Elucidating the Pharmacokinetics/Pharmacodynamics of Aerosolized Colistin against Multidrug-Resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* in a Mouse Lung Infection Model

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**ABSTRACT** The pharmacokinetics/pharmacodynamics (PK/PD) of aerosolized colistin was investigated against *Acinetobacter baumannii* and *Klebsiella pneumoniae* over 24 h in a neutropenic mouse lung infection model. Dose fractionation studies were performed over 2.64 to 23.8 mg/kg/day, and the data were fitted to a sigmoid inhibitory model. The area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC) in the epithelial lining fluid was the most predictive PK/PD index for aerosolized colistin against both pathogens. Our study provides important pharmacological information for optimizing aerosolized colistin.

**KEYWORDS** polymyxin, respiratory tract infections, pulmonary administration, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, Gram-negative bacteria

Colistin is a poly peptide antibiotic that has been increasingly used as a last-line therapy for pulmonary infections caused by multidrug-resistant (MDR) Gram-negative bacteria (1–6). Inhalation of colistin has been commonly used in the treatment of pulmonary infections caused by *Pseudomonas aeruginosa* in people with cystic fibrosis (3, 4). However, the pharmacokinetics/pharmacodynamics (PK/PD) of aerosolized colistin has not been examined against *Acinetobacter baumannii* or *Klebsiella pneumoniae* (7, 8). Our present study is the first to investigate the PK/PD of aerosolized colistin against both pathogens.

All animal experiments were approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Female Swiss mice (8 to 10 weeks of age) were obtained from Monash Animal Research Platform (Clayton, Victoria, Australia) or Australian Resources Centre (Perth, Western Australia, Australia). A neutropenic mouse lung infection model was employed as described previously (7, 8) with *A. baumannii* (ATCC 19606, 248-01-C.248, and N-16870.213; MICs, 2.64 to 23.8 mg/kg/day) were fitted to a sigmoid inhibitory model. The area under the concentration-time curve over 24 h in the steady state divided by the MIC (AUC/MIC) in the epithelial lining fluid was the most predictive PK/PD index for aerosolized colistin against both pathogens. Our study provides important pharmacological information for optimizing aerosolized colistin.

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Colistin is a polypeptide antibiotic that has been increasingly used as a last-line therapy for pulmonary infections caused by multidrug-resistant (MDR) Gram-negative bacteria (1–6). Inhalation of colistin has been commonly used in the treatment of pulmonary infections caused by *Pseudomonas aeruginosa* in people with cystic fibrosis (3, 4). However, the pharmacokinetics/pharmacodynamics (PK/PD) of aerosolized colistin has not been examined against *Acinetobacter baumannii* or *Klebsiella pneumoniae* (7, 8). Our present study is the first to investigate the PK/PD of aerosolized colistin against both pathogens.

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For other strains (107 CFU/lung). This higher bacterial inoculum of \( n \) was observed for bacterial killing and regrowth following treatment with aerosolized colistin (Fig. 1). Although the colistin MICs were different among the isolates, they were increased with increasing inflammation (10). Even though high colistin exposure is needed to establish a reliable lung infection over 24 h. Interstrain variability was observed for bacterial killing and regrowth following treatment with aerosolized colistin is shown in Fig. 1. Minor differences in bacterial loads were observed between the control and the treated groups (2.64 and 5.28 mg base/kg) for \( A. \) baumannii and \( K. \) pneumoniae (Fig. 1). Even though regrowth was observed at 24 h across all strains (except \( A. \) baumannii ATCCC 19606), bacterial killing by colistin was observed against \( A. \) baumannii 248-01-C.248 and \( A. \) baumannii N-16870.213 within 6 h of the pulmonary administration, while within 1 h against all three strains of \( K. \) pneumoniae. For \( A. \) baumannii ATCCC 19606, both killing and regrowth were not evident over 24 h. One potential reason for the lack of bacterial killing against \( A. \) baumannii ATCCC 19606 was probably due to the polymyxin inoculum effect; a much higher inoculum (\( \sim 10^6 \) CFU/lung) was used for \( A. \) baumannii ATCCC 19606 as opposed to that for other strains (\( \sim 10^7 \) CFU/lung). This higher bacterial inoculum of \( A. \) baumannii ATCCC 19606 was needed to establish a reliable lung infection over 24 h. Interstrain variability was observed for bacterial killing and regrowth following treatment with aerosolized colistin (Fig. 1). Although the colistin MICs were different among the isolates, they were not able to fully predict the efficacy of aerosolized colistin against each isolate. For example, the killing effect against \( K. \) pneumoniae KP1 (MIC, 0.25 mg/liter) was similar to or even worse than that against other strains with higher MICs (0.5 to 1 mg/liter) (Fig. 1). Surprisingly, doubling the dose only marginally improved the killing efficacy, and the regrowth was evident (Fig. 1). A possible explanation is that some bacterial cells not directly exposed to aerosolized colistin in the lungs were able to proliferate (4, 10). This was evidenced in a piglet study, wherein after inhalation, the drug penetration decreased with increasing inflammation (10). Even though high colistin exposure is achieved in the epithelial lining fluid (ELF) after inhalation (8), the current single-dose PD profiles show that it is still not possible to completely eradicate bacteria in the lungs (Fig. 1). Regrowth was evident with all isolates (except \( A. \) baumannii ATCCC 19606), even though the ELF concentration remained well above the MIC over the 12-h sampling period (8). This regrowth could also be due to adaptive resistance, whereby colistin resistance was developed by \( A. \) baumannii through the loss or modification of lipopolysaccharides (LPS) (11, 12), while in \( K. \) pneumoniae, through the modification of LPS or the upregulation of capsule polysaccharides (13, 14). The exact mechanisms for the regrowth after aerosolized colistin remain unknown, and further studies are warranted.

To optimize the PK/PD of aerosolized colistin, a dose fractionation study was conducted to delineate the PK/PD indices. As mice were not able to tolerate \( >7.92 \) mg base/kg aerosolized colistin in a single dose or a cumulative dose of \( >23.8 \) mg base/kg, dose fractionation studies were performed over 2.64 to 23.8 mg/kg/day, including once-daily 2.64, 5.28, and 7.92 mg base/kg, twice-daily (12 hourly) 5.28 and 7.92 mg base/kg, and thrice-daily (8 hourly) 2.64, 5.28, and 7.92 mg base/kg doses (\( n = 3 \) or more for each group). Each pulmonary administration was performed under light anesthesia. At 0 or 24 h after the initiation of the first inhaled colistin dose, mice were...
FIG 1 In vivo time-kill kinetics of aerosolized colistin (2.64 and 5.28 mg base/kg) in a neutropenic mouse lung infection model. (A) *A. baumannii* ATCC 19606, (B) *A. baumannii* 248-01-C.248, (C) *A. baumannii* N16870.213, (D) *K. pneumoniae* ATCC BAA 2146, (E) *K. pneumoniae* 17, and (F) *K. pneumoniae* KP1. The limit of detection is 164 CFU/lung. Data points represent means ± standard deviations (n = 3 or more per time point).
humanely killed and the lungs were harvested for viable counting. Subsequently, the data were fitted to a sigmoid inhibitory model (7). For *A. baumannii* and *K. pneumoniae*, the ratios of the area under the colistin concentration-time curve over 24 h in the steady state to the MIC in ELF (AUCELF/MIC) and in plasma (fAUCplasma/MIC) were the most predictive PK/PD indices for the efficacy of aerosolized colistin in neutropenic mice (Table 1 and Fig. 2 and 3; see also Fig. S1 to S4 in the supplemental material); a much better correlation was observed than with the maximum concentration of colistin divided by the MIC (Cmax/MIC) and the cumulative percentage of time that the colistin concentration exceeds the MIC at steady-state PK (%TAU11022MIC) in both ELF and plasma (Table 1 and Fig. 2 and 3). Unlike the previous observations with *P. aeruginosa* (8), a higher degree of interstrain variability in AUCELF/MIC and fAUCplasma/MIC was observed with *K. pneumoniae* (Table 2). Even with the highest tolerable aerosolized colistin dose, it was not possible to achieve /H9253 2-log10 kill at 24 h against two isolates of *K. pneumoniae* (Table 2). A lower efficacy (i.e., inability to achieve a 2-log10 kill) for aerosolized colistin against *K. pneumoniae* than against *A. baumannii* and *P. aeruginosa* might be related to the capsule which has been reported as a mechanism of polymyxin resistance in *K. pneumoniae* but not the other two species (14–16). A lower therapeutic response against *K. pneumoniae* was also noted for systemic polymyxin B in a neutropenic mouse thigh infection model (17). The exact mechanism for the differences in the in vivo efficacy of colistin against different bacterial species requires further investigations.

Pulmonary administration of colistin was much more effective than systemic delivery for the treatment of respiratory tract infections caused by Gram-negative pathogens (7, 8). To achieve the antibacterial stasis against *A. baumannii 248-01-C.248*, the fAUCplasma/MIC required with subcutaneous administration (11.6) (7) is 14-fold higher than that required with pulmonary administration (0.86) (Table 2). This is consistent with the finding from a study by Chiang et al. (18), in which bacterial load, survival rate, wet lung/body weight ratio, and histopathological changes were examined in neutropenic mice with lung infections of *A. baumannii*, where the pulmonary administration of colistin (75,000 IU/kg thrice daily) was more efficacious than intraperitoneal administration (150,000 IU/kg thrice daily). Likewise, Bowers et al. reported a lack of responsiveness for intraperitoneally administered polymyxin B (10 mg/kg) for treating lung

### Table 1 PK/PD model parameters for AUC/MIC of aerosolized colistin in the ELF and plasma against *A. baumannii* and *K. pneumoniae*

<table>
<thead>
<tr>
<th>Strain</th>
<th>AUCELF/MIC</th>
<th>fAUCplasma/MIC</th>
<th>Emax</th>
<th>E0</th>
<th>E50</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. baumannii</em></td>
<td>248-01-C.248</td>
<td>2.51 (15)</td>
<td>7.70 (3.0)</td>
<td>628 (8.1)</td>
<td>10 (65)</td>
<td>0.61</td>
</tr>
<tr>
<td>ATCC 19606</td>
<td>5.80 (22)</td>
<td>7.96 (5.9)</td>
<td>1419 (13)</td>
<td>5.0 (55)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>17N-16870</td>
<td>2.65 (13)</td>
<td>7.83 (2.7)</td>
<td>1.94 (9.3)</td>
<td>10 (53)</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>ATCC BAA 2146</td>
<td>2.84 (31)</td>
<td>8.97 (4.6)</td>
<td>421 (33)</td>
<td>2.12 (72)</td>
<td>0.64</td>
</tr>
<tr>
<td>17</td>
<td>3.22 (12)</td>
<td>8.45 (3.2)</td>
<td>554 (5.3)</td>
<td>10 (59)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>KP1</td>
<td>3.49 (22)</td>
<td>9.90 (4.55)</td>
<td>1581 (22)</td>
<td>2.38 (49)</td>
<td>0.68</td>
<td></td>
</tr>
</tbody>
</table>

*Emax* is the maximal effect.

*E0* is the effect without colistin treatment.

*EI50* is the value of AUC/MIC required to achieve 50% of *Emax*.

\(r^2\) is the Hill coefficient.

*Data in parentheses are the percentage relative standard error (%RSE).*
infections caused by *A. baumannii* in neutropenic mice (19). The superior efficacy of aerosolized colistin as opposed to parenteral administration against lung infections was also reported with *K. pneumoniae*. In a neutropenic mouse lung infection model, the subcutaneous administration of colistin was not able to achieve bacteriostasis against...
In the present study, mild killing against *K. pneumoniae* was evident with aerosolized colistin and bacteriostasis was achieved against all three isolates (Table 2). The superior efficacy of aerosolized colistin was not unexpected due to the high exposure in the ELF, and recent PK studies in animals (8, 10, 20–25) and patients.

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have also demonstrated this. Well-designed clinical studies are needed to evaluate nebulized colistin for the management of life-threatening respiratory tract infections caused by MDR Gram-negative pathogens.

Against *A. baumannii*, *K. pneumoniae* (except for *K. pneumoniae* KP1), and *P. aeruginosa* (8), the AUCELF/MIC targets for bacteriostasis generally fell within the same order of magnitude (Table 2). The high AUCELF/MIC targets (Table 2) were most likely due to the heterogeneous distribution of the colistin aerosol in the respiratory tracts (4) and the binding of colistin to mucin (26) and surfactants in the lungs (27). The calculated PK/PD targets in the ELF can be employed in the Monte Carlo simulation to derive the probability of target attainments to optimize the inhalational dosage regimens of CMS in the clinic (28, 29). However, due to the lack of a robust population PK model in humans after inhalation, it is not possible to perform simulations to compare the AUCELF/MIC targets from the current animal studies with the AUCELF/MIC targets achieved in critically ill patients receiving aerosolized CMS (4, 30). The calculated AUC/MIC targets must be used with a recognition of the potential limitations associated with animal PK/PD studies. First, the plasma protein binding (7, 31) and the abundance of potential transporters (e.g., PEPT2) in lung epithelial cells are species dependent (32–34). Second, the Penn-Century MicroSprayer may have a lower delivery efficiency, as it generates aerosol droplets with an approximately 10-μm diameter, which is larger than those generated by clinically available nebulizers (<10 μm in diameter depending on the types of nebulizer) (22).

To the best of our knowledge, this is the first study to reveal that AUCELF/MIC is the most predictive PK/PD index for the in vivo efficacy of aerosolized colistin against *A. baumannii* and *K. pneumoniae*. Our preclinical results provide important pharmacological information for future clinical studies to optimize the dosage regimens of inhaled colistin for treating respiratory tract infections caused by MDR *A. baumannii* and *K. pneumoniae*.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01790-17.

**SUPPLEMENTAL FILE 1,** PDF file, 0.7 MB.

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REFERENCES


