Inhaled amiloride and tobramycin solutions fail to eradicate *Burkholderia dolosa* in patients with cystic fibrosis

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Abstract

Background: *Burkholderia dolosa* can result in chronic airway infection and rapid decline in lung function in patients with cystic fibrosis (CF). Amiloride has antibacterial properties that may be synergistic with aminoglycosides against other species belonging to the *Burkholderia cepacia* complex (Bcc). We attempted to eradicate *B. dolosa* using a combination of nebulized tobramycin and nebulized amiloride in infected CF patients.

Methods: A 6-month, open-label trial of continuous inhaled amiloride, delivered via nebulization four times daily, and continuous inhaled tobramycin (TIS or TOBI®) nebulized twice daily, was offered to all CF patients at our institution who are chronically infected with *B. dolosa*.

Results: Twenty-two of 27 patients with *B. dolosa* were eligible and twelve elected to participate. Eradication of *B. dolosa* was not noted in any study subject. While patients tolerated treatment with no adverse effects, there was also no apparent impact on other secondary outcome measures.

Conclusions: Concurrent, continuous inhalation of amiloride and tobramycin for 6 months was not effective for the eradication of chronic *B. dolosa* airway infection in CF patients.

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Keywords: Cystic fibrosis; *Burkholderia dolosa*; *Burkholderia cepacia* complex; Amiloride; Tobramycin

1. Introduction

Cystic Fibrosis (CF) is a chronic, progressive, and ultimately fatal genetic disease [1]. The main physiological defect is related to abnormalities in ion and water transport across epithelial cells, leading to viscous secretions with low water content. The primary clinical manifestations include disruption of mucociliary clearance in the lung resulting in persistent lung infection and inflammation with progressive lung damage, as well as exocrine pancreatic insufficiency with consequent malabsorption and poor weight gain. The prognosis for patients with cystic fibrosis has gradually improved from a median life expectancy of 5 years of age in the United States during the 1930’s to the 2008 projection of 37.4 years of age based on Cystic Fibrosis registry data [2], but lung damage due to chronic airway infection and subsequent respiratory failure remains the primary cause of death.

*Burkholderia cepacia* complex (Bcc) bacteria are comprised of at least 17 species of gram-negative organisms recognized as important pathogens in the airway of patients with CF. Chronically infected patients may have an accelerated clinical decline, and some have experienced “cepacia syndrome,”...
characterized by recurrent fevers, bacteremia, necrotizing pneumonia, and accelerated progression of pulmonary disease resulting in death within weeks to months [3–7]. Bcc bacteria are often highly antibiotic resistant, notoriously difficult to treat and implicated in numerous infectious epidemics within CF centers. Although initially identified as a single bacterium, more recent genomic analysis has allowed Burkholderia to be further speculated [8]. The relative virulence and clinical impact of these different species remain unclear; however, a recent report describing the clinical impact of an outbreak of *B. dolosa* (genomovar VI) documented a markedly accelerated decline in lung function as compared to patients colonized with *B. multivorans* and patients without Bcc infection [9]. The clinical impact of this epidemic strain of multiply antibiotic resistant *B. dolosa* led us to investigate novel therapies, such as combined treatment with nebulized amiloride and Tobramycin solution for inhalation (TIS) or TOBI®.

TOBI®, is an established treatment for managing airway infection in CF [10] Amiloride, however is best known as a mild, potassium sparing diuretic that inhibits Na+ channel activity. As a diuretic, it exerts this effect in the distal convoluted tubule, but it also blocks Na+ channel activity on the epithelial cell surface. In cystic fibrosis (CF) a defect in Cl− transport at the apical cell membrane is thought to secondarily lead to excessive Na+ reabsorption resulting in viscous airway secretions prone to impaction, airflow obstruction and bacterial infection. Amiloride has therefore been studied in CF in an effort to reverse this process by reducing the viscosity and improving clearance of airway secretions. The effect of amiloride on the CF airway has been substantiated in vitro in respiratory epithelial cell culture [11–13] and in vivo [14] by nasal potential difference measurements across the epithelia of CF patients, where it corrects the abnormal potential difference.

Nebulized amiloride has also been demonstrated in numerous trials to have an excellent safety profile [15–19], though studies of efficacy have rendered mixed results. No statistically significant effect on various outcomes including FVC, FEV1, number of hospitalizations, colonization status, and pulmonary exacerbations was demonstrated in CF patients chronically colonized with *Pseudomonas aeruginosa* treated with nebulized amiloride in combination with standard therapy vs. placebo (nebulized saline) control [19,20]. However, other trials have demonstrated improved mucociliary clearance [15,21].

Although more studies exist evaluating the role of amiloride as a mucolytic in CF, amiloride also has in vitro antimicrobial properties [22,23] and antimicrobial synergy exists between amiloride and tobramycin [22–25]. However, the mechanism of action for the synergy remains speculative. Extracellular sodium antagonizes tobramycin-related inhibition of Bcc bacteria proliferation and this effect is reversed by amiloride suggesting that amiloride may act directly through sodium channels on the surface of the bacteria [25]. Additionally, several substituted amine compounds have demonstrated synergy with tobramycin against Bcc isolates, suggesting the amine group in amiloride may be an important moiety in the observed synergy [23]. Interestingly, the synergy between amiloride and tobramycin appears to be more pronounced against *Burkholderia cepacia* complex bacteria than other common CF airway flora such as *P. aeruginosa* and *Stenotrophomonas maltophilia* [24,25] which may explain mixed results in clinical efficacy in trials involving patients infected with *P. aeruginosa*.

A recent case report series provided additional preliminary support of the synergy seen in vitro. Middleton, Kidd, and Williams studied the in vivo antimicrobial effect of amiloride (5 mL of 1 mM solution nebulized 3 times a day) and tobramycin (80 mg in 2 mL nebulized 3 times a day, immediately following amiloride inhalation) for up to 6 months in 4 CF patients colonized with Bcc bacteria (genomovars III, VII, and unknown) [17]. Eradication of the bacteria from the airways was noted in 3 of the 4 patients studied, and reportedly persisted for at least 2 years. This result is remarkable because it appears to be rare for Bcc bacteria to be eliminated from the airway in CF once infection is established. In another study, for example, only 6% of patients with one respiratory culture positive for *B. cenocepacia* had subsequent negative cultures [26]. Middleton’s group defined baseline colonization as more than 3 positive cultures, thus it might be expected that their rates of spontaneous resolution would be even lower, making the report of eradication in 3 out of 4 patients more dramatic. All isolates in the Middleton study were tobramycin resistant, which lends credence to the in vitro synergy data noted above.

Given the inherently ill and medically unstable lung disease in patients chronically infected with *B. dolosa*, as well as the established safety profile of nebulized amiloride and nebulized tobramycin in the CF patient population, we elected to undertake an open label trial of concurrent administration of amiloride for inhalation (ASI) and TIS. Our hypothesis was that the use of these combined therapies could eradicate *B. dolosa* infection.

2. Materials and methods

We conducted a 24-week, prospective open label trial of continuous treatment with ASI and TIS in CF patients infected with *B. dolosa*. The trial was approved by Children’s Hospital Boston’s (CHB) Internal Review Board Committee on Clinical Investigation and informed consent was obtained. An independent Data and Safety Committee monitored the study.

Eligibility required two *B. dolosa* respiratory cultures separated by ≥2 weeks, with the second culture within 30 days of enrollment. Patients were ineligible if they: had known sensitivity to amiloride or TIS; used other investigational drugs within 4 weeks; or had an unstable clinical diagnosis that, in the Principal Investigator’s opinion, compromised patient safety.

The dose of TIS, 300 mg twice daily, is that commonly used clinically in the United States. The amiloride dose of 4.5 mg in 4.5 mL (3.3 mM solution) via nebulization four times daily was chosen to provide the maximal dose for which there was efficacy and stability data. This dose and formulation was found to meet appearance (no precipitate) and chemical purity/stability guidelines (<1% w/w of related compound impurities by HPLC) when stored at room temperature in light-protected vials for 3 months (reference: Chemistry, Manufacturing, and Control Section; Serial # 006; August 26, 1991). The dosing
proposed in this study thus differs from that used by Middleton et al. who administered amiloride (5 mL of a 1 mM solution) and tobramycin (80 mg) three times daily for 6 months. The higher doses proposed in this study were thought to potentially increase the likelihood of efficacy. We also chose a length of therapy consistent with the greatest prior length of therapy resulting in eradication. Adherence was monitored by counting empty amiloride and TIS vials and consistently demonstrated good compliance. Patients completed study visits on days 7(±1), 28(±3), 84(±7), 168(±7) and 182(±7).

Primary endpoint: eradication of B. dolosa, defined as 3 consecutive negative respiratory cultures. Respiratory cultures were obtained at each visit and included sputum cultures or oropharyngeal cultures when sputum was unavailable. Following a negative culture, additional cultures were to be obtained after day 182, no less than 14 days apart, until three negative cultures or a positive culture was obtained.

Secondary endpoints: study visits included a physical exam, pulmonary function tests, sputum cultures and additional laboratory testing. These evaluations were completed at the screening visit and days 7(±1), 28(±3) 84(±7), 168(±7) and 182(±7). Spirometry was performed in compliance with ATS guidelines on the Morgan Scientific ScreenStar Spriometer using Knudsen reference values. Blood work included: hematology (hematocrit, hemoglobin, RBC count, WBC count and differential, platelets); chemistry (sodium, potassium, chloride, bicarbonate, total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), LDH, BUN, creatinine, uric acid, calcium, phosphate, total protein); serum inflammatory markers (total IgG and C-reactive protein); tobramycin serum level. Urine was sent for total protein; serum inflammatory markers (total IgG and C-reactive protein); tobramycin serum level. Urine was sent for urinalysis (color, specific gravity, pH, protein, glucose, ketones). Urine pregnancy test was done at the screening visit for subjects of childbearing potential. Audiology was performed at baseline and the end of the study using conventional pure tone audiometry looking specifically for sensorineural hearing loss. Quality of life was assessed by age appropriate Cystic Fibrosis Questionnaire-Revised (CFQ-R) [27] on day 7(±1), 84(±7), and 182(±7). Spirometry was performed in compliance with ATS guidelines on the Morgan Scientific ScreenStar Spriometer using Knudsen reference values. Blood work included: hematology (hematocrit, hemoglobin, RBC count, WBC count and differential, platelets); chemistry (sodium, potassium, chloride, bicarbonate, total bilirubin, alkaline phosphatase, ALT (SGPT), AST (SGOT), LDH, BUN, creatinine, uric acid, calcium, phosphate, total protein); serum inflammatory markers (total IgG and C-reactive protein); tobramycin serum level. Urine was sent for urinalysis (color, specific gravity, pH, protein, glucose, ketones). Urine pregnancy test was done at the screening visit for subjects of childbearing potential. Audiology was performed at baseline and the end of the study using conventional pure tone audiometry looking specifically for sensorineural hearing loss. Quality of life was assessed by age appropriate Cystic Fibrosis Questionnaire-Revised (CFQ-R) [27] on day 7(±1), 84(±7), and 182(±7). Spirometry was performed in compliance with ATS guidelines on the Morgan Scientific ScreenStar Spriometer using Knudsen reference values.

Table 1 shows baseline patient characteristics for enrolled study patients, non-study patients who did not receive the study treatment (“non-amiloride”) and the three patients who received study treatment through compassionate use protocols. Compared with subjects who did not receive the study treatment, the study cohort was generally younger and had a longer interval since first infection with B. dolosa.

3. Results

Table 1 shows baseline patient characteristics for enrolled study patients, non-study patients who did not receive the study treatment (“non-amiloride”) and the three patients who received study treatment through compassionate use protocols. Compared with subjects who did not receive the study treatment, the study cohort was generally younger and had a longer interval since first infection with B. dolosa.

Table 1: Baseline characteristics. Data are expressed as mean (±SD) or number (%).

<table>
<thead>
<tr>
<th>Baseline characteristic</th>
<th>Amiloride (N=12)</th>
<th>Non-amiloride (N=10)</th>
<th>Compassionate use (N=3)</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>19.1 (±4.9)</td>
<td>29.9 (±11.4)</td>
<td>22.7 (±0.3)</td>
<td>.008</td>
</tr>
<tr>
<td>Male gender</td>
<td>9 (75%)</td>
<td>6 (60%)</td>
<td>1 (33%)</td>
<td>.65</td>
</tr>
<tr>
<td>Body mass index</td>
<td>20.9 (±2.8)</td>
<td>21.9 (±2.3)</td>
<td>20.0 (±3.3)</td>
<td>.37</td>
</tr>
<tr>
<td>Pancreatic insufficiency</td>
<td>12 (100%)</td>
<td>10 (100%)</td>
<td>3 (100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>FEV1, % predicted b</td>
<td>67.0 (±15.4)</td>
<td>57.1 (±19.3)</td>
<td>50.0 (±8.0)</td>
<td>.20</td>
</tr>
<tr>
<td>Months since first positive B. dolosa culture</td>
<td>68.3 (±25.8)</td>
<td>45.0 (±13.9)</td>
<td>40.1 (±4.7)</td>
<td>.02</td>
</tr>
</tbody>
</table>

* P-values compare amiloride vs. non-amiloride cohorts.

b FEV1 at the time of screening in study patients and the best FEV1 over the first 3 months of the study enrollment period in those patients who did not participate.
All study subjects had respiratory cultures positive for *B. dolosa* at baseline, as required for eligibility in the study. One subject had no repeat culture during the study and a second subject had only one because he dropped out of the trial. 10 of 12 subjects had ≥ 3 culture results. All follow-up cultures were also positive. Therefore, no patient met the primary endpoint for eradication of *B. dolosa* from their airways.

We also analyzed quantitative respiratory cultures of *B. dolosa*. To maximize the sample size for this analysis, we used data from either the Week 24 or Week 26 visits as the “end of treatment” value, and averaged the two values if both were available (Table 2). The number of organisms per mL at both baseline and end of treatment was approximately 7 logs (10,000,000) and there was no significant change from baseline to end of treatment (n=5 subjects with data at both time points, paired t-test p = .63). There was also no trend over time evident when on-treatment time points (n=6 at 4 weeks; n=6 at 12 weeks) were considered.

Not including normal flora, bacterial organisms other than *B. dolosa* were present in sputum cultures from 3 of 12 (25%) patients at baseline and the prevalence of other organisms remained stable at 20–45% throughout the study.

Two inflammatory markers (IgG and C reactive protein), were analyzed similarly to quantitative cultures. There was no suggestion of a change from baseline for these outcomes. Table 2 summarizes the baseline and end of treatment results.

There were no significant differences in quality of life scale subscale scores between baseline and end of treatment. However, the treatment burden subscale was marginally significant (p = .07, Table 2) with a mean decline of 12.5 points on a 0–100 scale. The change from baseline to 24 weeks was slightly larger (16.7 point decline, p = .05). In the study cohort, with no appreciable change in the rate from before to after baseline in either cohort. P-values for comparing rates of change between cohorts and for changes in rates of decline from before to after baseline were all >.15. In the study cohort, the estimated rates of decline are 6.6 and 6.4 percentage points per year before and after baseline, respectively.

As shown in Fig. 1, the rate of change in percent predicted FEV\(_1\) is similar for study patients and for the non-amiloride cohort, with no appreciable change in the rate from before to after baseline in either cohort. P-values for comparing rates of change between cohorts and for changes in rates of decline from before to after baseline were all >.15. In the study cohort, the estimated rates of decline are 6.6 and 6.4 percentage points per year before and after baseline, respectively.

Recognizing that the slopes of the four line segments in Fig. 1 are similar, we fit a simplified model which assumes equal slopes in order to estimate an overall rate of decline in lung function. The estimated rate of decline is 5.1 percentage points per year (95% CI 3.1 to 7.1). For comparison, our original report documented a decline of 7.1 points per year (95% CI 4.5 to 9.6) during the 18 months following initial colonization with *B. dolosa* [9].

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>End of treatment a</th>
<th>N b</th>
<th>P c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative <em>B. dolosa</em> culture (log(_10) organisms/mL)</td>
<td>7 7.01 (±0.85)</td>
<td>7 6.89 (±0.70)</td>
<td>5</td>
<td>.63</td>
</tr>
<tr>
<td>IgG (mg/dL)</td>
<td>12 1511.2 (±431.0)</td>
<td>10 1560.8 (±506.7)</td>
<td>10</td>
<td>.99</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>12 2.01 (±3.92)</td>
<td>10 4.43 (±8.85)</td>
<td>10</td>
<td>.46</td>
</tr>
<tr>
<td>Treatment burden d</td>
<td>8 54.2 (±9.3)</td>
<td>8 41.7 (±16.5)</td>
<td>8</td>
<td>.07</td>
</tr>
</tbody>
</table>

a Week 24 or Week 26 (averaged if data available at both time points).

b Number of patients with data at both baseline and end of treatment.

c Paired t-test.

d Subscale of CFQ-R.

![Fig. 1. Mean percent predicted FEV\(_1\) before and after start of treatment in patients enrolled in the trial (or corresponding “baseline” date in non-amiloride cohort). Error bars are ±1 SE. Estimates and SEs are based on a random effects model [9].](image-url)
5. Discussion

In this limited cohort of CF patients we were not able to demonstrate eradication of *B. dolosa* from airway cultures during concurrent therapy with inhaled amiloride and tobramycin. We also did not demonstrate improvement in any other health outcomes after 24 weeks of treatment.

It is notable that patients who enrolled in this trial were generally younger as compared to non-participants, and they tended to have been infected with *B. dolosa* for a longer period of time. There was also a trend toward better baseline lung function in the amiloride group though this did not reach statistical significance. The decline in FEV₁ over time during the study period was very similar in study participants and non-participants.

Treatment burden associated with our study was cited as the reason two of the 12 subjects withdrew. Similarly, the CFQ-R subscale score associated with treatment burden suggested that the trial contributed to the treatment burden, though this measure also did not reach significance.

Our results are in contrast to the previous case report series from Middleton et al. [17] in which 3 out of 4 patients treated with nebulized tobramycin and amiloride experienced eradication of a *Burkholderia cepacia* complex organism. However, it is notable that none of the patients in that series was known to be infected with *B. dolosa*. *B. dolosa* appears to be a particularly drug resistant bacteria among species of Bcc organisms. The dosage of medications also differed in our trial as compared to this earlier report. The Middleton study used tobramycin 80 mg/2 mL TID while we administered TIS 300 mg (60 mg/ml×5 mL) BID. Amiloride in the prior study was given at a dose of 5 mL of a 1 mM solution TID while we provided patients with 4.5 mL of a 3.3 mM solution QID. Thus our total dose of each medication exceeded that administered in the Middleton report. While these modifications in dosage and timing seemed reasonable it is possible that we would have had greater therapeutic efficacy if we had replicated the Middleton trial more exactly.

In a more recent report of clinical experience with TIS and amiloride (1.5 mg in 5 mL 0.45% sodium chloride) twice daily in the treatment of *B. cepacia*, eradication was achieved in one of 7 patients [28]. This patient cultured *B. cepacia*, genomovar IV for less than two months. The remaining six patients who did not eradicate *B. cepacia* were infected for longer periods of time. It is notable that in Middleton’s study the period of chronic infection prior to the eradication attempt was also relatively brief while our patients were all infected for years prior to amiloride/TIS therapy. Thus length of colonization may be a key factor influencing the prospect of eradication and it may be reasonable to expect reduced eradication in our population compared to these reports, independent of *Burkholderia* genovar status.

Use of inhaled as opposed to systemic antibiotics may also limit efficacy, since drug may not reach affected areas of lung. However, orally administered amiloride at clinically acceptable doses does not result in measurable sputum levels of drug [19], and evidence suggests that absorption of amiloride from the airway is an active process, with an airway half-life of only 20 min [7]. Thus systemic administration of amiloride is not a realistic option. And while parenteral administration of tobramycin is common, the toxicity of this associated with long term administration makes it prohibitive. Of note, the three patients who received inhaled amiloride outside of the protocol on an individualized basis were all on intravenous aminoglycosides and *B. dolosa* was also not eradicated in these patients.

Our initial report documented a decline in FEV₁ of 7.1% per year following *B. dolosa* infection as compared to an annual average FEV₁ decline of 2.3% per year prior to infection [9]. In the current analysis, we estimate a decline of 5.1% per year after the start of amiloride (or after a similar baseline date for the non-study patients), suggesting that patients chronically infected with *B. dolosa* continue to suffer rapidly advancing disease.

Finally, our study was fundamentally limited in several important ways. First, we have an inherently small patient population, which limits the power of our results. Additionally the characteristics of nebulized ASI produced by the nebulizer were not studied, thus inadequate airway deposition is a potential explanation for lack of efficacy. Furthermore this study was designed without a control group. The lack of a control group was an effort to provide a safe and potentially beneficial treatment option to a group of patients with very few therapeutic choices who have a high potential for rapid decline in lung function and death. The cohort of patients who elected not to participate is self-selected and any comparison with the study group is subject to bias.

Future evaluation should include a randomized prospective trial in patients with less severe lung disease, less well established airway infection, and other *B. cepacia* genovars.

References

[10] Ramsey BW, Pepe MS, Quan JM, Otto KL, Montgomery AB, Williams-Warren J, et al. Intermittent administration of inhaled tobramycin...


