A Proof-of-Concept Study of the Efficacy of Systemically Administered Polymyxins in Mouse Burn Wound Infection Caused by Multidrug-Resistant Gram-Negative Pathogens

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ABSTRACT The efficacy of subcutaneously administered polymyxins against burn wound infections caused by Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae was examined in a murine infection model. Subcutaneously administered colistin and polymyxin B (30 mg/kg thrice daily) achieved a ≥2-log_{10} reduction in the bacterial load for P. aeruginosa and A. baumannii infections, whereas wound infections by K. pneumoniae were less responsive (<1-log_{10} reduction). This study highlights the potential therapeutic benefits of parenteral polymyxins for treating burn wound infections.

KEYWORDS polymyxins, thermal injury, multidrug resistance, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae

Thermal injury is a major global public health problem that is associated with high mortality and morbidity rates (1–4). Approximately 50% of deaths resulting from thermal injury are related to secondary bacterial infections caused by Gram-negative pathogens, such as Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumoniae (2, 5–7). Treatment of burn wound infection is often complicated due to the rapid development of resistance. Over the last decade, clinicians have encountered infections caused by multidrug-resistant (MDR) isolates of P. aeruginosa, A. baumannii, and K. pneumoniae that are resistant to almost all available antibiotics (8, 9). A class of “old” antibiotics, polymyxins, has undergone clinical resurgence as a last resort (10, 11).

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 (age, 8 to 10 weeks; weight, 25 to 30 g) were obtained from Monash Animal Services (Clayton, Victoria, Australia). A neutropenic mouse burn wound infection model was established as previously described with P. aeruginosa (PAO1 and 19056muc; colistin and polymyxin B MIC, 1 and 0.5 mg/liter, respectively), A. baumannii (ATCC 19606 and 248-01-C.248; colistin and polymyxin B MIC, 1 mg/liter for both isolates), and K. pneumoniae (ATCC BAA 2146 and KP1; colistin and polymyxin B MIC, 0.5 mg/liter for...
both isolates) (13, 14). MICs were determined by broth microdilution (15). Mice were rendered neutropenic by subcutaneous injection of two doses of cyclophosphamide on days −4 and −1 (16). While under anesthesia with gaseous isoflurane, a third-degree thermal injury was induced by pressing a heated block (92°C to 95°C; diameter, 1.4 cm) to the shaved back of a neutropenic mouse for ~5 seconds. Mice were then immediately challenged by an intradermal injection of 50 μl of an early logarithmic-phase bacterial suspension (~10^6 CFU) directly into the wound. Colistin sulfate (lot 081M1526V; ≥15,000 IU/mg; Sigma-Aldrich, MO, USA) and polymyxin B sulfate (lot BCBF8382V; ≥6,000 IU/mg; Sigma-Aldrich) were subcutaneously administered at 2 h postinoculation. Colistin and polymyxin B 30 mg/kg thrice daily (every 8 hours; maximum daily dose, 90 mg/kg) were subcutaneously injected. Thermally injured mice treated with sterile saline were included as growth controls. Mice were humanely killed (n = 3 or more for each group), and the bacterial load in the burn wound tissue was measured at 0 and 24 h after the start of treatment. The entire burn wound tissue was aseptically collected and individually processed as previously described (16). The bacterial load of each wound tissue sample was expressed as log_{10} CFU per wound tissue sample. The lower limit of the colony count was 170 CFU per wound (equivalent to 1 colony per plate). Statistical analysis was performed using Student’s t test (Graph Pad Prism version 7.00, San Diego, CA, USA).

The dosage regimens of the polymyxins used were chosen based on their plasma pharmacokinetics (PK) in critically ill patients and animal scaling (17–19). With the currently recommended dosage regimens, parenteral administration of colistin (as colistin methanesulfonate [CMS]) and polymyxin B results in average steady-state unbound concentrations (f_{CSS,avg}) of 1 to 3 mg/liter (17–22). Based on our previously published single-dose PK studies of polymyxins in neutropenic mice (16, 23), a subcutaneous dose of 30 mg/kg administered thrice daily would result in the area under the concentration-time curve of unbound polymyxins over 24 h (fAUC) of ~20 to 33 mg · h/liter, which is equivalent to an f_{CSS,avg} of ~0.8 to 1.5 mg/liter for both polymyxins (16, 23). A limitation of the present study is that single-dose PK of polymyxins was not studied in thermally injured mice due to the reduction principle in animal ethics. The complex pathology of thermal injury may alter the PK of many drugs in humans (24). Recently, the effects of thermal injury on the PK of intravenously administered CMS was investigated in burn patients (25, 26), and it appeared that the percentage of thermally injured area relative to the total body surface area affected the PK of colistin (26). In our burn wound infection mouse model, the thermal injury was mild and the area (1.4 cm diameter) was only ~1.92% of the total body surface area of the mice (24, 27). Therefore, it is unlikely that the PK of both polymyxins was substantially altered in our burn wound infection mouse model. Nevertheless, our current study revealed that subcutaneously administered polymyxins (30 mg/kg thrice daily) were able to effectively decrease the bacterial load by ~2 log_{10} in thermally injured mice infected with P. aeruginosa or A. baumannii (Fig. 1). This is consistent with the previous observations in thermally injured patients, in which parenterally administered CMS (5 mg/kg daily; maximum, 160 mg every 6 h) displayed positive clinical outcomes and prevented sepsis in thermally injured patients by reducing endotoxin levels (28, 29).

The antibacterial efficacy of colistin versus polymyxin B for treating burn wound infections caused by Gram-negative pathogens is shown in Fig. 1. There were no significant differences in the antibacterial efficacy between colistin and polymyxin B for all isolates (P > 0.05). These results are similar to those from our previous in vivo study, in which equimolar daily doses of each polymyxin resulted in similar antibacterial efficacy for K. pneumoniae ATCC BAA 2146 and FADDI-KP032 isolates in a mouse thigh infection model (23). Our recent PK/PD study of aerosolized polymyxins also revealed that aerosolized polymyxin B displayed in vivo PK/PD characteristics similar to those of aerosolized colistin (30, 31). Likewise, both polymyxins displayed similar in vitro PD properties (Table 1) (12). These findings have major clinical implications, as colistin is administered in the form of the inactive prodrug CMS, whereas only formed colistin is the antibacterial entity (10, 32). A clinical PK study in healthy subjects showed that the...
CMS-to-colistin conversion was relatively slow and low after intravenous administration (33). In contrast, polymyxin B is administered in its pharmacologically active form (11, 34). This rapid attainment of the target polymyxin B concentration was linked to the superior bacterial killing in a one-compartment in vitro model (35). Future clinical

TABLE 1 \( \text{fAUC/MIC} \) values of subcutaneous colistin and polymyxin B at 30 mg/kg/8 h

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC (mg/liter) of:</th>
<th>( \text{fAUC/MIC} ) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colistin</td>
<td>PMB</td>
</tr>
<tr>
<td>( P. \text{aeruginosa PAO1} )</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( P. \text{aeruginosa 19056muc} )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( A. \text{baumannii ATCC 19606} )</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( A. \text{baumannii 248-01.C248} )</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>( K. \text{pneumoniae ATCC BAA 2146} )</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>( K. \text{pneumoniae KP1} )</td>
<td>0.5</td>
<td>0.5</td>
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</table>

\( ^a \)Colistin and polymyxin B (PMB) dosage regimens involved 30 mg/kg thrice daily to achieve \( \text{fAUC} \) of \( \approx 33 \) (16) and \( \approx 20 \) (23) mg · h/liter, respectively.

\( ^b \)\( \text{fAUC/MIC} \), area under the unbound polymyxin concentration-time curve over 24 h divided by the MIC.

Values are \( \text{fAUC/MIC} \) achieved after treatment with 30 mg/kg thrice daily and were calculated based on the assumption that mild thermal injury had no significant effect on the PK of polymyxins in mice (25).
studies are needed to examine which of the two clinically available polymyxins is superior in treating burn wound infections.

\( \text{fAUC/MIC} \) was previously identified as the most predictive PK/PD index to describe the antibacterial efficacy of polymyxins against \( P. \ aeruginosa, A. \ baumannii, \) and \( K. \ pneumoniae \) in a mouse thigh infection model (16, 23). Polymyxin B and colistin share similar PK/PD targets against the same isolate because of similar in vivo PK/PD properties and molecular weights (16, 23). A good correlation between the previously established \( \text{fAUC/MIC} \) targets for thigh infections and the PD outcome for treating burn wound infections was demonstrated in the present study (16). Assuming that the small-area thermal injury has no major effect on the PK of polymyxins in mice, 90 mg/kg/day polymyxin B and colistin would achieve a polymyxin \( \text{fAUC/MIC} \) value of 66 to 40 for strains with an MIC of 0.5 mg/liter or 33 to 20 for strains with an MIC of 1 mg/liter (Table 1), which were much higher than the \( \text{fAUC/MIC} \) targets of 7.4 to 17.6 against \( P. \ aeruginosa \) and \( A. \ baumannii \) for a 2-log\(_{10}\) reduction in a neutropenic mouse thigh infection model (16, 23). In agreement with these PK/PD targets, \( \geq 2 \text{-log}_{10} \) reductions were achieved in the present study for the burn wound infections caused by \( P. \ aeruginosa \) and \( A. \ baumannii \) (Fig. 1). Our results suggest that the current standard dosage regimens for polymyxins are likely effective for burn wound infections caused by \( P. \ aeruginosa \) and \( A. \ baumannii \). In contrast, PK/PD studies on systemically administered polymyxin B in a neutropenic mouse thigh infection model revealed that even with the highest tolerated polymyxin B dosage regimen (120 mg/kg/day), a 2-log\(_{10}\) reduction was not achieved against \( K. \ pneumoniae \) (23). Similarly, our present study demonstrated that a 2-log\(_{10}\) reduction was not achieved against \( K. \ pneumoniae \) in thermally injured mice with 30 mg/kg/8 h colistin or polymyxin B (Fig. 1 and Table 1). Consistent with our previous PK/PD studies, the magnitude of antibacterial killing of polymyxins against \( K. \ pneumoniae \) strains was lower than that against \( P. \ aeruginosa \) and \( A. \ baumannii \); this may be partially due to the capsule in \( K. \ pneumoniae \) (9). The exact mechanism for the differences in the in vivo efficacy of polymyxins against different bacterial species remains unclear, and further studies are warranted.

To the best of our knowledge, this is the first preclinical study to demonstrate the therapeutic efficacy of systemically administered polymyxins for treating burn wound infections caused by MDR \( P. \ aeruginosa, A. \ baumannii, \) and \( K. \ pneumoniae \). Furthermore, we demonstrate that previously established PK/PD targets against mouse thigh infection are useful in guiding the selection of the optimal dosage regimens for the treatment of burn wound infections. Well-designed clinical studies are warranted to investigate the likely impact of thermal injury on the PK of polymyxins and their potential therapeutic efficacy in burn patients.

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We have no conflicts of interest to declare.

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