Long-term, low-dose azithromycin treatment reduces the incidence but increases macrolide resistance in \textit{Staphylococcus aureus} in Danish CF patients

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Abstract

Background: Since 2001, long-term, low-dose azithromycin treatment has been used for CF patients chronically infected with \textit{Pseudomonas aeruginosa} in the Copenhagen CF centre. Our study investigates changes in incidence of colonization with \textit{Staphylococcus aureus}, \textit{Streptococcus pneumoniae}, \textit{Haemophilus influenzae} and \textit{Moraxella catarrhalis} and changes in macrolide sensitivity in these microorganisms during azithromycin treatment.

Methods: CF patients treated continuously with azithromycin for at least 3 months were included. Results of microbiological examination, including phage typing results of \textit{S. aureus}, obtained during treatment were compared to results obtained 2 years before treatment.

Results: 70 patients (median age 29.1 years) treated for a median of 4 years (range 0.7–5.1) were included. Before treatment, 44 patients had at least one culture positive for \textit{S. aureus} compared to 25 patients during treatment ($p < 0.01$). Mean percentage of sputum samples with growth of \textit{S. aureus} decreased from 12.1% (range 0–82.6%) before treatment to 6.1% (range 0–93.2) during treatment ($p < 0.0006$). Prevalence's of \textit{H. influenzae} and \textit{S. pneumoniae} also decreased significantly. Fifteen of 214 isolates (7%) of \textit{S. aureus} were macrolide resistant before treatment, increasing to 95 of 181 isolates (52.5%) during treatment ($p < 0.001$). Macrolide resistant strains were found in 3 of 44 \textit{S. aureus} colonized patients before treatment and in 11 of 25 patients at some time during treatment ($p < 0.03$), all belonging to different phage types. First resistant \textit{S. aureus} isolate was isolated after a median treatment duration of 1.5 years (range 0.3–2.9). No MRSA were isolated. Only 1 macrolide resistant isolate of \textit{M. catarrhalis} was found during treatment. No macrolide resistance was found in \textit{H. influenzae} or \textit{S. pneumoniae}.

Conclusion: Long-term, low-dose treatment with azithromycin in CF patients leads to reduced prevalence of \textit{S. aureus}, \textit{S. pneumoniae}, and \textit{H. influenzae}, but increased macrolide resistance in \textit{S. aureus}. Reduction in the prevalence of \textit{S. aureus} will make increasing macrolide resistance clinically insignificant in these patients.

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Keywords: Azithromycin; Cystic fibrosis; Macrolide resistance; Prevalence; Staphylococcus aureus

1. Background

In Cystic Fibrosis (CF), chronic pulmonary infection caused by \textit{Pseudomonas aeruginosa} is the main complication leading to increased morbidity and mortality. Diffuse pan-bronchiolitis, a disease well described in Japanese patients, shares some similarities with CF, including chronic airway infection caused by \textit{P. aeruginosa}. In the late 1980s a beneficial effect of long-term, low-dose treatment with the macrolide erythromycin in these patients was shown [1]. This led to the first studies on the effect


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of macrolides in CF patients. The results were encouraging [2,3]. To date, several studies in CF patients using long-term, low-dose azithromycin have shown similar, positive effects on change in lung function, number of exacerbations and nutrition expressed as body mass index (BMI). Randomized, placebo controlled, double blinded multi-centre studies and retrospective studies of the effect of this treatment both in CF patients chronically infected with P. aeruginosa and in CF patients without chronic infections have agreed on the positive effect [2,4–10].

The mechanisms of this effect are not fully understood: azithromycin can inhibit quorum sensing in P. aeruginosa, which controls formation of several virulence factors [11–16], azithromycin shows anti-inflammatory effects [17–21] and inhibits the production of alginate [22].

Continuing this low-dose treatment for several years has raised the concern of possible appearance of macrolide resistant microorganisms. CF patients are characterized by infections caused by Staphylococcus aureus, Haemophilus influenzae and also by Streptococcus pneumoniae and Moraxella catarrhalis — microorganisms also found in healthy individuals. A few studies have found an increase in macrolide resistance in these microorganisms isolated from CF patients in long-term, low-dose azithromycin treatment [23,24].

In the Copenhagen CF centre, azithromycin has been used as a standard treatment since 2001 for patients chronically infected by P. aeruginosa [25]. Treatment has been discontinued only at time of lung transplantation or on the occurrence of side effects such as tinnitus.

The present study was done to investigate if azithromycin treatment leads to changes in the incidence of colonization with S. aureus, H. influenzae, S. pneumoniae, and M. catarrhalis and/or changes in the occurrence in macrolide resistance in these microorganisms.

2. Materials and methods

2.1. Study design

A descriptive, retrospective study.

2.2. Patients

All 297 CF patients followed in the Copenhagen CF centre were evaluated for inclusion in the study. All patients are seen every month in the out patient clinic, and at each visit a sputum sample or a sample of lower respiratory tract secretions obtained by endo-laryngeal suction is examined microbiologically [26]. All patients treated with low-dose azithromycin continuously for at least 3 months prior to November 1st 2005 were identified, and all microbiological culture results obtained for a period of 2 years prior to start of azithromycin and during treatment were used for the study.

2.3. Treatment

Long-term, low-dose azithromycin treatment is tried in all patients with established chronic, Gram-negative pulmonary infections, and in patients with recurrent P. aeruginosa isolates despite continuous inhaled antibiotic therapy. Treatment is discontinued if unacceptable side effects occur. Azithromycin dose in patients >40 kg was 250 mg every day, in patients <40 kg 250 mg every other day. Smaller children received half the normal dose as syrup.

2.4. Microbiological results

Number of positive cultures and number of all samples for microbiological investigation was counted in the pre-treatment and treatment period, and positive cultures expressed as the percentage of all sputum samples taken for microbiological investigation. Number of patients with at least 1 positive culture of the individual microorganisms was counted in the pre-treatment and treatment period.

Growth on 7.5% NaCl plates was used to detect growth of Staphylococcus species. Final identification of S. aureus was based on conventional phenotypic characters; a colistin-zone diameter of <16 mm, the ability to clot plasma (coagulase production test) and positive Staphaurex Latex Kit (Remel Europe Ltd.). S. pneumoniae, H. influenzae and B. catarrhalis were identified based on conventional phenotypic characteristics.

2.5. Susceptibility to antibiotics

Primary sensitivity testing was done using disc-diffusion (Neo-Sensitabs®, Rosco, Taastrup, Denmark) on blood-agar plates (Statens Serum Institute, Copenhagen, Denmark). Resistance to either of the macrolides tested (erythromycin and azithromycin and/or clarithromycin) was evaluated.

Number of macrolide resistant strains for each microorganism was counted and compared to total number of positive cultures for that specific microorganism, and number of patients with at least 1 macrolide resistant strain was counted in the pre-treatment and treatment period.

Bacteriological investigations include phage typing of all S. aureus isolates at the Statens Serum Institute, Copenhagen, as reported previously [27]. These results were used to investigate if clonal spread of macrolide resistant S. aureus had occurred.

2.6. Statistics

Percentage of sputum samples with growth of individual microorganisms was compared using Student’s paired t-test. Number of patients harbouring specific microorganisms and number of macrolide resistant strains were compared using chi-square test. Level of significance: p <0.05.

3. Results

3.1. Patients

70 patients were included in the study. Median age at onset of treatment was 29.1 years (range 1.5–53.2 years). Sixty-one of treated patients had well-established chronic airway infection caused by P. aeruginosa, 5 of treated patients were developing
chick infection with *P. aeruginosa* and 4 patients had chronic airway infections caused by *A. xylosoxidans* or *B. multivorans*. Median duration of azithromycin treatment per November 1st 2005 was 4 years (range 0.7–5.1 years).

### 3.2. Microbiology

Microbiological results from 6298 sputum samples were evaluated: 2240 samples taken in the 2 years prior to treatment, median 30 samples/patient (range 9–54) and 4058 samples taken during treatment, median 59 samples/patient (range 14–142).

The number of patients with at least one culture positive for *S. aureus* decreased from 44 of 70 patients (63%) in the pre-treatment period to 25 of 70 patients (36%) in the treatment period (p<0.01). The prevalence of *S. aureus* decreased from a mean of 12.1% of samples (median 4, range 0–82.6) before treatment to a mean of 6.1% (median 0, range 0–93.2) during treatment (p<0.0006). Although the numbers were small, the prevalence of *H. influenzae* decreased significantly from a mean of 1.5% (median 0, range 0–16.7) before treatment to a mean of 0.2% (median 0, range 0–3.2) during treatment (p<0.003). The prevalence of *S. pneumoniae* also decreased from a mean of 1% (median 0, range 0–16.7) before treatment to a mean of 0.3% (median 0, range 0–4.3) (p<0.05). The prevalence of *M. catarrhalis*, and the number of patients with at least one culture positive for *H. influenzae, S. pneumoniae* and *M. catarrhalis* did not change (Table 1).

#### 3.3. Macrolide resistance

Prior to treatment, three patients had at least one macrolide resistant isolate of *S. aureus*. During treatment, this number increased to 11 patients (p<0.03). Two patients harboured resistant isolates before and during treatment, and one patient with macrolide resistant *S. aureus* prior to treatment lost the macrolide resistant strain during treatment. The 9 patients with no resistant isolates prior to Azithromycin treatment had been treated for a median of 1.5 years (range 0.3–2.9) at the time of first resistant isolate.

Prior to treatment, 15 of 214 isolates (7%) of *S. aureus* were macrolide resistant. During treatment, this number increased to 95 of 181 isolates (52.5%) (p<0.001).

Phage typing showed that phage types found in CF patients were the same phage types found in Denmark in general. Development of resistance occurred in phage types previously harboured by the patient. No spread of resistant clones was found.

One macrolide resistant isolate of *M. catarrhalis* was found during treatment. No macrolide resistant strains of *H. influenzae* or *S. pneumoniae* were found at any time.

We found no methicillin resistant isolates of *S. aureus* (MRSA) in the present study.

### 4. Discussion

Since the first results showing the positive effect of azithromycin on progression on CF lung disease [2], long-term, low-dose azithromycin has been used to treat an increasing number of CF patients with chronic *P. aeruginosa* infection. Lately the increase of macrolide resistance in *S. aureus* and *H. influenzae* have been reported following this treatment regimen [23,24]. These reports inspired us to undertake the present study, since azithromycin has been used for several years now in the Copenhagen CF centre. Median treatment duration in the study was 4 years.

6298 samples of lower airway secretion were included in the study. The percentage of sputum cultures positive for *S. aureus* was very low prior to treatment, median 4% (range 0–82.6%) and decreased significantly (p<0.0006) during treatment, median 0% (range 0–93.2%). Treatment of *S. aureus* is very aggressive in the Copenhagen CF centre [28] and <10% of patients are chronically infected with *S. aureus*, explaining the very low prevalence of *S. aureus* in sputum cultures. Number of patients with at least one culture positive for *S. aureus* decreased significantly from 44 before azithromycin treatment was started to 25 after initiation of treatment.

It seems that long-term, low-dose azithromycin treatment can provide some protection from *S. aureus* infections in CF, but with increasing prevalence of macrolide resistance this may change in the future. Saiman et al. [8] found significantly fewer azithromycin treated patients with new *S. aureus* colonization compared to patients receiving placebo; 2 of 84 patients in the treatment group had new *S. aureus* colonization after 168 days, while this was the case in 12 of 92 patients in the placebo group. However, no difference was found when comparing proportion of patients clearing *S. aureus* infection during the 168 days of azithromycin treatment — in the azithromycin group, 18% cleared the infection and in the placebo group, 12% of patients cleared the *S. aureus* infection. The duration of *S. aureus* infection, and whether these patients were chronically infected, was not reported.

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**Table 1** Percentage of sputum samples with positive cultures of individual microorganisms and number of patients with at least one positive culture of a microorganism (N)

<table>
<thead>
<tr>
<th>Species</th>
<th>Before treatment</th>
<th></th>
<th></th>
<th></th>
<th>During treatment</th>
<th></th>
<th></th>
<th>p-value</th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean %</td>
<td>Median %</td>
<td>Range</td>
<td>N</td>
<td>Mean %</td>
<td>Median %</td>
<td>Range</td>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>12.1</td>
<td>4.0</td>
<td>0–82.6</td>
<td>44</td>
<td>6.1</td>
<td>0</td>
<td>0–93.2</td>
<td>25</td>
<td>&lt;0.0006</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>1.5</td>
<td>0</td>
<td>0–16.7</td>
<td>15</td>
<td>0.2</td>
<td>0</td>
<td>0–3.2</td>
<td>7</td>
<td>&lt;0.003</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>1</td>
<td>0</td>
<td>0–16.7</td>
<td>10</td>
<td>0.3</td>
<td>0</td>
<td>0–4.3</td>
<td>9</td>
<td>&lt;0.05</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>M. catarrhalis</em></td>
<td>0.6</td>
<td>0</td>
<td>0–20</td>
<td>5</td>
<td>0.1</td>
<td>0</td>
<td>0–6.5</td>
<td>2</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
Phaff et al. [23] studied 156 CF patients followed in the Rotterdam CF centre. Forty-one % of patients were chronically infected with P. aeruginosa, but it was not reported how many of these patients were treated with azithromycin. The observation time was 5 years, and 41% of the patients followed in the centre received azithromycin at some time. Indications for treatment were progression of clinical symptoms and/or worsening of pulmonary function, and poor response to conventional treatment. Median duration of azithromycin treatment was 1.1 years (range 0.01–5). All isolates of S. aureus and H. influenzae from patients receiving azithromycin treatment at the time of microbiological examination were compared to isolates from patients not receiving azithromycin treatment at the time. Significantly fewer isolates of S. aureus were found in the azithromycin treated group. A significantly larger proportion of macrolide resistant isolates of S. aureus and H. influenzae were cultured, not only when comparing samples from azithromycin treated patients to samples from patients not receiving azithromycin at the time, but also when comparing isolates from all CF patients to isolates from non-CF patients. Furthermore, the proportion of S. aureus and H. influenzae resistant to azithromycin increased during the observation time. It was not reported whether the included patients were chronically infected with S. aureus. It was found that P. aeruginosa carriage was associated with reduced isolation of S. aureus and H. influenzae. The present study is not directly comparable to the Rotterdam study by Phaff et al., since the majority of our patients were chronically infected with P. aeruginosa, the prevalence of S. aureus is very low and our patients acted as their own controls: comparing sputum cultures prior to azithromycin treatment to sputum cultures during azithromycin treatment. In the study by Phaff et al. [23], azithromycin was discontinued in the Rotterdam CF centre due to the increase in macrolide resistance.

Tramper-Stranders et al. [24] studied CF patients chronically infected with P. aeruginosa in the Utrecht CF centre. Clinical and microbiological data for each year were compared from the year prior to treatment start and during all years of treatment. Median treatment duration was 3.5 years (range 0.9–7). A slight, though not significant, decrease in S. aureus colonization in azithromycin treated patients and significantly increasing macrolide resistance in these patients were found. In this study, around 50% of patients were colonized with S. aureus, which is higher than in our study. In the present study, percentage of H. influenzae and S. pneumoniae in lower airway samples decreased significantly during treatment (Table 1). Percentage of cultures positive for M. catarrhalis did not change during treatment. Number of patients with at least one culture positive for any of the 3 microorganisms did not change during azithromycin treatment. The number of cultures positive for these 3 microorganisms was generally very low.

We found a significant increase in macrolide resistant S. aureus strains from 7% to 52.5% of isolates (p<0.001). Furthermore, the number of patients harbouring resistant strains increased significantly from 3 to 11 of 70 patients (p<0.03).

Compared to the Danish population, phage types found in CF patients did not differ from phage types found in general [29,30]. Occurrence of macrolide resistance is considerably higher than the general macrolide resistance of almost 5%. In individual patients we found development of resistance in phage types harboured by the patient prior to treatment start. Some patients continued to have intermittent occurrence of macrolide sensitive strains after first appearance of resistance.

Tramper-Stranders et al. [31] investigated the possibility of household transmission of macrolide resistant S. aureus from CF patients to household contacts, and found that this could not be proven. Possible transmission between CF patients was not reported. In our study we found no sign of transmission between patients when comparing phage types.

We found no increase in macrolide resistance in H. influenzae, S. pneumoniae or M. catarrhalis.

No strains of methicillin resistant S. aureus (MRSA) were found in our study, consistent with the low incidence of MRSA in Denmark [28,32,33].

Because of our very aggressive treatment of S. aureus colonization and infection and following a very low prevalence of this microorganism in CF patients at the Copenhagen CF centre [28], the occurrence of macrolide resistant S. aureus has no impact on treatment of this infection, and long-term, low-dose azithromycin treatment is continued.

The present study is a retrospective study. Further, preferably prospective, studies are needed for effects of long-term azithromycin on occurrence of macrolide resistance in household contacts of treated patients, in CF patients not treated with azithromycin, and of short-term azithromycin treatment in CF patients and other patient groups.

4.1. Conclusion

Long-term, low-dose azithromycin treatment reduces the prevalence of S. aureus, H. influenzae and S. pneumoniae in CF patients, but leads to an increase in macrolide resistance in S. aureus isolates from these patients. Treatments strategies resulting in a reduction of prevalence of S. aureus in CF populations are important. Reduction in the prevalence of S. aureus will make increasing macrolide resistance clinically insignificant. The azithromycin treatment strategy in chronically infected CF patients has been shown to be beneficial in this patient population and should not be discontinued.

References


