In Vitro Efficacy of High-Dose Tobramycin against Burkholderia cepacia Complex and Stenotrophomonas maltophilia Isolates from Cystic Fibrosis Patients

Anina Ratjen,a Yvonne Yau,b Jillian Wettlaufer,b Larissa Matukas,c James E. A. Zlosnik,d David P. Speert,d John J. LiPuma,e Elizabeth Tullis,f Valerie Watersa

Division of Infectious Diseases, Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; Division of Microbiology, Department of Pediatric Laboratory Medicine, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; Division of Laboratory Medicine, St. Michael’s Hospital, University of Toronto, Toronto, Ontario, Canada; Division of Infectious Diseases, Department of Pediatrics, Centre for Understanding and Preventing Infection in Children, Child and Family Research Institute, University of British Columbia, Vancouver, British Columbia, Canada; Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan, USA; Division of Respirology and Keenan Research Centre of Li Ka Shing Knowledge Institute, Department of Medicine, St. Michael’s Hospital, University of Toronto, Toronto, Ontario, Canada

Burkholderia cepacia complex and Stenotrophomonas maltophilia infections are associated with poor clinical outcomes in persons with cystic fibrosis (CF). The MIC50 based on planktonic growth and the biofilm concentration at which 50% of the isolates tested are inhibited (BIC50) of tobramycin were measured for 180 B. cepacia complex and 101 S. maltophilia CF isolates and were 100 μg/ml for both species. New inhalation devices that deliver high tobramycin levels to the lung may be able to exceed these MICs.

As individuals with cystic fibrosis (CF) age, they are increasingly infected in their lungs with multidrug-resistant Gram-negative organisms such as Burkholderia cepacia complex and Stenotrophomonas maltophilia, which are associated with poor clinical outcomes (1–5). Treatment of these infections is difficult (6–9) due to their numerous mechanisms of antimicrobial resistance, including efflux pumps, chromosomally encoded β-lactamases, decreased outer membrane permeability, intracellular survival, and biofilm formation (10–12). However, newer inhalational antibiotic therapies have the ability to deliver very high concentrations of drug to the lung, which may be able to overcome some of these mechanisms. One of the new inhalational antibiotics available is tobramycin inhalation powder (TIP), delivered by the Podhaler device, which can target this drug to the lung, which may be able to overwhelm the efficient efflux pumps known to be present in B. cepacia complex and S. maltophilia (14–16).

In order to determine whether the known pulmonary concentrations of inhaled high-dose tobramycin powder can overcome these inhibitory concentrations, the aim of this study was to measure the inhibitory concentrations of tobramycin for a large collection of B. cepacia complex and S. maltophilia isolates, grown planktonically and in a biofilm, from pediatric and adult CF patients.

B. cepacia complex isolates (n = 180) were prospectively collected from sputum samples from CF patients from four study sites, The Hospital for Sick Children (n = 10), St. Michael’s Hospital (n = 36), the Cystic Fibrosis Foundation Burkholderia cepacia Research Repository at the University of Michigan (n = 16), and the Canadian Burkholderia cepacia Complex Research and Referral Repository at the University of British Columbia, Vancouver (n = 118). S. maltophilia isolates (n = 101) were obtained from pediatric CF patients at The Hospital for Sick Children in Toronto (n = 67) and from adult CF patients (n = 34) at St. Michael’s Hospital in Toronto. All the isolates used in this study were independent strains (1 isolate/patient). Antimicrobial susceptibility testing was performed on isolates grown planktonically by broth microdilution using Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Antimicrobial susceptibility testing was also performed on isolates grown as a biofilm using a modified form of the Calgary biofilm technique (18, 19). The antibiotic panels contained tobramycin at concentrations of 0, 10, 100, 200, 400, 800, 1,600, and 3,200 μg/ml. The MIC based on planktonic growth and the biofilm inhibitory concentration (BIC) of tobramycin for each isolate were determined by visually assessing the turbidity of each well (see Supplementary Methods in the supplemental material for more detail).

The tobramycin MIC50 and BIC50 (the BIC at which 50% of isolates were susceptible) were 100 μg/ml for a large collection of CF B. cepacia complex isolates (Table 1), largely consistent across most species of the B. cepacia complex. Burkholderia vietnamiensis, previously shown to be more susceptible to aminoglycosides (15), had an MIC50 of 10 μg/ml. Burkholderia dolosa isolates, responsible for an outbreak at a U.S. CF care center (20), demonstrated a higher MIC50 of 200 μg/ml. Similarly, the tobramycin MIC50 and BIC50 for S. maltophilia were 100 μg/ml. The distrib-
tion of the tobramycin MICs and BICs for *B. cepacia* complex and *S. maltophilia* is shown in Fig. 1. A significant proportion of *B. cepacia* complex isolates had tobramycin MICs ($n/11005 \leq 10 \mu g/ml$, 11%) and BICs ($n/11005 \leq 10 \mu g/ml$, 18%) that were $\leq 10 \mu g/ml$, as did *S. maltophilia* isolates, with 34% ($n/11005 = 34/101$) of MICs and 29% of BICs ($n/11005 = 29/101$) that were $\leq 10 \mu g/ml$. Conversely, the MIC$_{90}$ and BIC$_{90}$ for *B. cepacia* complex isolates were 400 $\mu g/ml$ and for *S. maltophilia* isolates were 1,600 $\mu g/ml$ and 3,200 $\mu g/ml$ (Table 1), respectively, suggesting that in these cases, TIP administration may not be capable of exceeding these high inhibitory concentra-

tions.

The correlations between the two methods (planktonic and biofilm) of antimicrobial susceptibility testing was calculated using the Spearman correlation coefficient and were found to be statistically significant for *B. cepacia* complex isolates ($r = 0.5549$, $P < 0.0001$) and for *S. maltophilia* isolates ($r = 0.3638$, $P = 0.0002$), suggesting that tobramycin can function well against organisms grown in a biofilm state, as expected in the CF lung. The agreement between the two methods of antimicrobial susceptibility testing is illustrated in a Bland-Altman plot for *B. cepacia* complex (Fig. 2A) and *S. maltophilia* (Fig. 2B) isolates.

To date, this is the largest in vitro study of a contemporary collection of clinical CF isolates to determine the tobramycin concentrations required to inhibit the planktonic and biofilm growth of *B. cepacia* complex and *S. maltophilia*. Although traditionally considered to be intrinsically resistant to systemically attainable aminoglycoside concentrations based on CLSI breakpoints (17), our data suggest that TIP treatment can achieve a maximal drug concentration ($C_{\text{max}}$)/MIC ratio of up to 20-fold for the majority of *B. cepacia* complex and *S. maltophilia* isolates from CF patients. It is unknown what $C_{\text{max}}$/MIC ratio is required to successfully suppress bacterial growth in the CF lung, but there is a relationship between the $C_{\text{max}}$ and the MIC required to inhibit *Pseudomonas aeruginosa* growth, with higher ratios associated with greater reduction in bacterial density (21).

We also demonstrated that tobramycin inhibitory concentrations were similar regardless of whether the organisms were grown planktonically or as a biofilm, suggesting that at these high levels, tobramycin may be effective in the CF lung environment. Different classes of antimicrobials have various degrees of efficacy against dense slow-growing matrix-enveloped bacterial commu-

### TABLE 1

<table>
<thead>
<tr>
<th>Organism (no. of isolates)</th>
<th>MIC$_{50}$ ($\mu g/ml$)</th>
<th>BIC$_{50}$ ($\mu g/ml$)</th>
<th>MIC$_{90}$ ($\mu g/ml$)</th>
<th>BIC$_{90}$ ($\mu g/ml$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. cepacia</em> complex (180)</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td><em>B. cenocepacia</em> (83)</td>
<td>100</td>
<td>100</td>
<td>800</td>
<td>800</td>
</tr>
<tr>
<td><em>B. multivorans</em> (41)</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td><em>B. stabilis</em> (16)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>400</td>
</tr>
<tr>
<td><em>B. vietnamiensis</em> (19)</td>
<td>10</td>
<td>10</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>B. dolosa</em> (14)</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>400</td>
</tr>
<tr>
<td><em>B. cepacia</em> (7)</td>
<td>100</td>
<td>100</td>
<td>800</td>
<td>400</td>
</tr>
<tr>
<td><em>S. maltophilia</em> (101)</td>
<td>100</td>
<td>100</td>
<td>1,600</td>
<td>3,200</td>
</tr>
</tbody>
</table>

FIG 1 Distribution of tobramycin biofilm inhibitory concentrations (BICs) measured by biofilm antimicrobial susceptibility testing and MICs measured by planktonic antimicrobial susceptibility testing for *Burkholderia cepacia* complex (A) and *Stenotrophomonas maltophilia* (B) cystic fibrosis isolates.

FIG 2 Bland-Altman plots of average inhibitory concentrations versus difference between biofilm inhibitory concentrations (BICs) and MICs for *Burkholderia cepacia* complex (A) and *Stenotrophomonas maltophilia* (B) cystic fibrosis isolates, with points on the x axis ($y = 0$) indicating complete agreement.

712 aac.asm.org

Antimicrobial Agents and Chemotherapy

January 2015 Volume 59 Number 1

Downloaded from http://aac.asm.org on July 5, 2017 by guest
nities based on their ability to penetrate biofilms and their mechanism of action (22). Aztreonam, for example, is not as effective as tobramycin at reducing P. aeruginosa biofilm mass on airway epithelial cells, and tolerance to aztreonam may develop secondary to biofilm exopolysaccharide production (23). In our study, however, high-dose tobramycin overcame mechanisms of biofilm resistance and inhibited bacterial protein synthesis in stationary-phase organisms.

Despite these results, however, in vitro susceptibility testing, whether by the planktonic or biofilm method of growth, does not necessarily predict clinical response in CF patients, and it is unclear whether TIP, which delivers a sputum tobramycin concentration 1.5- to 2-fold higher than TIS, will translate into improved efficacy in the treatment of these infections. Clinical trials of TIP therapy in this patient population are under way to assess this question (Clinical Trials.gov identifier NCT02212587).

In conclusion, TIP administration may deliver pulmonary drug concentrations in excess of what is required to inhibit the majority of B. cepacia complex and S. maltophilia CF isolates, even when grown as a biofilm. This offers a potential therapeutic option to a CF population for whom there is no effective chronic suppressive antimicrobial treatment.

ACKNOWLEDGMENTS

We acknowledge the work of Danuta Kovach and Carlos Costano in the laboratory and all the staff at the microbiology laboratories involved in this study. We also thank CF Canada for funding the Canadian Burkholderia cepacia Complex Research and Referral Repository and the U.S. CF Research Repository.

This study was funded through an unrestricted investigator-initiated grant from Novartis Pharmaceutical Canada, Inc. A.R. was supported by a grant from CF Canada. Neither Novartis nor CF Canada had any involvement in the study design, interpretation of the data, writing of the manuscript, or the decision to submit the manuscript for publication.

E.T. has received consultancy and speaking fees from Novartis. All authors declare no conflict of interests.

REFERENCES


