous organ transplantation; receipt of macrolide antibiotics or oral steroids for more than 14 days, or a different research medication in the 2 months before recruitment; or having begun rhDNase as a new treatment in the 2 months before recruitment (long-term users were included).

Procedures

The trial was a randomised double-blind, placebo-controlled, crossover design of 15 months' duration. There were four periods: recruitment, run-in, and randomisation (1 month); treatment period one (6 months); washout (2 months); and finally treatment period two (6 months). There were nine specified visits, at 0, 1, 3, 5, 7, 9, 11, 13, and 15 months. Patients were randomly assigned azithromycin in treatment period one, followed by placebo in treatment period two, or placebo in treatment period one followed by azithromycin in treatment period two (figure 1).

Between recruitment and randomisation, the stability criteria were a variation in FEV_1 of less than 10% ([randomisation FEV_1−recruitment FEV_1]/recruitment $FEV_1 \times 100\%$) and no use of extra antibiotics or oral steroids. Randomisation was deferred if necessary until stability was re-established for a minimum period of 1 month. The codes for treatment and randomisation were supplied by the statistics department of Pfizer USA (Groton, USA), and the pharmacy department at the Royal Brompton Hospital allocated the next patient to a prespecified set of codes. After stratification for FEV_1 greater than or less than 60% of that predicted by age, sex, and height, the randomisation number generated a treatment code that allocated tablet bottles matching the treatment code. The pharmacy, the patients, and all the trial supervisors were unaware of treatment allocation at all times. Code-breaking sheets for emergency use were kept in the hospital safe but were never used.

Azithromycin and placebo were supplied in identical 250 mg pink tablets by Pfizer Pharmaceuticals. Dosage was 250 mg azithromycin or one placebo tablet per day if the patient's weight was 40 kg or lower, and 500 mg or two placebo tablets if greater than 40 kg.

At every visit, a history was taken and physical examination undertaken. The patient's weight in underwear was measured to the nearest 0·1 kg , and height in bare feet was measured with a stadiometer to the nearest mm. Spirometry was done with American Thoracic Society criteria[12] and sputum or cough swab cultures were then obtained. All changes in symptoms and treatment were recorded. Any administration of oral or intravenous antibiotics was recorded with a note about whether the reasons for their use conformed with a standard definition of a pulmonary exacerbation.[13] Great care was taken to ensure that all usual treatments remained entirely the responsibility of the clinicians, with the proviso that macrolides were not to be prescribed without permission of the trial supervisor. No patient received macrolides as rescue or specific therapy during the trial.

Pure tone audiometry was undertaken before, during, and on completion of each treatment period with an Amplivox 2150 audiometer (Amplivox, Oxford, UK). Any minor failure (falling below –20 db at any frequency) led to a repeat test 1 month later with no change in medication. Further failure or initial major failure (to below −40 db) led to urgent formal audiology testing.

Patients underwent a 3-min standardised 15 cm step exercise test[14] before and on completion of each treatment period. Changes in visual analogue scores, heart rate, and oxygen saturation were recorded between onset and completion of the test. Patients also completed a quality-of-life questionnaire validated in patients with cystic fibrosis[15] before and on completion of each treatment period. Additionally, every patient was invited to speculate at the end of the trial as to the order in which the treatments were given—ie, active/placebo or placebo/active.

Sputum or cough swab cultures were sent to the microbiology department at the Royal Brompton Hospital, and were cultured for the common cystic fibrosis pathogens on several media: heated blood agar, MaConkey, Burkolderia cepacia, Difco pseudomonas, Sabaurauds dextrose, and mannitol salt agar. Plates were incubated at 37°C and assessed at 24 and 48 h.

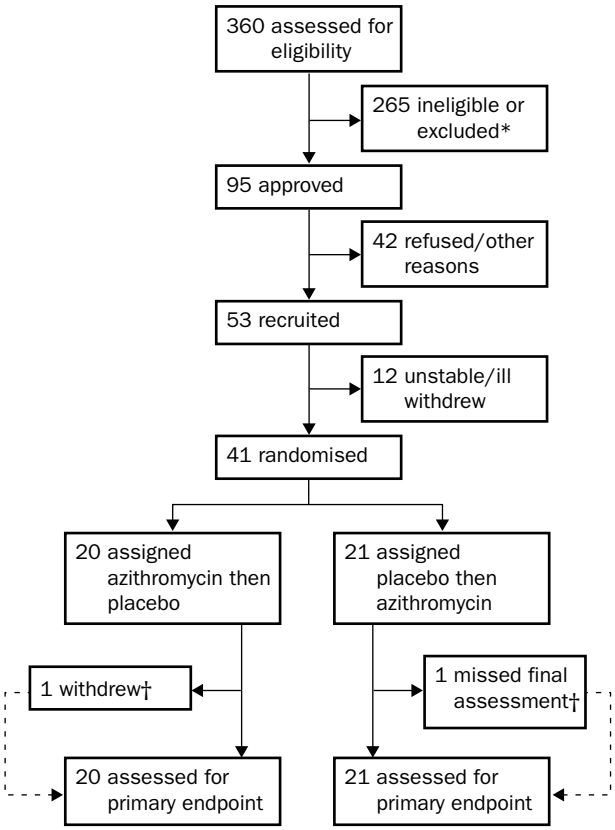

Figure 2: **Trial Profile**
*Ineligible or excluded almost exclusively because children were younger than 8 years old, lived too far away, or were part of a shared care programme where visits to the hospital occurred only once a year, or could not yet swallow pancreatic enzymes whole. †Patients had sufficient data to be included in analysis.

Spontaneously expectorated sputum was collected into a sterile container and immediately placed in liquid nitrogen and stored at –80°C. The samples were thawed on ice and the tester was unaware of the trial code for every patient. Excess saliva was removed from the sputum and phosphate-buffered saline (Gibco, UK) was added (1 mL per 1 mg sputum). The samples were mixed thoroughly with a whirlymixer for 30 s; then on ice on an orbital plate mixer for 10 min; then for a further 30 s on the whirlymixer. The samples were then centrifuged for 20 min at 20 000 *g*, 4°C. From the supernatant, interleukin-8 and neutrophil elastase activity were measured. Total interleukin-8 was measured in samples diluted 1:500 in phosphate-buffered saline with a commercially available ELISA kit (Pelikine kit, Eurogenetics, UK). Activity of neutrophil elastase was measured in samples diluted 1:10 in assay buffer (0·3 mol/L TRIS-HCl, containing 1·5 mol/L NaCl, pH 8·0). Sputum samples (10 μL) and neutrophil elastase standards (human leukocyte elastase [Sigma, Poole, UK]; 10, 50, and 100 mU in 10 μL assay buffer) were placed in the wells of a 96-well plate and preincubated for 1 min at 37°C. Then 90 μl substrate (0·555 mmol/L N-methoxysuccinyl-ala0pro-val-p-nitroanilide; Sigma) was added to every well and the plate incubated for 5 min at 37°C. The colour change was read as an increase in absorbance at 410 nm on a microtitre plate reader (Dynatech, UK). With these conditions, the standard curve was linear and elastase activity in the samples was calculated against a standard curve on every plate.

Outcome measurements

The primary outcome measure was the relative change in FEV_1 between the azithromycin and placebo treatment periods. This change was defined as the difference between the average of the 4-month and 6-month spirometry values for each treatment period (months 5 and 7, and 13 and 15); divided by the FEV_1 at randomisation (month 2) for treatment period 1, or the end of the washout period (month 9) for treatment period 2 (expressed as a percentage) to reduce any period effect. This can be written as (azithromycin [(month 4 + month 6)/2]/baseline [months 2 or 9]×100%)–(placebo [(month 4+month 6)/2]/baseline [months 2 or 9]×100%). This calculation was done for every patient and an overall median and CI was calculated.[16] The two patients with either a month 4 or month 6 value missing had their remaining result used as a single value.

The spirometric secondary outcome measures were changes in forced vital capacity (FVC) and in mid-expiratory flow between 25% and 75% of forced vital capacity ($MEF_{25-75\%}$) calculated as above. The definition of a clinically relevant change in FEV_1 depended on the within-patient variability.[17,18] A change of more or less than 13% in FEV_1 and FVC and greater than 20% for mid-expiratory flow was regarded as clinically relevant.

The other secondary endpoints were pulmonary exacerbation rate, antibiotic use, changes in exercise tolerance, quality of well-being, bacterial culture densities, and changes in total interleukin-8 and neutrophil elastase.

	Placebo then active treatment (n=21)	Active then placebo treatment (n=20)	Total (n=41)
Male/female	8/13	10/10	18/23
Age at start of trial (med,range)	14·3 (8·1–18·6)	13·6 (8·1–17·8)	13·8 (8·1–18·6)
Genotypes			
ΔF508/ΔF508	15	9	24
ΔF508/G551D	1	1	2
ΔF508/G542X	1	0	1
Others and unknown	4	10	14
Season of trial start: (Oct–March/April–September)	9/12	10/10	21/20
Three positive cultures of *P aeruginosa* in year before trial (Yes/No)	10/11	11/9	21/20
Three positive cultures of *S aureus* in year before trial (Yes/No)	8/13	4/16	12/29
Previous symptoms of wheeze >1/week (Yes/No)	14/7	15/5	29/12
Receiving nebulised anti-pseudomonal prophylaxis (Yes/No)	20/1	18/2	38/3
Receiving oral anti-staphylococcal prophylaxis (Yes/No)	15/6	10/10	25/16
Receiving rhDNAse Yes/No	8/13	7/13	15/26
% predicted FEV1 at start of trial (mean [SD]; median [range])	61 (14); 61 (33–80)	59 (12); 58 (37–78)	60 (13); 60 (33–80)
% predicted FVC at start of trial (mean [SD]; median [range])	80 (13); 80 (53–99)	80 (9); 81 (64–99)	80 (11); 81 (53–99)
% predicted MEF at start of trial (mean [SD]; median [range])	37 (20); 32 (11–82)	36 (20); 28 (13–75)	36 (19); 29 (11–82)

Table 1: **Characteristics of trial participants**

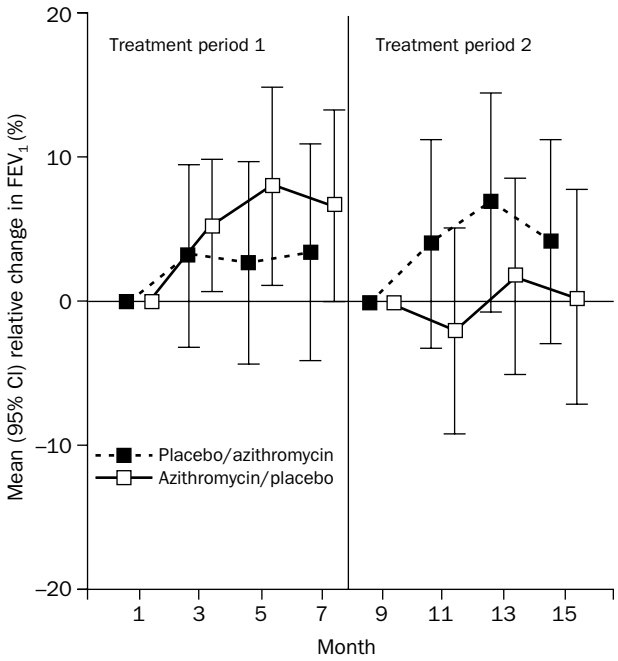

Figure 3: **Forced expiratory volume in 1 s**
Mean (95% CI) change from baseline visit for each treatment group at every treatment visit. Note visit at end of washout period (month 9) has been restandardised to zero.

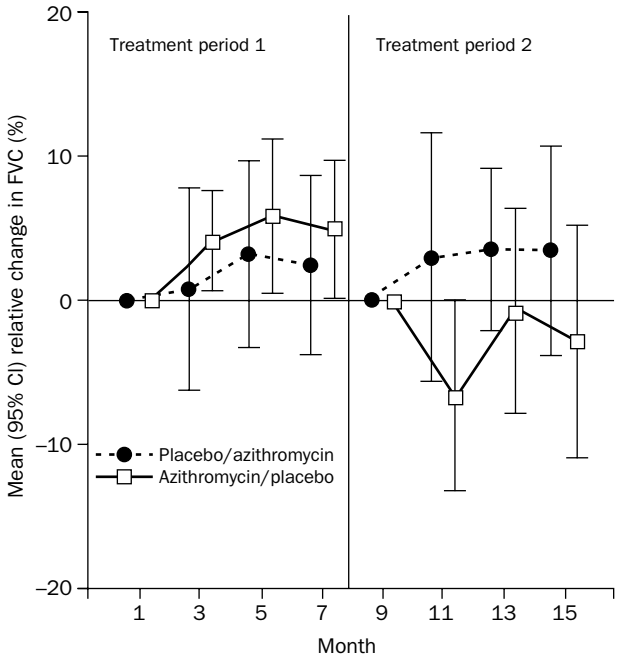

Figure 5: **Forced vital capacity (FVC)**
Mean (95% CI) change from baseline visit for each treatment group at every treatment visit. Note visit at end of washout period (month 9) has been restandardised to zero.

Statistical analysis

The study was designed so that 40 evaluable patients would be sufficient to detect, with 85% certainty at the 5% level, a 7% relative difference in FEV_1 (assuming an SD of this difference to be 15%).[17,18] This difference is about the same as the average beneficial effect of DNase on FEV_1 in the US multicentre trial.[13] To be eligible for analysis, a patient had to be recruited, randomised, and to have completed all the first treatment and at least 2 months of the second treatment. All randomised patients in this study completed the whole of the first treatment and at least 4 months of the second.

All data were entered before treatment codes were broken, and were assessed on an intention-to-treat basis. Individual differences for every patient between azithromycin and placebo were summarised as median and 95% CI.[16] The total number of patients benefiting (or not) from treatment was assessed with the binomial test. A cross-sectional analysis of group means at every treatment time point (three in each treatment group, six in total) allowed us to determine by the sign test whether there was an overall effect for azithromycin or placebo for all spirometry variables. Other differences were tested for with the Mann-Whitney and χ^2 tests. $p<0{\cdot}05$ was regarded as significant.

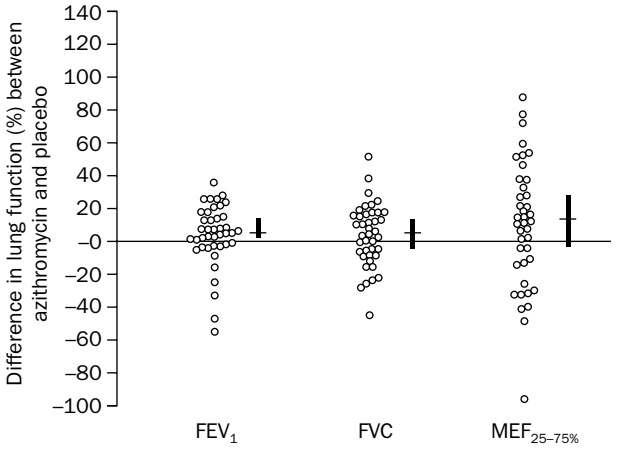

Figure 4: **Differences between average lung function at fourth and sixth month treatment visits in each treatment group**
Overall median and 95% CI are shown. Positive value indicates that average lung function for that patient when on azithromycin was greater than when receiving placebo.

Role of the funding source

Pfizer Pharmaceuticals (Groton, USA) supplied azithromycin and placebo medications and provided the code sequences for randomisation. One design suggestion was made. No salaries or honoraria were paid. The company had no role in the collection, analysis, or interpretation of the data. It has not sought nor been offered sight of the manuscript.

Results

Figure 2 shows the trial profile for recruitment and randomisation. 41 of the 53 patients recruited conformed to the stability criteria to undergo randomisation, and all were evaluable patients. Randomisation was done between August, 1999, and March, 2000; the study was completed in June, 2001. One patient in the azithromycin/placebo group withdrew after 4 months of the second treatment period because of fears that she was taking placebo, and one other patient in the placebo/azithromycin group missed the 6-month final assessment of the first treatment period because she had been admitted to another hospital elsewhere in the UK; these two patients had sufficient data to be included in analyses. Table 1 shows the demographic characteristics.

Figure 3 shows the mean (95% CI) change in FEV_1 from baseline (randomisation) in each treatment group. The mean FEV_1 was always greater at any time point in the group receiving azithromycin (Sign test $p=0{\cdot}031$). The median relative difference between azithromycin and

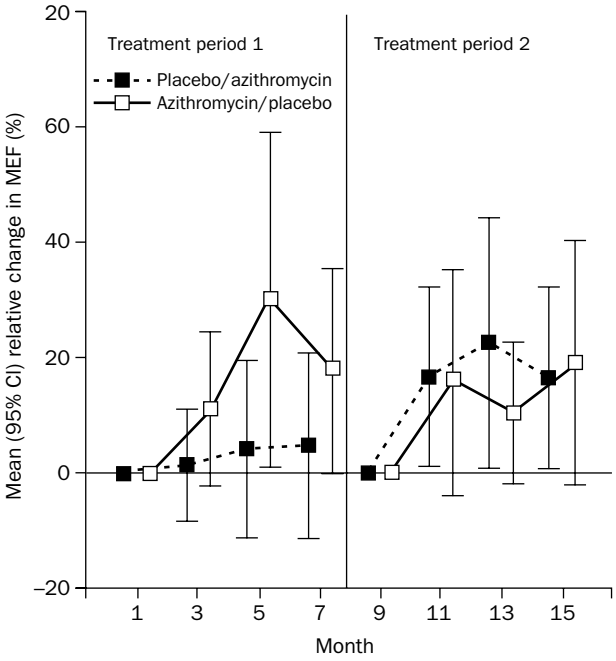

Figure 6: **Changes in mid-expiratory flow**
Mean (95% CI) change from baseline visit for each treatment group at every treatment visit. Note visit at end of washout period (month 9) has been restandardised to zero.

placebo was 5·4% (95% CI 0·8–10·5). FEV_1 was improved by more than 13% in 13 of 41 patients, and deteriorated by more than 13% in five patients (p=0·059). Individual differences are shown in figure 4.

Since there were only two permitted doses (250 or 500 mg), dose per kg ranged from 6·2 to 12·3 mg/kg/day. There was no correlation between dose per kg and change in FEV_1 for the whole group or when only 22 patients who were homozygous for ΔF508 were assessed.

The median difference in FEV_1 between the start of the two treatment periods was 0·0% (−4·8 to 4·2). 15 of 20 patients benefited from azithromycin in treatment period 1, and 13 of 21 in treatment period 2 (p=0·51). The overall median benefit in FEV_1 for sequence azithromycin/placebo was 6·4%, and for sequence placebo/azithromycin was 5·3% (Mann-Whitney p=0·6). There were no sequence effects when patients who did not take rhDNAse were assessed (p=0·9).

The median relative difference in percentage of predicted forced vital capacity between active and placebo treatment was 3·9% (95% CI −2·5 to 9·2). 14 of 41 patients improved by more than 13%, and seven deteriorated by more than 13% (p=0·127, figure 4). However, at every treatment time point, mean FVC was greater in the group receiving azithromycin (figure 5, sign test, p=0·032). Median difference for mid-expiratory flow (figure 6) was 11·4% (−1·19 to 23·7 %). 15 of 41 patients improved by more than 20%, and nine deteriorated by greater than 20% (p=0·221, figure 4).

To allow for any effect of azithromycin to develop, data from the last 4 months of each treatment group were assessed, and the results are summarised in table 2. Significantly fewer patients received additional oral antibiotic courses when on azithromycin than on placebo (p=0·005). 17 patients had 24 fewer courses when on azithromycin than with placebo, and five patients had six extra courses. The number of pulmonary exacerbations and intravenous antibiotic courses did not differ overall between placebo and azithromycin.

17 of 41 patients produced sputum at least once during the last 4 months of each treatment group, and only these specimens were assessed for interleukin-8 and neutrophil elastase. Differences between active and placebo period were assessed for every patient. The median absolute difference in interleukin-8 between azithromycin and placebo was 9041 pg/g sputum (95% CI −70 889 to 73 800) and for neutrophil elastase was 2·94 mU/mL sputum (95% CI −12 to 6); neither difference was significant. There were no associations between interleukin-8 or neutrophil elastase and changes in lung function.

Frequency of positive cultures for all organisms did not differ between azithromycin and placebo. 17 of 41 patients never produced samples that grew *P aeruginosa* during the last 4-month period of either treatment. Of the rest, there were equal colony densities between treatments for 11, seven had higher colony densities when on placebo than when on azithromycin, and six had lower colony densities when on placebo. Non-tuberculous mycobacteria were tested for, but were never isolated during the study.

The drug was very well tolerated with no subjective reports of side-effects. 12 of 190 patients failed hearing tests in minor ways, all of whom passed on retesting 2 months later. There were no abnormal clotting results. The maximum recorded alanine aminotransferase concentration was 135 IU/L and aspartate aminotransferase 87 IU/L on one occasion in the same individual at the end of 6 months' treatment with azithromycin. Since this measurement was made at the end of the trial, no drug was taken subsequently, and the liver enzyme concentrations had halved 2 months later.

The median difference in the visual analogue score (range 0–100) for well being between the end of the azithromycin and placebo treatment periods was 0, as was the change in the total quality of well being score. The baseline heart rate and oxygen saturation, together with the minimum oxygen saturation and maximum heart rate during the 3-min step test, was no different between that at the end of placebo and that at the end of active treatment; median difference for every patient for all variables of 0%.

	Number with pulmonary exacerbations* when on placebo	Number with pulmonary exacerbations* when on azithromycin	Number receiving IV antibiotics when on placebo	Number receiving IV antibiotics when on azithromycin	Number receiving oral antibiotics when on placebo	Number receiving oral antibiotics when on azithromycin
Courses or exacerbations						
0	24/41	23/41	31/41	28/41	14/41	23/41
1	15/41	13/41	9/41	13/41	16/41	15/41
2	2/41	5/41	1/41	0/41	10/41	3/41
3 or more	0/41	0/41	0/41	0/41	1/41	0/41
p	0·06		0·4		0·005	

*Four or more from the following 12 criteria: change in sputum; new or increased haemoptysis; increased cough; malaise, fatigue or lethargy; temperature >38°C; anorexia or weight loss; sinus pain or tenderness; change in sinus discharge; change in physical examination of the chest; decrease in pulmonary function by ⩾10% of previously recorded value; radiographic changes consistent with pulmonary infection. IV=intravenous.

Table 2: **Summary of antibiotic usage and pulmonary exacerbation rates**

For treatment sequence azithromycin/placebo, 15 of 18 patients correctly identified the sequence and three were uncertain. For the sequence placebo/azithromycin, only nine of 20 identified the sequence correctly, 11 were incorrect, and one was uncertain. Use of RhDNAse did not affect the outcome.

The median relative difference between azithromycin and placebo for FEV_1 was 11·5% (95% CI 5·3–16·5) for the 26 patients not receiving concurrent treatment with rhDNAse, and –3·6% (−22 to 3·9) for the 15 receiving rhDNAse. The median relative difference for FVC was 7·3% (1·2–13·7) in patients not receiving rhDNAse, and –4·5% (−16·2 to 11·0) when receiving rhDNAse. For mid-expiratory flow, the median relative difference was 21·9 (8·6–36·7) in patients not receiving rhDNAse, and –9·5% (–32 to 14) in those that did. Of the 12 of 15 children on concurrent rhDNase, 11 of 15 received their intravenous antibiotics while on azithromycin, and six of 15 when on placebo.

Discussion

We have shown a significant though modest group response in FEV_1 (the primary endpoint) to azithromycin; 32% (13 of 41) of patients had a clinically meaningful improvement of more than 13%, and five of 41 a clinically important deterioration. The overall benefits on FVC and mid-expiratory flow are questionable. Our findings did not show a mechanism by which the effect on lung function occurred, possibly because of inadequate power for some of the secondary outcomes.

The overall improvement in FEV_1 (5·4%) with azithromycin over 6 months is similar to that achieved by the US DNAse study,[13] which showed 5·8% improvement over 6 months. Thus, faced with a child in whom conventional therapy is failing, the clinician could choose to try treatment with rhDNAse or azithromycin. The advantages of rhDNAse are safety and a response within 2 weeks in most children. However, in the most severely affected individuals a response can be delayed by up to 3 months.[19] The disadvantages are that it requires nebulisation, many children deteriorate on treatment,[20] and its expense (UK£3750–7500 for alternate day or daily treatment per year, respectively). The advantages of azithromycin are its ease of use and its low cost (UK£700–1300 for 250 mg or 500 mg daily per year, respectively) compared with rhDNAse. The disadvantages are its slow onset of action (2–4 months) and its unknown efficacy and safety profile after 6 months. Treatment with azithromycin leads to a reduction in the number of additional oral courses of antibiotics prescribed, but does not reduce the number of intravenous courses.

An in-vitro study[21] showing substantial inhibition of rhDNAse by macrolides, especially azithromycin, is noteworthy. Our provisional findings of better results in children who did not take concurrent rhDNAse merits further study, since this subgroup analysis was neither specified nor powered for in the design protocol, but arose from a suggestion during peer-review of the paper.

The absence of a dose–lung function response might reflect the presence of a dose-response plateau, given the high dose used in this trial compared with that used for diffuse panbronchiolitis, for which azithromycin is given twice weekly. Our study was done entirely on an intention-to-treat basis, and although compliance was assessed by counting tablets at every clinic visit and was greater than 80% in each group (data not shown), true compliance is clearly not accurately known. However, azithromycin's long half-life (about 40 h) renders the patient less vulnerable to missing doses.

The significant change in FEV_1 applies to the group as a whole and not necessarily to an individual. Thus, even though the overall improvement was 5·4%, 13 of 41 individuals deteriorated on treatment, five of 13 by greater than 13%. Since any beneficial effect of azithromycin might not be seen until 4 months, a trial period of treatment ought to last 4–6 months.

No evidence for a mechanism of effect was noted. Nevertheless, this situation is unsurprising and should not automatically imply that the effect was not anti-inflammatory. However, discussion of mechanisms is inevitably speculative. The study was powered only to detect changes in lung function, all other measurements being secondary endpoints. Inflammatory markers in sputum are prone to wide variability even in stable patients measured repeatedly,[22,23] since every cough can produce sputum from a different part of the lung. Additionally, differences in collection technique, and the technical difficulties of assessing viscous sputum, add to the increased variability. Thus a particular sputum sample might not be at all representative of the total inflammatory status of the lung. Blood markers for pulmonary inflammation are, however, even less reliable than sputum, and were not used in this study.

If we accept that microbiological colony counts are a crude measure of overall pulmonary status, azithromycin did not seem to have any microbiological effect. The striking reduction in the use of additional oral antibiotics when azithromycin was given is worth noting, but unfortunately the study did not address whether *Chlamydia pneumoniae*, against which azithromycin would be effective, had a role in the cystic fibrosis patient's pulmonary status.[24] An antimicrobial effect on *Haemophilus influenzae* or non-tuberculous mycobacteria is unlikely since neither were isolated in the year before or during the study in any patient. No changes in frequency of isolation or colony density of *S aureus* was seen, and no effect on lung function showed an association with previous oral Staphyloccocal prophylaxis. As with diffuse panbronchiolitis,[4] regular growths of *P aeruginosa* were not a prerequisit for benefit.

A third and intriguing potential mechanism is increased expression of the cystic fibrosis transmembrane conductance regulator (CFTR). In monkeys, erythromycin has been reported to upregulate expression of P-glycoproteins,[25] of which CFTR is a member. High intracellular concentrations of azithromycin might alter the function of this P-glycoprotein, although there is no evidence to lend support to this notion.

Thus, azithromycin might have a role in reduction of the effects of minor changes in pulmonary status, but might not affect the main exacerbations. However, since most patients had no exacerbations or intravenous antibiotics during the study, any small beneficial effect is likely to have been unnoticed. The decision to prescribe oral or intravenous antibiotics remained entirely in the hands of the patient's usual clinician, and this situation led to the discrepancy between the number of defined pulmonary exacerbations and number of courses of antibiotics given (table 2). For example, a reduction in lung function and change in sputum colour, in the absence of any other clinical features, would normally lead to the prescription of additional antibiotics, but would not be defined as a pulmonary exacerbation[13] because only two and not four criteria were met.

There are several potential explanations for azithromycin's apparent slow onset of action. First, any anti-inflammatory effects of azithromycin could take a long time to influence the enormous inflammatory

load in cystic fibrosis. Second, substantial intracellular accumulation of azithromycin might be required to trigger an effect, despite its long half-life. The slight but non-significant decline in mean FEV_1 and FVC between the fourth and sixth month of treatment (figures 3 and 5) might be a chance finding, but could indicate either a maximum response level or that continued accumulation of azithromycin at this dose might become toxic. Further studies should aim to find out whether the benefit of azithromycin is sustained beyond 6 months, and whether we used the optimum or necessary dose.

In conclusion, a 4–6 month trial of azithromycin is safe, comparatively inexpensive, and could greatly benefit the lung function of patients who do not respond adequately to conventional treatment for cystic fibrosis. The mechanism of benefit remains unknown.

Contributors

A Equi recruited participants and obtained data. I Balfour-Lynn, A Bush, and M Rosenthal supervised the design, collection, and analysis of the data and preparation of the manuscript.

Conflict of interest statement

None declared.

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