Effectiveness of inhaled tobramycin in eradicating \textit{Pseudomonas aeruginosa} in children with cystic fibrosis

Sanja Stanojevic a,⁎,1, Valerie Waters b,1, Joseph L. Mathew a, Louise Taylor a, Felix Ratjen a

a Division of Respiratory Medicine, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto M5G 1X8, Canada
b Division of Infectious Diseases, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, 555 University Avenue, Toronto M5G 1X8, Canada

Received 8 August 2013; received in revised form 5 September 2013; accepted 11 September 2013
Available online 3 October 2013

Abstract

Background: Inhaled tobramycin therapy has been shown to be efficacious in clinical trials for the eradication of initial \textit{Pseudomonas aeruginosa} infection in children with cystic fibrosis (CF). However, the effectiveness of different regimens in eradicating \textit{P. aeruginosa} and preventing the development of chronic infection in actual clinical settings has yet to be determined.

Methods: This was an observational study of children (<18 years of age) with CF with incident \textit{P. aeruginosa} infection from 2005–2012 based on data collected from the Toronto CF Database and medical charts. Patients who received inhaled tobramycin (80 mg/2 ml twice daily for 365 days) were compared to those who received tobramycin inhalation solution (TIS) (300 mg/5 ml twice daily for 28 days) with respect to eradication and development of chronic infection. We also examined the risk factors for recurrence of infection.

Results: During the study period, 65 patients were identified with incident \textit{P. aeruginosa}, of which 7 (11%) failed eradication therapy. Eradication failure was similar between the two treatment groups. A total of 4 patients (6%) developed chronic \textit{P. aeruginosa} infection in the 12 months following the end of therapy with no differences between treatment groups. Female gender, older age, pancreatic insufficiency, lower lung function and worse nutritional status were identified as risk factors for recurrence of \textit{P. aeruginosa} infection.

Conclusions: Both regimens of inhaled tobramycin have similar effectiveness in eradicating \textit{P. aeruginosa} and preventing chronic \textit{P. aeruginosa} infection in CF patients in clinical practice. Further work is needed, however, to identify patient characteristics and bacterial factors that play a role in eradication failure, in order to develop more effective antimicrobial rescue treatment strategies.

© 2013 European Cystic Fibrosis Society. Published by Elsevier B.V. All rights reserved.

Keywords: \textit{Pseudomonas aeruginosa}; Tobramycin; Eradication; Pediatrics

1. Introduction

Children with cystic fibrosis (CF) typically develop pulmonary infection with \textit{Pseudomonas aeruginosa} early in life and without treatment go on to develop chronic infection, typically within a year of the first infection [1,2]. Chronic pulmonary infection with mucoid \textit{P. aeruginosa} is associated with accelerated decline in lung function and earlier mortality in CF [3–5]. As a result, much effort has been made to prevent the development of chronic \textit{P. aeruginosa} through various antibiotic regimens [6]. Since the early 1980s, eradication programs for \textit{P. aeruginosa} have been attempted in younger patients with CF, with the goals to eliminate the organism, prevent the establishment of persistent infection and prolong the time to subsequent infection [7,8]. In addition, although the risk factors for initial \textit{P. aeruginosa} infection have been defined, factors affecting the risk of re-infection have yet to be determined [9–11]. Failure to eradicate \textit{P. aeruginosa} has been shown to be associated with an increased risk of subsequent pulmonary exacerbations [12], which can affect the rate of lung function decline [13].
While multiple antibiotic strategies have been studied for the eradication of \( P. \ aeruginosa \), many protocols include inhaled tobramycin as the backbone of the regimen. Randomized control trials of inhaled tobramycin have shown both tobramycin 80 mg twice daily and tobramycin 300 mg twice daily to be more effective than placebo for eradicating \( P. \ aeruginosa \) from the airways of children with CF [14,15]. However, there have not been any studies evaluating the effectiveness of these eradication protocols within the clinical setting. This is particularly important because not all antimicrobial agents are equally available to all patients due to costs and health insurance coverage, and not all patients fully comply with the protocol, resulting in varying treatment durations.

The objectives of this study were thus to compare the effectiveness of two inhaled tobramycin regimens (inhaled tobramycin (80 mg/2 ml twice daily, typically for 365 days) or tobramycin inhalation solution (TIS) (300 mg/5 ml twice daily, typically for 28 days) with respect to 1) \( P. \ aeruginosa \) eradication and 2) development of chronic infection. In addition, we sought to identify risk factors for recurrence of \( P. \ aeruginosa \) following eradication therapy.

2. Materials and methods

2.1. Subject population and data collection

This was a retrospective observational study based on data collected from the Toronto Cystic Fibrosis Database as previously described [16]. Pediatric patients (<18 years of age) followed at the Hospital for Sick Children Cystic Fibrosis clinic from 2005–2012 were eligible for this analysis (n = 453). A detailed description of the inclusion/exclusion criteria is presented in Fig. 1. We focused on incident \( P. \ aeruginosa \) cases during the study period, thus patients were excluded if they had a positive \( P. \ aeruginosa \) culture prior to 2005, if they did not have a positive \( P. \ aeruginosa \) culture before 2012, or had a lung transplant. All microbiology data were based on culture results from sputum, bronchoalveolar lavage samples or throat swabs. To be included in these analyses, patients had to have at least 3 negative cultures documented in the previous 12 months, have received inhaled antibiotics within 180 days of the incident positive \( P. \ aeruginosa \) culture, and had at least one culture after the end of treatment. Patients with incomplete exposure and outcome data in the database were excluded from the study. This study was approved by the Research Ethics Board at the Hospital for Sick Children (REB# 1000013759).

2.2. Definitions of variables

Height and weight measurements from all clinic visits were used to calculate body mass index (BMI), which were then converted to age-standardized z-scores using the WHO 2006 growth charts for children <2 years of age, and the CDC 2000 growth charts for children ≥2 years of age [17,18]. Children older than 5 years of age routinely performed spirometry at our clinic. Absolute values of forced expiratory volume in 1 s (FEV\(_1\)) were corrected for height, age and sex and analyzed as percent predicted and z-scores [19]. Thereafter FEV\(_1\) was summarized as 1) FEV\(_1\) at the time of first \( P. \ aeruginosa \) infection and 2) the rate of FEV\(_1\) change in the year preceding the first \( P. \ aeruginosa \) infection, calculated using all available observations for each patient separately. A pulmonary exacerbation was defined as a hospitalization for respiratory symptoms requiring intravenous antibiotics [20]. Burkholderia cepacia complex, Haemophilus influenzae, Staphylococcus aureus and Stenotrophomonas maltophilia infections were classified as any positive sputum or throat swab culture in the year preceding first \( P. \ aeruginosa \) infection. MRSA infection was not included due to its low prevalence in the CF population in Canada and in this center [21].

2.3. Treatment categorization

Patients were categorized according to treatment received: inhaled tobramycin (80 mg/2 ml twice daily, typically for 365 days) or tobramycin inhalation solution (TIS) (300 mg/5 ml twice daily, typically for 28 days). TIS was introduced in this CF clinic in 2007 and the 1 month TIS regimen was thus preferentially used from 2007 onwards when medical insurance was available to cover the costs; prior to 2007, inhaled tobramycin (80 mg/2 ml) was used. The treatment received was verified in the patient medical records. Inhaled antibiotics had to be given within 180 days of the incident \( P. \ aeruginosa \) positive culture to be considered as treatment associated with the first positive culture.

2.4. Outcomes

We compared the two tobramycin regimes with respect to two outcomes 1) \( P. \ aeruginosa \) eradication based on the microbiological results of the first culture after the patient stopped treatment and 2) development of chronic infection defined as >50% of cultures were positive for \( P. \ aeruginosa \) [22] in the year after the patient stopped initial treatment.

We also investigated risk factors for \( P. \ aeruginosa \) recurrence, both for 1) time to next \( P. \ aeruginosa \) infection and 2) \( P. \ aeruginosa \) recurrence in the 12 months following antimicrobial treatment.

2.5. Statistical analysis

The proportion of eradication failures and the proportion of patients that developed chronic \( P. \ aeruginosa \) infection (>50% of cultures positive in the year after treatment ended) were compared using Fisher’s Exact test. The risk factors for time to subsequent \( P. \ aeruginosa \) after the end of treatment were assessed using univariable Cox proportional hazard analysis, whereas the risk factors for becoming \( P. \ aeruginosa \) positive in the year following the end of treatment were assessed using univariable logistic regression analysis.
3. Results

3.1. Study population characteristics

Of the patients who developed incident *P. aeruginosa* infection (n = 65, Table 1), the median age of incident *P. aeruginosa* was 7.4 years (IQR (3.2; 10.3)), the mean baseline FEV$_1$% predicted was 89% (SD 16.1) and the mean BMI was within the normal range (zBMI 0.05, SD(0.8)). The majority of patients were culture positive for *Staphylococcus aureus* and *Haemophilus influenzae* in the year prior to incident *P. aeruginosa* infection; none were infected with *B. cepacia* complex and few were infected with *S. maltophilia*. Baseline characteristics of the two treatment groups were similar, with the exception that the TIS group had more respiratory cultures taken in the year prior to incident *P. aeruginosa* infection. The TIS group also had more respiratory cultures taken after the end of treatment, and was more likely to be cultured sooner after stopping treatment. Of note, of the 37 patients who received inhaled tobramycin 80 mg/2 ml, 24 did so before 2007 and 13 received tobramycin 80 mg after 2007.

3.2. Success of eradication

Eradication was evaluated in each treatment regime by examining the first respiratory tract culture after stopping treatment (Fig. 2). Of the 28 subjects treated with TIS 300 mg, 3 (11%) were positive for *P. aeruginosa* on the first culture
following the end of therapy (median treatment time 27 days; median culture time 21.5 days after end of TIS). In the 37 subjects treated with inhaled tobramycin 80 mg, 4 (11%) were positive for *P. aeruginosa* after stopping therapy (median treatment time 347 days; median culture time 83 days). The difference in the proportion of patients who achieved successful eradication of *P. aeruginosa* following inhalation therapy was not statistically different between the two groups (p = 0.99). Of the patients treated with inhaled tobramycin 80 mg, 5 were culture positive during treatment: 4 became negative by the end of therapy and 1 remained positive.

### 3.3. Development of chronic infection

The development of chronic *P. aeruginosa* infection, as defined by the Leeds criteria (>50% of cultures positive) [22], was determined in the 12 months following the end of treatment in both groups. There was no significant difference in the proportion of subjects who achieved successful eradication of *P. aeruginosa* following inhalation therapy was not statistically different between the two groups (p = 0.99). Of the patients treated with inhaled tobramycin 80 mg, 5 were culture positive during treatment: 4 became negative by the end of therapy and 1 remained positive.

### 3.4. Risk factors for recurrence of *P. aeruginosa* infection

A total of nine of 28 (32%) patients treated with TIS had at least one positive respiratory culture for *P. aeruginosa* in the year following treatment, compared to six of 37 (16%) patients treated with inhaled tobramycin 80 mg; these proportions were not significantly different (p = 0.150). Table 2 compares the risk factors for recurrence of *P. aeruginosa* positive culture in the 12 months after the end of treatment, irrespective of treatment. Female patients, older patients, pancreatic insufficient patients, those with lower lung function and lower body
mass index were more likely to have a positive culture. A longer interval between infection and start of treatment also increased the likelihood of a positive culture, as did the frequency of respiratory cultures after the end of treatment. The limited sample size precluded multi-variable analysis of these risk factors. When the risk factors of age, FEV1 and BMI were examined using values from the beginning of the 12 months post-eradication treatment and in a time varying manner, they were not significantly associated with recurrence of *P. aeruginosa* infection.

These risk factors for re-infection were similar to risk factors for initial acquisition. The risk factors for initial infection were assessed by comparing the study cohort of 65 patients to children followed in the clinic who did not develop *P. aeruginosa* infection. A total of 96 of the 133 patients who met the same inclusion/exclusion criteria were used for this study analysis (i.e. transplant patients excluded, at least one year of observation etc.). Compared to this sample of patients that were negative for *P. aeruginosa* during the same time period, patients who acquired *P. aeruginosa* for the first time...

### Table 2

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Univariable OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female gender</td>
<td>2.39 (1.9; 3.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Older age at first infection</td>
<td>1.1 (1.06; 1.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pancreatic insufficient</td>
<td>4.61 (3.4; 6.31)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lung function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline FEV1% pred at time of first infection</td>
<td>0.90 (0.89; 0.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in FEV1% pred in the year prior to first infection</td>
<td>1.01 (0.97; 1.04)</td>
<td>0.768</td>
</tr>
<tr>
<td>Body mass index (z-score for age)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline zBMI at time of first infection</td>
<td>0.72 (0.62; 0.83)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Change in zBMI in the year prior to first infection</td>
<td>0.14 (0.07; 0.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Culture rate after end of treatment</td>
<td>1.07 (1.05; 1.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to start of treatment</td>
<td>1.02 (1.01; 1.02)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mucoid status</td>
<td>1.32 (0.77; 2.27)</td>
<td>0.305</td>
</tr>
</tbody>
</table>
were older (7.4 years vs 4.4 years, \( p = 0.003 \)), were more likely to be pancreatic insufficient (90.8% vs 76.0%, \( p = 0.021 \)), and were less likely to be infected with \( S. \) aureus (75% vs 95%, \( p = 0.001 \)).

3.5. Time to subsequent \( P. \) aeruginosa infection

Twelve months after the end of treatment, more than 70% of all patients remained free of \( P. \) aeruginosa infection (Fig. 3). Of those that did reacquire \( P. \) aeruginosa, the median time to next infection was 117 days (IQR 31.5; 143). Pancreatic sufficient patients were more likely to remain \( P. \) aeruginosa free in the year after the end of treatment (HR 5.30; 95%CI 1.7; 16.7). No other risk factors emerged using univariable Cox proportional hazard models.

4. Discussion

To our knowledge, this study is the first to evaluate the clinical effectiveness of two regimens of inhaled tobramycin therapy in the eradication of \( P. \) aeruginosa from the airways of young children with CF. It demonstrates that treatment of CF patients with inhaled tobramycin at a concentration of 80 mg/2 ml for 1 year or with TIS of 300 mg/5 ml for 1 month appears to have similar effectiveness in achieving \( P. \) aeruginosa eradication with low rates of chronic infection in both groups. The eradication rates achieved in clinical practice compare favorably to those seen in previously reported controlled clinical trials.

Antimicrobial therapy for the eradication of \( P. \) aeruginosa has been used for almost 30 years and has been shown in several studies to be superior to placebo alone [14,15,23]. Many regimens include inhaled tobramycin as a major component of the treatment protocol. Eradication failure rates following treatment with inhaled tobramycin alone range from 7–21% [14,24–27] which is consistent with the eradication failure rate of 11% in our study. Studies have less commonly examined the development of chronic \( P. \) aeruginosa infection as an outcome measure but Proesmans et al. [26] have demonstrated a 5% rate of chronic infection at 1 year following inhaled tobramycin therapy, similar to that found in this current study. In addition, as with the ELITE study comparing 28 to 56 days of TIS, longer duration of therapy did not result in improved outcomes in this study, although it has to be noted that tobramycin doses differed between the two groups [27].

It is notable that the results of the present study, which occurred in a clinical setting, are comparable to the findings from those in controlled research studies. Examining the effectiveness of a therapy in an observational study rather than its efficacy within a randomized controlled trial, is prone to treatment selection bias because, in an observational study, patients are more likely to be treated if they have more severe disease, often resulting in worse clinical outcomes [28,29]. In addition, patients in this study were not as closely followed and monitored as those in a clinical trial [15,25]. Our patients were also not pre-selected according to stringent inclusion criteria including the presence of negative \( P. \) aeruginosa antibodies. Serum IgG antibodies to \( P. \) aeruginosa are known to rise in CF patients who develop chronic, mucoid \( P. \) aeruginosa infection and although they are not frequently used in North America for the diagnosis of \( P. \) aeruginosa infection in CF, in many European CF centers the presence of anti-\( P. \) aeruginosa antibodies is a criteria in the diagnosis of chronic infection [1,30,31]. Excluding subjects with positive \( P. \) aeruginosa antibodies, representing more established infection, may increase the success rate of eradication therapy in a study environment that is not replicated in a clinical setting [14,24,27]. Despite all of these factors, eradication therapy with as little as 1 month of inhaled tobramycin was shown to be effective in eradicating \( P. \) aeruginosa infection and preventing the development of chronic infection in the vast majority of patients in our clinic. These conclusions are clinically relevant given the additional treatment burden of 12 months of treatment compared to 1 month of treatment. Although the medical insurance coverage for each therapy may differ, the overall cost of both regimens is similar in most countries after taking into account the treatment duration. Given the apparent comparable effectiveness of these two eradication strategies, in settings where cost is not a factor, most patients and clinicians would likely favor the shorter treatment course (1 month).

Our study was also the first to identify risk factors for recurrence of \( P. \) aeruginosa infection following eradication therapy. Some of the variables such as older age, pancreatic insufficiency (as an indicator of CFTR function) and female gender have been recognized as risk factors for initial \( P. \) aeruginosa infection in CF either in analysis of this current dataset or in previous studies [9–11]. However, additional, potentially modifiable risk factors for \( P. \) aeruginosa recurrence were identified including worse lung function and nutritional status at the time of first infection and delay in initiating eradication therapy. As aerosol deposition is less homogeneous in patients with reduced lung function, this could suggest that these patients may benefit from additional systemic therapy, but further evidence is needed to support this hypothesis. In addition, we demonstrated that patients with \( S. \) aureus infection were less likely to have initial \( P. \) aeruginosa acquisition whereas previous studies identified it as a risk factor for \( P. \) aeruginosa infection, suggesting that further investigation of the role of the CF pulmonary microbiome in \( P. \) aeruginosa infection is required.

There were several limitations to this study. Compared to some other clinical trials of \( P. \) aeruginosa eradication [32,33], our sample size was smaller, limiting our ability to adjust for potential confounding factors. As this was a retrospective analysis of registry data and an evaluation of the effectiveness of anti-\( P. \) aeruginosa eradication protocols in an actual CF clinic, there was a considerable variation in the timing of initiation of therapy. In addition follow up sputum cultures were typically obtained in the first month following the end of TIS therapy whereas they were usually obtained 3 months following the end of inhaled tobramycin (80 mg/2 ml) treatment. This led to an intrinsic favoring of tobramycin 80 mg in any analysis of time to next positive \( P. \) aeruginosa infection. While we took these variations into account in the analyses, these differences in the
treatments and procedures precluded more detailed analysis of time to subsequent infection. Finally, TIS was introduced at our center in 2007 resulting in a bias towards more recent incident *P. aeruginosa* cases being treated with TIS.

In conclusion, this study demonstrates that inhaled tobramycin is effective in eradicating *P. aeruginosa* infection as well as preventing the development of chronic *P. aeruginosa* infection in CF patients in an actual clinic setting. Further work is needed, however, to be able to accurately identify which patients will fail initial inhaled tobramycin therapy, and what role the *P. aeruginosa* bacterium and the polymicrobial community of the CF lung play in this failure, in order to develop more effective antimicrobial rescue treatment strategies.

**Acknowledgments**

This study was funded by Sellers Chair of Cystic Fibrosis and Irwin Family Foundation.

**References**


