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Original Article

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.

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,"

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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 speciated [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, FEV_1, 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